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Molecular Diagnosis and Clinical Relevance of t(9;22), t(4;11) and t(1;19) Chromosome Abnormalities in a Consecutive Group of 141 Adult Patients with Acute Lymphoblastic Leukemia

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Over a time period of five years leukemic blast samples from 141 consecutive patients with adult ALL were referred to our laboratory, for molecular evaluation of chromosome abnormalities. The t(9;22), t(4;11) and t(1;19) which are most commonly found in adult ALL with a B-precursor phenotype were molecularly analyzed by similar RT-PCR based protocols. BCR-ABL transcripts generated by the t(9;22) translocation were demonstrated in 36 patients (25%) and were restricted to the 109 patients with B precursor ALL (33% of this group). Of 83 patients showing a common phenotype (CD10+), 34 were BCR-ABL positive (41%) whereas only 2 out of 26 with Null ALL (HLA-Dr+, CD19+, CD10–) were positive. Interestingly, the percent of BCR-ABL positive CD10+ ALL increases significantly with age being 20% in patients less than 30 years old and more than 50% in older patients. None of the T-ALL (24 patients) and B-ALL (8 patients) were positive. The majority of cases (67%) showed the p190 gene subtype.

The cytogenetic diagnosis of Philadelphia chromosome was always confirmed by the molecular analysis and this approach allowed for the detection of the presence of the BCR-ABL rearrangement in 26 patients when a negative result or no metaphases were obtained. The complete remission rate was similar among BCR-ABL positive and negative patients but a shorter remission duration was observed in those showing molecular evidence of t(9;22) and this finding was significantly evident in CD10+ ALL patients. By means of comparison, in most of the same adult ALL patients, we analyzed the yet unrecognized prevalence of the t(4;11) and t(1;19) translocations by the molecular analysis of their chromosomal breakpoints. Rearrangements of the ALL-1 gene on 11q23 band and ALL-1/AF.4 fusion transcripts specific for the t(4;11) were demonstrated in 7 out of the 21 Null ALL investigated, with no additional positive cases found among the other ALL subgroups. Overall the clinical behavior of t(4;11) positive patients was dismal with a very short CR duration. Chimeric E2A-PBX1 transcripts generated by the t(1;19) were found in only two of the 87 B-precursor ALL analyzed.

The presented results provide further evidence for the utility of RT-PCR based methods for the molecular diagnosis of chromosome translocations in ALL. The identification of such abnormalities can significantly contribute to the identification of more appropriate therapeutic options for standard and high risk ALL patients

KEY WORDS: ALL chromosome translocations molecular diagnosis

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INTRODUCTION

In acute lymphoblastic leukemia (ALL) chromosomal translocations are adverse prognostic indicators and karyotypic studies have emerged as major prognostic determinants in both adult and childhood ALL.[1,2] An abnormal karyotype is an adverse risk factor in adult ALL independent of age, immunophenotype and initial leukocytes.[3–5] Achievement and duration of complete remission as well as survival have been found different among chromosome groups, but cytogenetic analysis of ALL is, however, a difficult challenge because standard chromosome-processing techniques often yield ill-defined metaphases and several studies underscored the limitations of this approach for the diagnosis of chromosome abnormalities in ALL.[5] The genes involved in some of these chromosome abnormalities have been molecularly identified and some of them were shown to generate chimeric transcripts and proteins. Therefore new molecular tools became available for an accurate and rapid molecular diagnosis of chromosome abnormalities. In particular, the balanced translocation t(9;22) present in the Philadelphia chromosome confers an especially ominous outlook.[6] The use of Reverse Transcription Polymerase Chain Reaction (RT-PCR) recently suggested that the frequency of the t(9;22) chromosomal abnormality in adult ALL might be higher than established by conventional cytogenetics.[7] However, some discrepancies have been noted in the prevalence reported by different authors in B precursor ALL.[8–10] Similarly, a very aggressive clinical course characterizes those patients carrying the t(4;11) (q21;q23) abnormality[11] while at least in childhood ALL, patients with the t(1;19) (q23;p13.3)[12,13] also have an aggressive course. Since the breakpoints of both these translocations have been recently cloned[14–17] and RT-PCR based methods have been developed for the diagnosis and monitoring of the generated fusion transcripts,[18–22] we planned to investigate the prevalence of these three abnormalities in a series of 141 adult ALL patients consecutively referred to our laboratory over a period of five years.

MATERIALS AND METHODS

Patients

Bone marrow (BM) or peripheral blood (PB) samples obtained from 141 adult ALL patients, were consecutively sent to our laboratory from five different hematologic Institutions. The patients ranged in age from 14 to 80 years (median 33). Diagnosis was established according to standard morphologic, immunophenotypic and cytochemical criteria. Leukemic blast cells (more than 80% pure) for DNA and RNA extraction or cytogenetic analysis were obtained from BM or PB samples by Ficoll-Hypaque centrifugation. These patients were treated according to previously published chemotherapy regimens; 21 patients on HEAV'D protocols;[4,23] 107 on IVAP protocols.[24] These protocols included an intensive four-drug induction chemotherapy that included an anthracycline, with multidrug post-remission consolidation and prolonged standard maintenance chemotherapy. Thirteen patients received palliative treatment because of their advanced age or for poor clinical conditions.

Cytogenetics

Chromosome preparations were obtained from direct BM cell cultures and from BM cell cultures not stimulated for 24 hours. The preparations were stained with quinacrine mustard (QFQ-banding technique) and 40 metaphases were routinely analyzed.

RT-PCR amplification of chimeric transcripts

Total RNA was prepared from leukemic samples according to a modification of the guanidinium isothyocyanate/CsCl method and used for cDNA synthesis and PCR amplification.[18] In vitro reverse transcription to cDNA of approximately 1 μg of total RNA was performed using 2.5 units of cloned Moloney Murine Leukemia Virus (Mo-MLV) reverse transcriptase in a volume of 20 μl containing 50 pmol of random hexamer primers, 1 mmol/l dNTP, and 20 U of RNAse inhibitor using the commercial Gene AMP kit (Cetus Perkin Elmer, Norwalk, CT) for 45 minutes at 42°C, 5 minutes at 99°C and 5 minutes at 5°C. Oligonucleotides used for RT-PCR analysis (Table 1) were synthesized according to published sequences.[7,18,22] For the amplification of the cDNA products, PCR mixture containing a final concentration of 2 mM $MgCl_2$, 1 × PCR Buffer, 2.5 U/100μl of Taq DNA Polymerase and 0.15 pM of specific sense and antisense primers was added (primers A and D for M-BCR; F and D for m-BCR)[7] (primers E5 and P3 for E2A/PBX transcripts).[22] PCR reactions were performed on an automated heat block (PCR system 9600, Perkin Elmer-Cetus). For detection of t(9;22) and t(1;19) the follow program of amplification was used: after an initial denaturation at 95°C for 1 min., 35 cycles of amplification reactions were performed each consisting of a denaturation step at 95°C for 10 seconds and an annealing/extension step at 60°C for 15 seconds. A final extension at 60°C for 6 minutes (1 cycle) and cooling at 4°C was done at the end. The first PCR product could be used for a second round of amplification (for further 35 cycles, in 100 μl final volume) as follows: DNA 5 μl; $MgCl_2$ 2mM; PCR buffer 1 ×, dNTPs 1mM; Taq DNA polymerase 2.5 U/100

Table 1 Sequences of oligonucleotides used for RT-PCR analysis of t(9;22), t(4;11) and t(1;19).

t(9;22)	*t(4;11)*	*t(1;19)*
oligo A: (B1B2 sense) 5′ GAAGAAGTGTTTCAGAAGCTTCTCCC 3′ ***oligo B***: (B2 sense) 5′ GTGAAACTCCAGACTGTCCACACGCA 3′ ***oligo C***: (A2 antisense) 5′ TCCACTGGCCACAAAATCATACAGT 3′ ***oligo D***: (A3 antisense) 5′ TGTGATTATAGCCTAAGACCCGGAG 3′ ***oligo F***: (E1 sense) 5′ AGATCTGGCCCAACGATGGCGAGGGC 3′ ***oligo L***: (E1 sense inner) 5′ ATGGCGAGGGCGCCTTCCAT 3′ ***oligo ABL Ia***: (ABL Ia sense) 5′ ATCTGCCTGAAGCTGGTGGGCT 3′ ***oligo G***: (Probe B3A2) 5′ TGGATTTAAGCAGAGTTCAAAAGCCCTTCAGCGGCCAGTA 3′ ***oligo H***: (Probe B2A2) 5′ GCTGACCATCAATAAGGAAAGAAGCCCTTCAGCGGCCAGTA 3′ ***oligo I***: (probe B1A2) 5′ TCCATGGAGACGCAGAAGCCCTTCCAGCGGC 3′	***oligo Ex. 5***: (sense) 5′ GAGGATCCTGCCCCAAAGAAAAG 3′ ***oligo Ex. 6***: (sense) 5′ CGCCCAAGTATCCCTGTAAAAC 3′ ***oligo AF4.1***: (antisense) 5′ TGAGCTGAAGCTGGTCTTCGAGC 3′ ***oligo Ex7 AS***: (antisense) 5′ CTTAAAGTCCACTCTGATCCT 3′	***oligo E5***: (sense) 5′ TGCACAACCACGCGGCCCTC 3′ ***oligo P3***: (antisense) 5′ CGCCACGCCTTCCGCTAACA 3′ ***oligo EP***: (probe) 5′ CGACTCCTACAGTGTTTTGAGTATCC 3′ ***oligo E3***: (antisense) 5′ CTTCTCCTCCTCCGAGTGGT 3′

μl and nested primers (B and C for M-BCR and L and C for mBCR). As control, amplifications of the normal ABL or E2A transcripts were performed (primers Ia and D for ABL and E5 and E3 for E2A). The PCR reaction for detection of t(4;11) was similar but the final concentration of $MgCl_2$ was 1.5mmol/L. Moreover, after an initial denaturation at 94°C for 1 minute, 30 cycles of amplification were performed with specific sense and antisense primers (Ex5 and AF4.1).[18] One cycle of denaturation, annealing and extension consist of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 30 seconds. At the end 1 μl of the first PCR product was used for a second round of amplification for further 30 cycles using the nested primer Ex6. The amplification of the normal ALL-1 gene mRNA was performed with the same cDNA preparation and the same conditions used to identify ALL-1/AF4 junctions, by the primers Ex6 and Ex7AS. To detect the amplified fragments, 30 μl of PCR mixtures were run on a 2.5 Nusieve % agarose gel stained with ethidium bromide and visualized under a UV lamp. After fractionation by electrophoresis through agarose gel, PCR products could be transferred to nylon membranes (Genescreen Plus, New England Nuclear, Boston, MA) and hybridized to specific ^{32}P end-labeled oligonucleotides spanning the junctions. Membrane were washed according to standard procedures.

Southern analysis

High molecular weight DNA was obtained from leukemic samples following standard procedures. After digestion with Bgl II and BamHI, DNAs were electrophoresed on 0.8% agarose gels, denatured and blotted onto nylon membranes. The B859 cDNA probe spanning the ALL-1 gene from exon 5 to 11 (kindly provided by Dr. G. Cimino, University of Rome, Italy) was labeled with the ^{32}P random-priming method. Filters were hybridized overnight, washed and exposed for 48 to 72 hours for autoradiography as previously described.[18]

Statistical analysis

For definition of complete remission (CR) the bone marrow had to be normocellular with trilineage normal hematopoiesis and no identifiable leukemic cells. CR curves were plotted using the Kaplan-Meier method and compared using the log-rank method. Proportions of patients achieving a response were compared using the Fisher's exact test.[4] Because of the large number of variables and the small sample size, variables were screened by univariate analysis using the log Rank test for their potential association with CR duration. Little or no association with CR duration ($p \leq 0.15$) resulted in the exclusion of that variable from the multivariate model. The selected variables (age, number of blasts at diagnosis and BCR-ABL positivity) were then included in the final proportional hazards Cox regression equation.

RESULTS

Detection of BCR-ABL fusion transcripts

One hundred and forty one patients were molecularly evaluated by RT-PCR for the presence of chimeric transcripts generated by the t(9;22) chromosome translocation. Cumulative results of this analysis are summarized in (Table 2). Overall, BCR-ABL transcripts were found in 36 patients (25%) and were restricted to the 109 ALL cases with B precursor phenotype (33% of this group). Of 83 patients showing a common phenotype (CD10+), 34 were BCR-ABL positive (41% of this subgroup) whereas only 2 out of 26 Null ALL (HLA-Dr+, CD19+, CD10–, CD20–) were positive. Interestingly, in the group of CD10+ ALL, the percent of BCR-ABL positive patients increased significantly with age being 20% in patients less than 30 years old and more than 50% in older patients (Table 3). None of the T-ALL (24 patients) and B-ALL (8 patients) were found positive. The majority of BCR-ABL positive cases (61%) showed the p190 gene subtype. Moreover, most patients less than 41 years old had the chimeric m-BCR-ABL fragment whereas an increase in the proportion of patients with a M-BCR breakpoint was seen in older patients. Chromosome preparations were adequate in only 34 out of 109 B-precursor ALL (31%). The molecular analysis always confirmed the cytogenetic diagnosis and allowed to demonstrate the presence of BCR-ABL rearrangement in 26 additional patients in which the analysis was not performed or a negative result or no metaphases were obtained.

Table 2 Detection of BCR-ABL chimeric transcripts in adult ALL: correlation with the immunophenotype

Subtype (No)	*Immunophenotype*	*M-BCR*	*m-BCR*
Null (26)	HLA-DR+ CD19+	0	2
Common (83)	HLA-DR+ CD19+ CD10+ (CD20+/–)	12	22
B (8)	HLA-DR+ CD19+ CD10+ CD20+ Slg+	0	0
T (24)	cyt CD3+ (CD7+ CD2+ CD5+ etc.)	0	0

Molecular evaluation of t(4;11) and t(1;19) in adult ALL

We have recently shown that all ALL patients with cytogenetic evidence of the t(4;11) chromosome abnormality can be successfully analyzed by a molecular approach.[18] Since chromosome 1, 10 and 19 can also contribute to translocations involving the 11q23 region, we first planned to investigate the presence of 11q23 gene rearrangements by Southern blot analysis using a cDNA probe spanning the ALL-1 gene from exon 5 to 11. DNA samples were available for 80 patients with B-precursor ALL and an abnormal configuration of the ALL-1 gene was demonstrated only in 7 of the 21 patients with a Null phenotype (Table 4). Four of these cases had also cytogenetic evidence of t(4;11). On the contrary, none of the 59 CD10+ cases, showed either cytogenetic or molecular evidence of 11q23 gene rearrangement. By RT-PCR, ALL-1/AF4 transcripts were demonstrated in all the seven patients where rearranged bands of the ALL-1 gene had been previously found by Southern analysis (table 4). Moreover, RT-PCR allowed to demonstrate that the breakpoint sites on the ALL-1 gene were between exon 7 and 8 in 4 patients or between exon 8 and 9 in 3 patients.

In childhood ALL the t(1;19) chromosome translocation accounts for up to 6–10% of all cases of ALL. Since this figure has not been evaluated yet in large consecutive series of adult ALL patients we planned to determine the prevalence of the E2A-PBX1 fusion gene by of a RT-PCR based molecular approach in 87 B-precursor ALL. E2A-PBX1 chimeric transcripts where shown in two out of the 63 patients with CD10+ ALL analyzed (Table 5). For both these patients cytogenetics confirmed the molecular results. None of the 24 Null ALL had karyotypic or molecular evidence of t(1;19). (Table 5).

Correlation of molecular abnormalities with clinical outcome

One hundred twenty eight patients were suitable for statistical evaluation of BCR-ABL status as a clinical prognostic indicator. The main clinical and laboratory findings of these patients are summarized in Table 6. Of these patients, 21 were treated according to HEAV'D protocols and 107 according to IVAP based regimens.[4,23–24] These studies gave comparable long-term results in both standard and high risk patients as defined by age, immunophenotype and blast cells count. Ninety eight patients (76%) obtained complete remission and were eligible for remission duration evaluation. Twenty five patients did not achieved complete remission and five had too short follow up data. No major differences were seen in the CR rate observed among BCR-ABL positive and negative patients (70% and 78% respectively). As shown in Fig. 1, the majority of BCR-ABL positive patients relapsed within one year, and remission lasted two years or more in only five out of nineteen cases at risk. Interestingly, among these five patients, two had p190 and three p210 rearrangement. Within the CD10+ ALL subgroup, outcome was significantly worse in BCR-ABL positive patients (Fig. 2). By performing a multivariate analysis taking into account the potential confounding effect of age and number of blasts at diagnosis, BCR-ABL positive patients had a relative risk of 2.2 (95% confidence intervals 0.88–5.68; p = 0.087) of relapse as compared to BCR-ABL negative patients. The major clinical and hematologic characteristics of these CD10+ ALL patients by BCR-ABL status are reported in Table 7. Again, when stratified by treatment protocol, no significant differences were observed among HEAV'D or IVAP treated patients.

Since t(4;11) appears to be present only in the group of Null ALL, the clinical correlation analysis was restricted

Table 3 Molecular analysis of t(9;22) in CD10+ adult ALL: age distribution

Age Between	*N° Cases*	*M-BCR*	*m-BCR*
14–30	35	1	6
31–40	10	0	5
41–50	9	3	3
>51	29	8	8

Table 4 11q23 (ALL-1 gene) rearrangements and t(4;11) fusion transcripts detected in adult ALL: correlation with the immunophenotype

Subtype (No)	*Immunophenotype*	*11q23 (ALL-1 gene) Rearrangements*	*ALL-1/AF4 Transcript*
Null (21)	HLA-DR+ CD19+	7	7
Common (59)	HLA-DR+ CD19+ CD10+ (CD20+/–)	0	0
T (8)	cyt CD3+ (CD7+ CD2+ CD5+ etc.)	0	0

Table 5 Detection of E2A-PBX chimeric transcripts in adult ALL: correlation with the immunophenotype

Subtype (No)	*Immunophenotype*	*E2A-PBX*
Null (24)	HLA-DR+ CD19+	0
Common (63)	HLA-DR+ CD19+ CD10+ (CD20+/–)	2
B (2)	HLA-DR+ CD19+ CD10+ CD20+ SIg+	0

Table 6 Main characteristics of clinically evaluable ALL patients

	Total	*BCR/ABL+*	*ALL-1/AF4+*	*E2A/PBX+*	*Neg*
N°Cases	128	30	7	2	89
Median age (range)	34 (14–73)	49 (16–69)	33 (14–63)	(35–22)	25 (14–73)
Hemoglobin (gr/100 ml)	9.5 (3–14.6)	9.5 (3–13.3)	9.6 (5.4–11.7)	7.4	9.8 (4.5–14.6)
Blasts at diagnosis (× 109/L)	5.6 (0–999)	5.2 (0–140)	27 (0.6–999)	(3.4–1.2)	5.6 (0–675)
Male:Female	64/64	17/13	3/4	0/2	48/41
Liver	39	7	2	0	30
Spleen	44	8	2	1	33
Lymphadenopathy	34	7	2	1	25
HEAV'D Protocol	21	4	1	0	16
IVAP Protocol	107	26	6	2	73
FAB 1:2:3	27:95:6	2:28:0	2:5:0	0:2:0	23:60:6

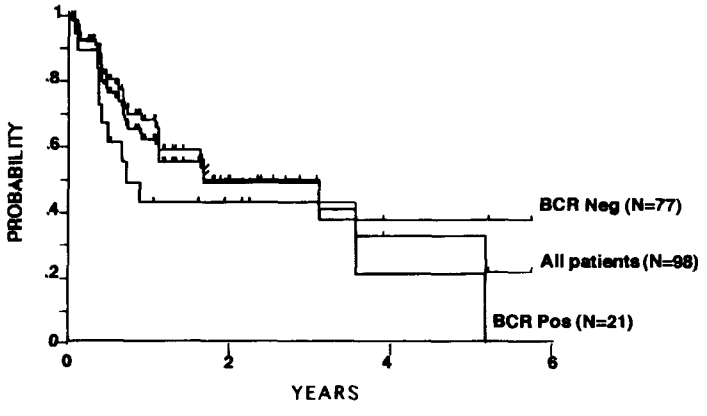

Figure 1 Complete remission duration for all patients and BCR-ABL positive and negative subgroups. Patients were treated according to HEAV'D and IVAP protocols as detailed in Results. Statistical analysis of remission duration between the presented groups showed no significant differences. The analysis used was the Kaplan-Meyer procedure and the difference was compared by the log rank test.

to this specific diagnostic subset. Nineteen out of twenty-six patients were valuable for remission duration according to the presence of ALL-1/AF4 chimeric transcripts. As shown in Figure 3, the outcome of t(4;11) positive patients was extremely poor. Finally, the two patients carrying the t(1;19) translocation are both in first CR after 10 and 26 months respectively.

DISCUSSION

There is a general consensus that patients carrying the t(9;22) and t(4;11) chromosome translocations are at the highest risk of leukemia relapse when treated accordingly to conventional chemotherapy.[25] Moreover, in childhood ALL the presence of the t(1;19) correlates significantly with several recognized adverse prognostic features including higher leukocyte counts and higher serum LDH.[12,13] The aim of this study was therefore the identification of such leukemias which may with some certainty be expected to be incurable without allogeneic bone marrow transplantation or other novel experimental approaches. Since all these chromosome aberrations can now be analyzed by rapid and sensitive molecular techniques we planned to investigate the prevalence of these translocations on a large series of adult ALL cases consecutively referred to our laboratory. Although the molecular analysis was performed on a consecutive group of patients en-

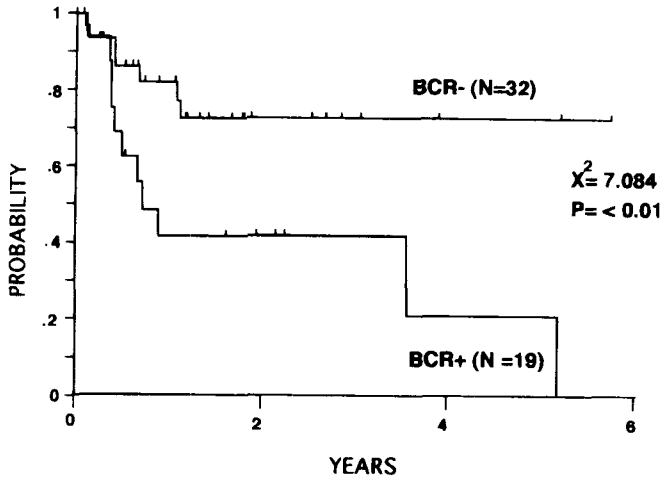

Figure 2 Complete remission duration of CD10+ ALL patients. A comparison of remission duration between the BCR-ABL positive and negative showed a significant different clinical outcome (P = 0.01; X^2 = 7.084) by the stratified log rank test.

Table 7 CD10+ ALL patients evaluable for CR duration: clinical and laboratory data at diagnosis

	BCR/ABL + *(n = 19)*	*BCR/ABL–* *(n = 32)*	*P Value**
Median age (range)	47 (16–63)	20 (14–73)	0,05
Hemoglobin (gr/100 ml)	9.2 (7.1–13.7)	9.2 (5–13.3)	
Blasts at diagnosis (× 109/L)	5.2 (0–73)	4.4 (0–73)	
Male:Female	8:11	15:17	
Liver:Spleen	5:5	6:11	
IVAP/HEAV'D Protocol	16/3	22/10	

*P = by Fisher exact test

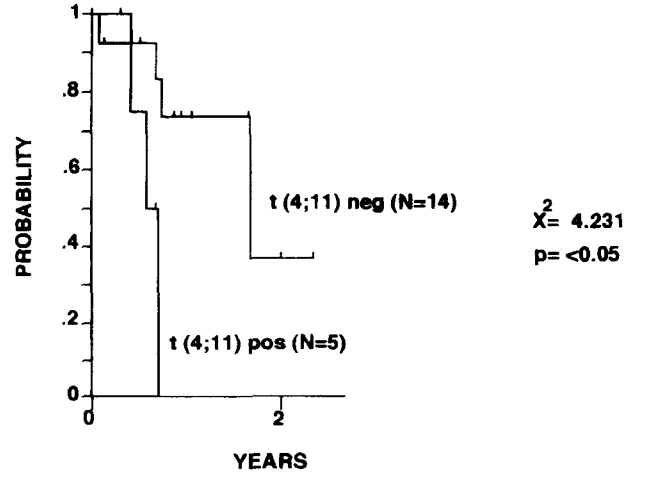

Figure 3 Complete remission duration for t(4;11) positive and negative Null-ALL B-ALL patients. The P value is indicated (X^2 = 4.231).

rolled to specific clinical trials,[23,24] samples were sent to the laboratory from different clinical institutions and we cannot rule out that some degree of selection might have occurred. Because in most of our patients cytogenetic analysis was not performed or insufficient metaphases were obtained the molecular analysis was of crucial importance to diagnose a relevant number of high risk ALL patients.[26] We found that BCR-ABL fusion gene is a common finding in adult ALL even though in our study the overall prevalence of t(9;22) positive cases, was slightly lower than reported by Maurer and Westbrook in their studies[7,9] and more similar to what was reported by others.[8,10] As already shown,[9,28] the overall percent of BCR-ABL positive and negative patients who enter in complete remission is remarkably similar. However, when all ALL patients were analyzed by BCR-ABL status, there was an evident trend toward a longer remission duration of BCR-ABL negative cases. Nonetheless some Ph+ ALL cases were found to remain in prolonged complete remission for more than two years and this allow to speculate that the clinical behavior of such cases might reflect a yet unrecognized biologic heterogeneity. More importantly, although by multivariate analysis we could not demonstrate the independent prognostic significance of BCR-ABL positivity, we found that the lack of t(9;22) in patients with a CD10+ immunophenotype allows the definition of a subgroup of ALL cases with a remarkable good clinical outcome. The identification of such patients, biologically similar to childhood ALL, deserves particular attention because it may avoid the use of inappropriate treatment which is, in itself an advantage to the patient. Once again our results suggest that the different frequency of t(9;22) positive cases could be a major reason for the difference in outcome between adult and childhood ALL where the survival of Ph+ patients is equally poor but where the frequency of this abnormality is less than 5%.[5] The dismal long term clinical results obtained in Ph+ ALL strongly recommend the exploration of new experimental approaches. In this respect it might be of interest that with some intensive protocols the overall remission rate can probably be further improved.[24] The bulk of evidence demonstrating that Ph+ ALL is more frequent in patients with advanced age emphasize the importance that an increasing number of such patients could be enrolled into more aggressive protocols. The rapid development and improvement of the supportive therapy offered by the hematopoietic reconstitution with autologous or allogeneic transplants will certainly contribute in this field.

The t(4;11) chromosome translocation characterizes a subset of B precursor ALL with a very aggressive clinical course. Although almost always restricted to ALL patients with null phenotype, some cases have been found among other phenotypic subgroups. Our results provide further evidence that a molecular diagnosis of t(4;11) can be made in a significant proportion of null ALL cases and our results are in keeping with those recently reported by others.[29] Although the total number of the t(4;11) positive cases does not allow statistically conclusive results to be drawn the clinical behavior of these patients was confirmed to be very poor with a very modest median CR duration. Interestingly, the comparison of CR duration between t(4;11) positive and negative patients revealed a significant difference when the analysis was performed within the group of null ALL cases confirming what has been published previously.[11] This strongly emphasizes the importance of the molecular diagnosis of this abnormality which can allow for rapid identification of these patients who need to be enrolled, whenever possible, into allogeneic bone marrow transplantation programs. It is in fact worth noting that other groups have suggested the importance of the molecular approach in order to identify the presence of molecular rearrangements of the 11q23 breakpoint cluster region in patients not identified by conventional cytogenetics. The peculiar biology of these blast cells generates additional difficulties in obtaining good quality metaphases.

In our study we found only two patients carrying the t(1;19) chromosome translocation even though in childhood ALL this chromosome abnormality is the most common non random translocation occurring in 5% of all cases and in 25% of pre-B ALL (cytoplasmic immunoglobulin positive but surface Ig negative). Although the molecular analysis we used cannot detect all the cytogenetic positive cases,[22] RT-PCR analysis has facilitated the identification of a great majority of patients with both cIg+ and cIg– leukemic blasts.[19,22] Of 221 B-precursor childhood ALL, Izraeli and coworkers performed a comparative analysis between cytogenetics and RT-PCR[30] for the diagnosis of t(1;19). Twenty one of these patients (9%) had cytogenetic evidence of t(1;19). Although RT-PCR failed to demonstrate E2A-PBX1 chimeric transcripts in two patients with a typical t(1;19), it allowed the identification of 12 additional positive cases previously not recognised at the karyotypic analysis. Therefore the molecular approach was shown to be superior and combined with cytogentics demonstrated an overall percent of 15% of t(1;19) positive childhood ALL. Despite the total number of patients analyzed still does not allow firm conclusions to be drawn, our results might suggest that t(1;19) is likely to be less frequent in adult than childhood ALL. Interestingly, a recent and prospective large karyotypic analysis of 453 adult ALL cases allowed the identification of t(1;19) positive cases in only 2.5% of patients.[31] The clinical follow up of the two patients we found positive for E2A-PBX1 tran-

scripts is too short to be discussed. However, it is noteworthy that the more recent protocols used in childhood ALL failed to confirm the unfavorable prognosis previously associated with the diagnosis of t(1;19) ALL.[32]

In conclusion, our results confirm that the front line use of PCR has an important impact on the timely diagnosis of high risk adult ALL patients which also has crucial prognostic implications. The molecular diagnosis of chromosome abnormalities is likely to play a central role in defining new therapeutic strategies for adult ALL patients.[33]

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