The *PAX5* gene is frequently rearranged in *BCR-ABL1*-positive acute lymphoblastic leukemia but is not associated with outcome. A report on behalf of the GIMEMA Acute Leukemia Working Party

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background

Recently, in genome-wide analyses of DNA copy number abnormalities using single nucleotide polymorphism microarrays, genetic alterations targeting *PAX5* were identified in over 30% of pediatric patients with acute lymphoblastic leukemia. So far the occurrence of *PAX5* alterations and their clinical correlation have not been investigated in adults with *BCR-ABL1*-positive acute lymphoblastic leukemia.

Design and Methods

The aim of this study was to characterize the rearrangements on 9p involving *PAX5* and their clinical significance in adults with *BCR-ABL1*-positive acute lymphoblastic leukemia. Eighty-nine adults with *de novo BCR-ABL1*-positive acute lymphoblastic leukemia were enrolled into institutional (n=15) or GIMEMA (*Gruppo Italiano Malattie EMatologiche dell'Adulto*) (n=74) clinical trials and, after obtaining informed consent, their genome was analyzed by single nucleotide polymorphism arrays (Affymetrix 250K NspI and SNP 6.0), genomic polymerase chain reaction analysis and re-sequencing.

Results

PAX5 genomic deletions were identified in 29 patients (33%) with the extent of deletions ranging from a complete loss of chromosome 9 to the loss of a subset of exons. In contrast to *BCR-ABL1*-negative acute lymphoblastic leukemia, no point mutations were found, suggesting that deletions are the main mechanism of inactivation of *PAX5* in *BCR-ABL1*-positive acute lymphoblastic leukemia. The deletions were predicted to result in *PAX5* haploinsufficiency or expression of *PAX5* isoforms with impaired DNA-binding. Deletions of *PAX5* were not significantly correlated with overall survival, disease-free survival or cumulative incidence of relapse, suggesting that *PAX5* deletions are not associated with outcome.

Conclusions

PAX5 deletions are frequent in adult *BCR-ABL1*-positive acute lymphoblastic leukemia and are not associated with a poor outcome.

Key words: ALL, *BCR-ABL1, PAX5*.

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Introduction

PAX5 is a member of the highly conserved paired-box (PAX) domain family of transcription factors necessary for many types of cell differentiation. *PAX5* is the only *PAX* family member expressed within the hematopoietic system, and its expression is restricted to certain stages along the B-cell differentiation pathway.[1] Its expression is initiated in pre-pro-B cells and maintained throughout subsequent stages of B-cell development before it is down-regulated in plasma cells.[2,3] It may function both as a transcriptional activator and a repressor, because it positively controls target genes encoding essential components of (pre-) B-cell receptor signaling such as the signal-transducing chain *Igα* (also called *CD79a* and *mb-1*),[4] the co-stimulatory receptors *CD19* and *CD21*,[5] the inhibitory co-receptor *CD72*[5,6] and the central adaptor protein *BLNK* (also called *SLP65*).[7] It negatively controls *PD-1*, *NOTCH1* (which is necessary for the commitment of lymphoid progenitors to the T-cell pathway), and the transcription of *M-CSFR*, thus rendering B-cell precursors unresponsive to myeloid cytokines such as macrophage colony-stimulating factor (M-CSF).[1] The *PAX5* gene is located at 9p13 and is juxtaposed to *IGH@* in cases with t(9;14)(p13;q32), a rare but recurring translocation found in a subset of B-cell non-Hodgkin's lymphomas[8] and resulting in deregulated expression of the gene product because of the proximity of IgH enhancers. Recently, in genome-wide analyses of DNA copy number abnormalities and loss of heterozygosity (LOH) using single nucleotide polymorphism (SNP) arrays, genetic alterations (deletions, focal internal amplification, sequence mutations and translocations) targeting the B-lymphoid transcription factor *PAX5* have been identified in over 30% of cases of childhood B-progenitor acute lymphoblastic leukemia (ALL).[9,10]

The structural rearrangements included fusion of *PAX5* to *FOXP1* (3p13),[9,11] *AUTS2* (7q11),[11] *ELN* (7q11),[12] *ETV6* (12p13),[9,11,13] *PML* (15q24),[14] *ZNF521* (18q11)[9] and *C20orf112* (20q11),[11,15] *LOC392027* (7p12.1),[15] *SLCO1B3* (12p12),[15] *ASXL1* (20q11.1),[15] *KIF3B* (20q11.21),[15] *HIPK1* (1p13),[16] *POM121* (7q11),[16] *DACH1* (13q21)[16] and *BRD1* (22q13.33).[16] In each predicted fusion protein, the DNA-binding paired domain of *PAX5*, and a variable amount of the C-terminal transactivating domains are fused to functional domains of the partner genes. Using quantitative polymerase chain reaction (PCR) analysis, An *et al.*[15] demonstrated that both the deletion and gene fusion events resulted in the same under-expression of *PAX5*, which extended to the differential expression of the *PAX5* target genes, *EBF1*, *ALDH1A1*, *ATP9A* and *FLT3*, suggesting a potential pathogenic role of *PAX5* deletion in pediatric acute leukemias.[9]

The impact of *PAX5* alterations on clinical outcome was investigated for the first time by Mullighan *et al.*[17] in children with high-risk B-cell-progenitor ALL in whom it was found that abnormalities of *PAX5* copy-number failed to act as a predictor of poor outcome. However, the occurrence and the role of *PAX5* alterations have so far not been investigated in adult patients with *BCR-ABL1*-positive ALL, the most frequent and prognostically the most unfavorable subtype of ALL in adults.[18] The outcome of patients with *BCR-ABL1*-positive ALL has improved with current therapies that include tyrosine kinase inhibitors such as imatinib,[19] nilotinib[20,21] and dasatinib.[22] Complete hematologic remissions can be obtained in 98-100% of patients treated with a tyrosine kinase inhibitor alone[19] or in association with conventional chemotherapy,[23] but relapse is still an expected event in the majority of cases.[21]

Here, we used different molecular approaches, including high resolution determination of DNA copy number alterations (Affymetrix GeneChip® Human Mapping 250K NspI and Genome-Wide Human SNP 6.0 array GeneChip microarrays), fluorescence *in situ* hybridization (FISH) and re-sequencing to study rearrangements on 9p involving the *PAX5* locus in 89 adults with *BCR-ABL1*-positive ALL.

Table 1. Demographic and clinical characteristics of patients with *BCR-ABL1*-positive acute lymphoblastic leukemia.

Clinical characteristics	N. of patients (n=89)	%
Age, years		
Median (range)	53.7 (13.2-78.0)	
Blasts, %		
Median (range)	90 (28-99)	
Gender		
Male/Female	53/36	
Protocol		
LAL2000	15	16.85
LAL1205	43	48.31
LAL0201-B	16	17.98
Institutional	15	16.85
Leukocytes,(x10^9/L)	23.4 (1.4-302.0)	
Median (range)		
Molecular		
BCR-ABL1+ P210	23	26.75
BCR-ABL1 + P190	57	66.28
BCR-ABL1 + P210 and P190	6	6.98
Follow-up, months		
Median (range)	21 (0.8-148.1)	

Design and Methods

Patients

All patients gave their informed consent to blood collection and biological analyses, in agreement with the Declaration of Helsinki. The study was approved by the *Seràgnoli* Department of Hematology and Medical Sciences, University of Bologna, Italy. We analyzed 89 patients (53 males and 36 females; median age, 54 years; range, 13.2-78; median blast percentage, 90%; range, 28-99%) with *de novo BCR-ABL1*-positive ALL enrolled between 04/1996 and 08/2008 in different clinical trials of the GIMEMA AL Working party or in institutional protocols (Table 1). In detail, 74 patients (83%) were enrolled in GIMEMA clinical trials (16 patients in the GIMEMA LAL0201-B protocol, 15 in the LAL2000 protocol and 43 in the LAL1205 protocol), while 15 patients (17%) were enrolled into institutional protocols. Details of the treatments schemes have been previously reported.[24] Briefly, patients enrolled for the LAL0201-B protocol were elderly (>60 years), Philadelphia chromosome (Ph)-positive ALL patients who received imatinib 800 mg daily, associated with steroids as front-line treatment.[19] Patients enrolled in the LAL2000 study were adults (>18

years) with ALL, including Ph-positive cases, who received induction and consolidation chemotherapy followed by maintenance therapy with imatinib. The LAL1205 trial[25,26] enrolled adult Ph-positive ALL patients who received dasatinib 70 mg *bid* for 84 consecutive days, as induction therapy, initially associated with steroids, without further chemotherapy as frontline treatment.

Nineteen patients underwent allogeneic stem cell transplantation in complete hematologic response as consolidation therapy (5 had an HLA-identical donor, 9 had a matched unrelated donor, 1 had an allogeneic graft and 4 an autologous transplant). At diagnosis, all patients were found to be *BCR-ABL1*-positive. The percentages of *BCR-ABL* fusion transcripts corresponding to p210 *versus* p190 *versus* p190+p210 were 27%, 66% and 7%, respectively.

Single nucleotide polymorphism microarray analysis

All 89 *BCR-ABL1*-positive ALL patients were analyzed by SNP array for *PAX5* deletions. Genomic DNA was extracted using the DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) from mononuclear cells isolated from peripheral blood or bone marrow aspirate samples by Ficoll gradient centrifugation. DNA was quantified using the Nanodrop Spectrophotometer and quality was assessed using the Nanodrop and by agarose gel electrophoresis.

Samples were genotyped with GeneChip® Human Mapping 250K NspI (69 cases, 78%) and Genome-Wide Human SNP 6.0 (20 cases, 22%) arrays (Affymetrix, Santa Clara, CA, USA) as previously described.[27] Copy number aberrations were scored using the hidden Markov model and the segmentation approach available within the Partek Genomic Suite (Partek Inc, Saint Louise, MO, USA) software package as well as by visual inspection. Five consecutive markers for the Human Mapping 250K NspI and ten for the Genome-Wide Human SNP 6.0 arrays were considered as alterations. All aberrations were calculated by a comparison with a set of 270 HapMap normal individuals and a set of samples obtained from people with acute leukemia in remission (n=20) in order to reduce the noise of raw copy number data. Copy number aberrations were also analyzed and eventually confirmed using Genotyping Console 3.0 (Affymetrix, Santa Clara, CA, USA).

Fluorescence in situ hybridization and probes

FISH analysis for the *PAX5* locus was performed as previously described.[27] Overlapping bacterial artificial chromosome (BAC) probes specific for the *PAX5* gene [RP11-465P6 (36,899,192-37,065,371), RP11-243F8 (36,834,935-37,023,204), and RP11-344B23 (36,764,163-36,939,832)], as well as a BAC for BCR [RP11-164N13 (chr22:21,897,904-22,091,572)] and a fosmid probe specific for the *IKZF1* gene [G248P800745C8 (chr7:50,381,496-50,422,338)], were properly selected according to the March 2006 release of the University Santa Cruz (UCSC) Human Genome Browser (http://genome.ucsc.edu/). At least 15 metaphases were analyzed for each patient.

PAX5 genomic quantitative polymerase chain reaction

PAX5 genomic quantitative PCR (Q-PCR) was performed in order to confirm SNP results and to characterize the extension of the deletion in 20/29 (69%) patients with *PAX5* deletion and in three normal germline cases. It was performed as described by Mullighan *et al.*[18] using a 7900 Real-Time PCR system and 7900 System software (Applied Biosystems, Foster City, CA, USA).

PAX5 point mutation screening

Genomic re-sequencing of *PAX5* coding exons was performed in 34 cases of *BCR-ABL1*-positive ALL. One hundred nanograms of genomic DNA was amplified with 2 U of FastStart Taq DNA Polymerase (Roche Diagnostics, Mannheim, Germany), 0.8 mM dNTP, 1 mM MgCl2, and 0.2 M forward and reverse primers in 25 μL reaction volumes (*Online Supplementary Table S1*). PCR cycling parameters were: one cycle of 95°C for 5 min, 35 cycles of 95°C for 30 s, 62°C for 30 s and 72°C for 1 min, followed by one cycle of 72°C for 7 min. PCR products were purified using a QIAquick PCR purification kit (Qiagen) and then directly sequenced using an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems) and a Big Dye Terminator DNA sequencing kit (Applied Biosystems). In some cases the PCR products were sub-cloned into the PCR®2.1-TOPO vector using the TOPO TA Cloning Kit (Invitrogen, San Diego, CA, USA). The cloned PCR products were purified and sequenced as described above. All sequence variations were detected, using the BLAST software tool (*www.ncbi.nlm.nih.gov/BLAST/*), by comparison to reference genome sequence data obtained from the UCSC browser (*http://genome.ucsc.edu/cgibin/hgGateway?db=hg18;March 2006 release*).

PAX5 real-time polymerase chain reaction and gene expression analysis

Mononuclear cells from 31 samples were separated as previously described[27] and total cellular RNA was extracted using the RNeasy total RNA isolation kit (Qiagen, Valencia, CA, USA). One microgram of total RNA was reverse transcribed using the High Capacity cDNA Archive Kit (Applied Biosystems). PCR using primers specific for exon 1 and exon 10 (F1 and R1, respectively, in *Online Supplementary Table S1*) of *PAX5* and nucleotide sequencing were performed to identify the isoforms derived from focal deletions. RNA integrity was confirmed by PCR amplification of *GAPDH* mRNA, which is expressed ubiquitously in human hematopoietic cells. *PAX5* gene expression was analyzed using the Hs00277134_m1 assay, a 7900 real-time PCR system and 7900 System software (Applied Biosystems). Results were expressed as $2^{-\Delta\Delta Ct}$. *GAPDH* was used as a control gene (Hs99999905_m1).

Statistical analysis

The primary end-points of the study were achievement of complete remission, duration of first complete remission (in terms of disease-free survival and cumulative incidence of relapse), and overall survival. The median follow-up time was estimated by reversing the codes for the censoring indicator in a Kaplan-Meier analysis.[28] Differences in the distributions of prognostic factors in subgroups were analyzed by the χ^2 or Fisher's exact test, and by the Kruskal-Wallis test. Overall survival was defined as the time from diagnosis to date of death or date of the last follow-up. Disease-free survival and the cumulative incidence of relapse were calculated from the time of achieving complete remission to the date of first

relapse, death or date of last follow-up. The probabilities of overall and disease-free survival were estimated using the Kaplan-Meier method[28] and the probability of the cumulative incidence of relapse[29] was estimated using the appropriate non-parametric method, considering death in complete remission as a competing risk. The log-rank test was used to compare treatment effect and risk factor categories for the Kaplan-Meier curves and Gray's test was used for the incidence curves; 95% confidence intervals (95% CI) were estimated using the method of Simon and Lee.[30] Cox proportional hazard regression models[31] were performed to examine and check for treatment results and the risk factors affecting disease-free survival.

All tests were two-sided, with *P* values of 0.05 or less indicating a statistically significant difference. All analyses were performed using the SAS software (SAS Institute, Cary, NC, USA).

Results

Deletion of PAX5 *occurs in 33% of adult cases of* BCR-ABL1*-positive acute lymphoblastic leukemia*

We determined the occurrence of *PAX5* alterations in a large cohort of adult patients with *BCR-ABL1*-positive ALL (n=89). These patients were enrolled from 1996 to 2008 in different clinical trials of the GIMEMA AL Working Party or in institutional trials (Table 1). Overall, by SNP array analysis we identified a loss of *PAX5* in 29 patients (33%). In all cases, the deletion was heterozygous. Four patterns of *PAX5* deletion were observed: (i) focal deletion involving only the *PAX5* gene in one case (3%); (ii) deletions involving only a portion of *PAX5* and both telomeric and centromeric flanking genes in 11 cases (38%) with a median size of 310 kb (range, 101 kb-16,395 kb) and ranging from 9p13.3 to 9p21.3; (iii) broader deletions involving the entire *PAX5* locus and a variable number of flanking genes in ten patients (34%) with a median size of 1,999 kb (range, 567 kb-18,208 kb) and ranging from 9p13.3 to 9p21.3; (iv) deletion of all chromosome 9 or 9p in seven patients (24%) (Figure 1 and *Online Supplementary Table S2*).

Since *PAX5* point mutations have been described to occur in B-lineage ALL,[17,32] we sought to determine whether this could also be the case in adult *BCR-ABL1*-positive ALL. We, therefore, investigated both normal and deleted *PAX5* subgroups for point mutations by PCR and subsequent deep exon-sequencing analysis but we failed to find any nucleotide substitutions, indicating that deletions were the main mechanism of *PAX5* alterations in our cases (*Online Supplementary Table S2*).

Furthermore, we investigated a molecular association between *PAX5* and *IKZF1* deletions. In 23/29 cases (88% of *PAX5*-deleted patients and 26% of the whole cohort studied) both *PAX5* and *IKZF1* losses were detected whereas in six patients (7%), loss of *PAX5* was associated with a normal pattern of *IKZF1*. In 34/89 cases (38%) a deletion of *IKZF1* was not associated with loss of *PAX5*, while the remaining 26 patients (29%) were normal for both *PAX5* and *IKZF1* (Table 2 and *Online Supplementary Table S2*).

PAX5 *deletions were confirmed by fluorescence* in situ *hybridization analysis and quantitative polymerase chain reaction*

FISH analysis with three overlapping BAC probes encompassing the whole *PAX5* gene (Figure 2A) was performed in two patients with *BCR-ABL1*-positive ALL, both harboring homozygous *IKZF1* deletions and heterozygous *PAX5* deletions as determined by SNP arrays.

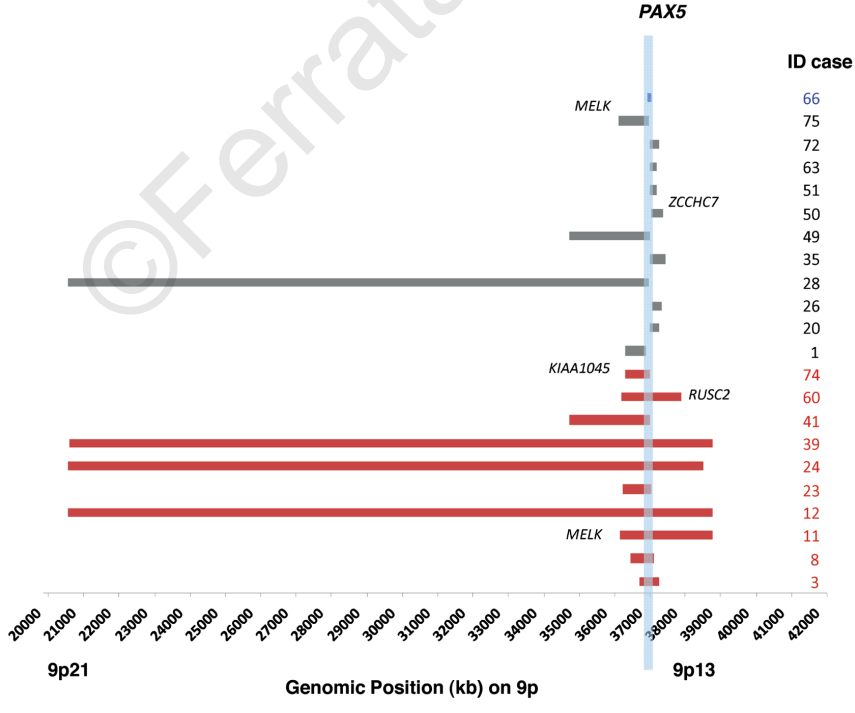

Figure 1. Diagrammatic representation of 29 *PAX5* deletions as assessed by SNP arrays. The size of the mono-allelic deletion is indicated by the horizontal bars. Each row represents a type of deletion: the focal deletion involving only the *PAX5* gene is in blue; deletions involving only a portion of *PAX5* and flanking genes are in gray; broader deletions involving *PAX5* and a variable number of flanking genes are in red. Deletions of the entire chromosome 9 are omitted. The genomic location of *PAX5* is shown by the vertical sky-blue box.

The results obtained indicate that in case #8 the breakpoint mapped within clone RP11-465P6, while in case #12 the deletion removed all the *PAX5* probes (Figure 2B).

Moreover, *PAX5* deletions, together with *IKZF1* losses, were only detected in Ph-positive cells, while no deletion was ever observed in Ph-negative cells (Figure 2C and *data not shown*).

Real-time genomic Q-PCR of *PAX5* was performed in order to confirm the SNP results and to characterize the extension of the deletions in 20/29 (69%) patients with deletions. There was complete concordance between the extent of the deletion previously identified by SNP array and the quantitative PCR results (*Online Supplementary Table S3*).

Furthermore, to investigate the consequences of genomic *PAX5* alterations in patients with *BCR-ABL1*-positive ALL, real-time quantitative PCR (RQ-PCR) was used to assess the expression of *PAX5* in cases with copy number changes on 9p13.2. RQ-PCR showed a down-modulation of *PAX5* transcript levels ($P<0.03$) in patients (n= 6) with deletion compared to those without (n=25) (*Online Supplementary Figure S1*). Interestingly, in one patient the deletion of *PAX5* involved only a subset of exons (2-6) resulting in an alternative transcript predicted to encode a prematurely truncated protein, due to a frame-shift, lacking key *PAX5* functional domains (*Online Supplementary Figures S2 and S3*).

PAX5 deletions and their clinical significance

PAX5 deletions were equally distributed between age, time (i.e. DNA sample stability-integrity) and between protocols (*Online Supplementary Table S4*). As previously reported,[24] all patients (100%) enrolled into protocol LAL1205 and all patients (100%) enrolled into protocol LAL-0201-B obtained a complete hematologic response and for this reason it was not possible to correlate this with other parameters. Therefore, only patients enrolled

Table 2. Frequency of *PAX5* and *IKZF1* copy number losses in 89 *BCR-ABL1*-ALL cases studied by SNP arrays.

IKZF1 and *PAX5* genomic status	N. of patients (%)
IKZF1 wt + *PAX5* wt	26 (29)
IKZF1 wt+ *PAX5* loss	6 (7)
IKZF1 loss + *PAX5* loss	23 (26)
IKZF1 loss + *PAX5* wt	34 (38)
Subtotal	89 (100)

Wt: wild-type.

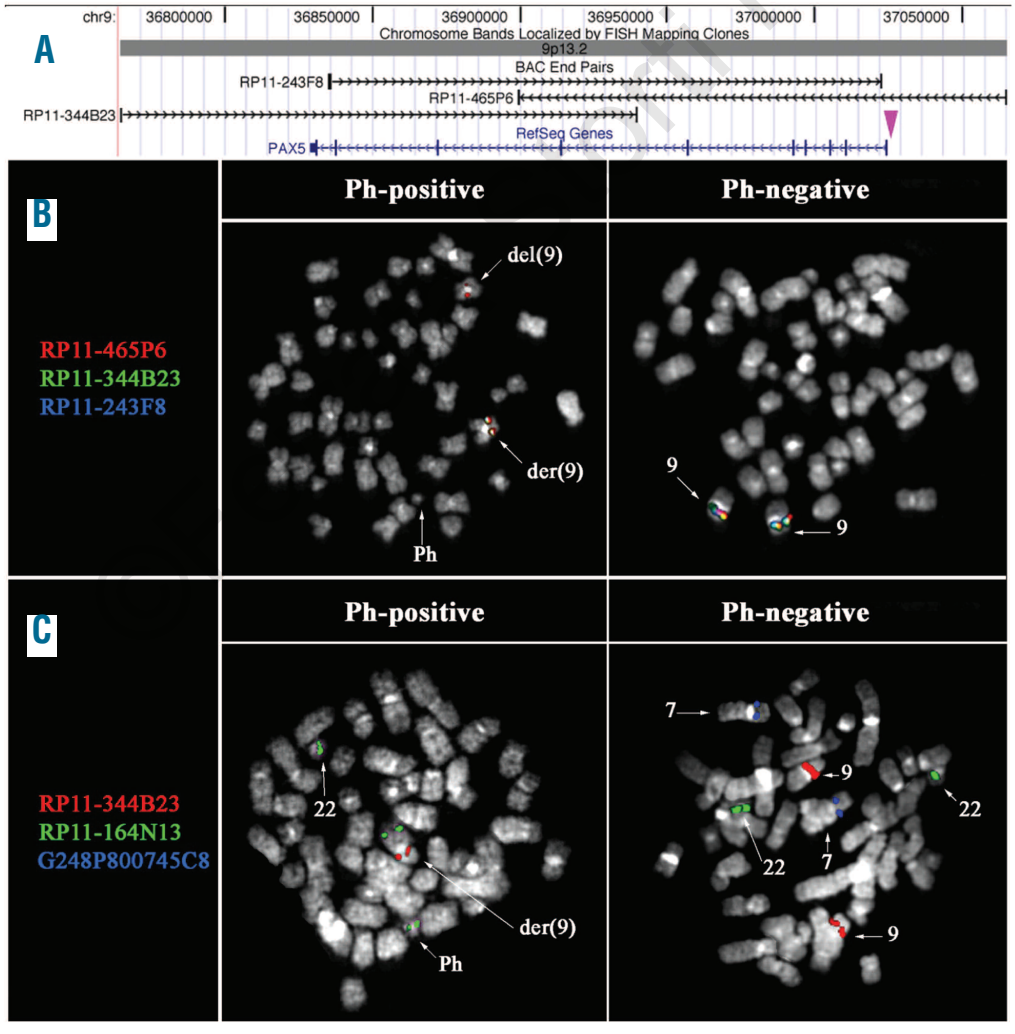

Figure 2. FISH results. (A) Map of the clones used in FISH experiments to detect *PAX5* deletions. The purple arrowhead indicates the breakpoint region mapped in case-#8 (B) and (C) FISH results obtained in case-#8 showing: (B) heterozygous deletion of clones RP11-344B23 (green) and RP11-243F8 (blue) and partial heterozygous deletion of RP11-465P6 (red) only in Ph-positive cells (middle column). Ph-negative cells showed co-localization of all three probes in both chromosome 9 homologs (right column); (C) revealing the occurrence of the *PAX5* heterozygous deletion (only one signal of RP11-344B23, in red) as well as *IKZF1* homozygous deletion (no signal of fosmid G248P800745C8, in blue) only in Ph-positive cells, as shown by the FISH pattern of the RP11-164N13 (*BCR*) probe (in green). Ph-negative cells did not show deletion of either *PAX5* or of *IKZF1*.

into the LAL2000 protocol and into institutional protocols (n=30) were evaluable for a correlation between complete hematologic response and *PAX5* deletion. Since one patient enrolled in LAL2000 never started therapy, 29 patients were evaluable (*Online Supplementary Table S5*). At the end of induction chemotherapy, 23/29 patients (79%) obtained a complete hematologic response whereas six (21%) were resistant to induction chemotherapy (ie, >25% blasts persisting in the marrow) (*data not shown*): 12/23 patients (52%) obtaining a complete hematologic response had wild-type *PAX5*, whereas 11/23 patients (58%) had a *PAX5* deletion (P=0.057), suggesting that *PAX5* deletion is not associated with a reduced probability of obtaining a complete hematologic response.

Deletions of *PAX5* were also not significantly correlated with overall survival (P=0.3294), disease-free survival (P=0.9249) or cumulative incidence of relapse (P=0.945), suggesting that *PAX5* deletions are not associated with outcome (Table 3 and Figure 3).

Discussion

In this study, we aimed at characterizing, by high resolution determination of genomic copy number alterations (Affymetrix GeneChip® Human Mapping 250K NspI and Genome-Wide Human SNP 6.0 microarrays), the rearrangements on 9p involving the paired box 5 gene (*PAX5*) and their clinical significance in adult *BCR-ABL1*-positive ALL. The rationale of this study was based

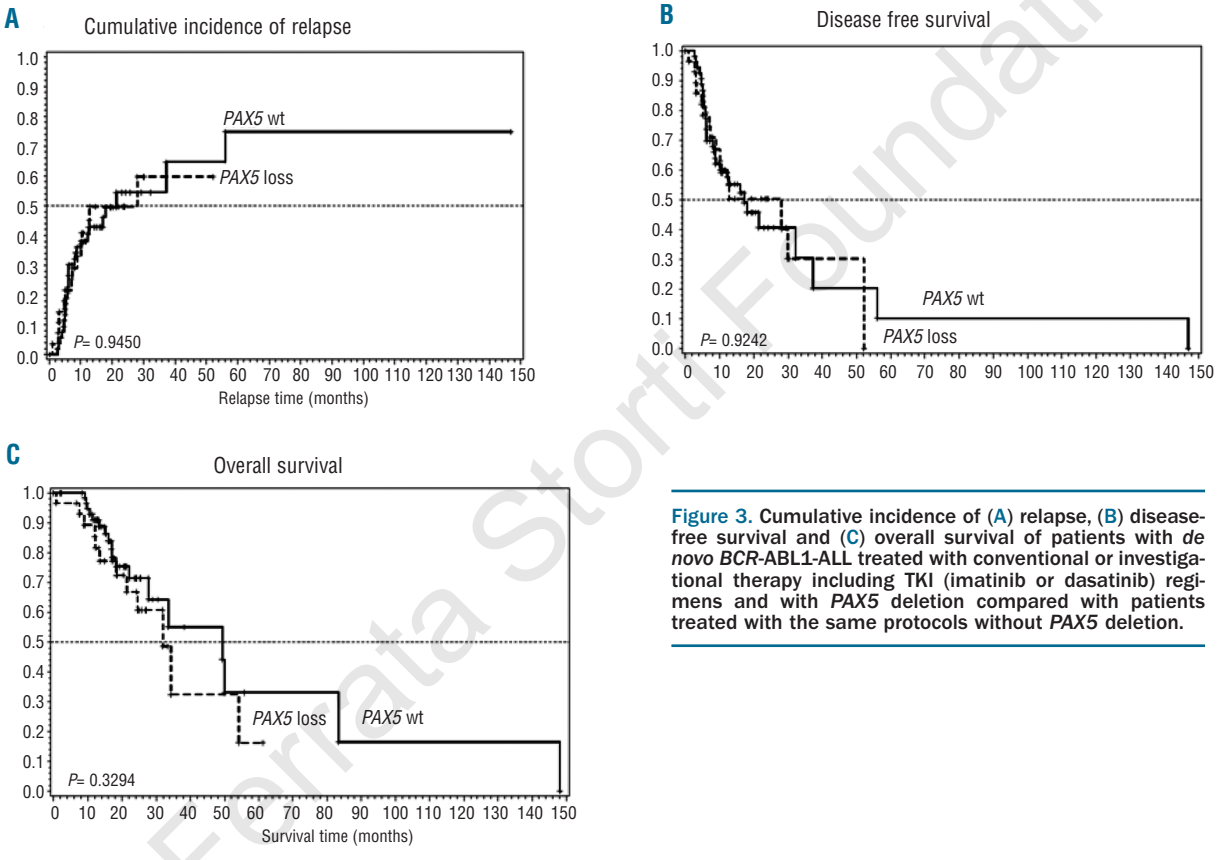

Figure 3. Cumulative incidence of (A) relapse, (B) disease-free survival and (C) overall survival of patients with *de novo BCR*-ABL1-ALL treated with conventional or investigational therapy including TKI (imatinib or dasatinib) regimens and with *PAX5* deletion compared with patients treated with the same protocols without *PAX5* deletion.

Table 3. Clinical outcome related to *PAX5* loss in univariate analysis.

			PAX5 status		
		ALL Patients N=89 % (C.I.95%)	*PAX5* wt N=28 % (C.I.95%)	*PAX5* deleted N=28 % (C.I.95%)	*P* value
Cumulative incidence of relapse	Patients at 24 months Median time	89 51.9% (51.1-52.7) 21.4 mo	28 54.5% (53-55.9) 21.4 mo	28 49.7% (47.5-51.8) 28 mo	0.9450
Disease-free survival	Patients at 24 months Median time	82 45% (39.8-50.8) 17.1 mo	54 40.7% (34.6-47.9) 17.1 mo	28 50.3% (41.4-61.2) 28.1 mo	0.9249
Overall survival	Patients at 24 months Median time	89 69.9% (62.3-78.5) 34.3 mo	60 71.4% (61.8-82.6) 49.3 mo	29 66.8% (55-81.1) 31.9 mo	0.3294

wt: (wild-type).

on the observation that alterations of *PAX5* occur in different B-cell malignancies, suggesting a role of *PAX5* as an oncogene. In non-Hodgkin's lymphoma *PAX5* is often over-expressed or targeted by aberrant somatic hypermutation,[33] whereas some cases of childhood ALL demonstrate monoallelic loss of *PAX5*, point mutations or even novel fusion genes.[9,11] Here, *PAX5* deletions affecting the entire gene or only some exons were identified in 33% of cases of adult *BCR-ABL1*-positive ALL. Deletions were predicted to result in either *PAX5* haploinsufficiency or expression of *PAX5* alleles with impaired DNA-binding. The latter was the case of a focal deletion affecting only a subset of exons (2-6) and resulting in an alternative isoform encoding a never previously described prematurely truncated protein lacking key PAX5 functional domains. Like other members of the *PAX* gene family, the *PAX* transcript is known to be alternatively spliced[34] to produce several distinct transcripts that modify the amino acid sequence of the putative PAX5 proteins.[35,36] Our results suggest that alternative splicing isoforms may derive from genomic deletion. Since, genomic deletions have been demonstrated to be important prognostic factors in ALL,[17,24] we sought to determine whether *PAX5* deletions affected outcome in adult patients with *BCR-ABL1*-positive ALL. However, when we investigated the association of *PAX5* deletions with clinical outcome, no association was detected. Thereafter, the contribution of both *PAX5* and *IKZF1* alterations to clinical outcome was assessed showing that only the following combinations, *PAX5* normal and *IKZF1* loss *versus PAX5* loss and *IKZF1* normal and *PAX5* loss and *IKZF1* loss *versus PAX5* loss and *IKZF1* normal, were associated with significant *P* values (P=0.027 and P=0.031, respectively) (*Online Supplementary Table S6*). These data confirmed that *IKZF1* status strongly affects the prognosis of adults with *BCR-ABL1*-positive ALL.[24] From a biological point of view, this difference could be attributed to the different roles the two transcription factors have during B-cell development, as hypothesized by Georgopoulos.[37] In multipotent hematopoietic stem cells, Ikaros promotes the priming of a cascade of lymphoid genes and at subsequent stages of lineage restriction and represses the expression of hematopoietic stem cell-specific genes.[17] In B-cell-restricted progenitors, *PAX5* is required for the transition from the pro-B cell to the pre-B cell.[1,38] It is likely that the loss of *PAX5* is not associated with a poor outcome because of a lack of deregulation in stem cell-associated genes.

In conclusion, *PAX5* deletions are frequent in adult *BCR-ABL1*-positive ALL and are often associated with a loss of *IKZF1*. A better understanding of the role of these genes in the development of *BCR-ABL1*-positive ALL may lead to the identification of new targets for novel therapies.

Authorship and Disclosures

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Appendix

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