Characterization of *ABL1* expression in adult T-cell acute lymphoblastic leukemia by oligonucleotide array analysis

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ABSTRACT

Background and Objectives

Recent data have highlighted an involvement of *ABL1* in T-cell acute lymphoblastic leukemia (T-ALL). Specifically, the presence of a fusion gene involving *ABL1* and *NUP214*, both located at 9q34, has been reported. We sought to evaluate whether T-ALL patients with overexpression of ABL showed a peculiar gene expression pattern and were characterized by having specific rearrangements.

Design and Methods

We previously assessed the expression profile of 128 adults with ALL by oligonucleotide arrays: 33 had T-ALL. In the current study, we evaluated the expression levels of *ABL1* in T-ALL cases and found three patients who had *ABL1* levels comparable to those detected in BCR/ABL$^+$ cases and one who had a significantly higher level of *ABL1* expression. In order to establish the incidence of *ABL1* overexpression in T-ALL, we evaluated 17 additional patients by quantitative (Q)-polymerase chain reaction (PCR) and reverse transcription (RT)-PCR.

Results

The three cases with *ABL1* expression levels comparable to those found in BCR/ABL$^+$ cases had a specific signature characterized by a high expression of genes involved in regulation of transcription. The fourth case, with the highest levels of *ABL1*, harbored the *NUP214-ABL1* rearrangement, which was confirmed by fluorescence *in situ* hybridization (FISH). Three of the four patients were refractory to induction chemotherapy. Of the 17 additional patients evaluated by Q-PCR and RT-PCR, none showed *ABL1* overexpression.

Interpretation and Conclusions

Overall, overexpression of *ABL1* was found in 8% of T-ALL cases. These results underline the value of microarray analyses for the identification of specific signatures associated with ABL1 overexpression, as well as rearrangements, e.g. *NUP214-ABL1*, in adult T-ALL.

Key words: *NUP214/ABL1*, T-lineage acute lymphoblastic leukemia, gene expression profile, ABL1 expression.

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Translocation t(9;22) (q34;q11) was the first identified non-random cytogenetic aberration in oncology. From a molecular standpoint, this translocation results in increased tyrosine kinase activity of the *ABL1* gene, which, in turn, disrupts several downstream pathways, namely cell cycle, apoptosis and cell adhesion.[1] The presence of the BCR-ABL fusion protein induces a very heterogeneous phenotype; in fact, not only can this aberration be found in different hematologic malignancies, e.g. chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), but at least three different fusion proteins have been described. In ALL, BCR-ABL is usually associated with a common/pre-B phenotype and more rarely with a pro-B phenotype; contrariwise, BCR-ABL involvement in T-lineage ALL is exceedingly rare and usually occurs in either blastic transformation of CML or true BCR-ABL$^+$ T-ALL.[2] Recent data support a novel role of BCR-ABL and/or *ABL1* also in T-ALL.[3] A rare fusion transcript, namely e6-a2 BCR-ABL, has been detected in a T-cell line.[4] Similarly, two new rearrangements involving the *ABL1* gene, *NUP214-ABL1* and *EML1-ABL1*, have recently been described in T-ALL.[5,6] In addition, amplification of the *ABL1* gene[7] was detected in 2.3% of children and 4.3% of adults with T-ALL in a study that evaluated 280 cases from a single institution[8] and in 6.8% in another study that enrolled only adult patients.[9] Thus, the use of newer technologies is revealing the role of *ABL1* also in T-ALL. In the present study, we evaluated the expression of *ABL1* in T-ALL using data derived from a series of 128 adult ALL patients analyzed by oligonucleotide arrays.

Design and Methods

Patients' characteristics

One hundred and twenty-eight adult patients with a diagnosis of ALL were studied at the onset of their disease. All patients were enrolled in the Italian multicenter clinical trial GIMEMA 0496[10] and gave their informed consent to biological studies according to the Declaration of Helsinki. The study was approved by the Institutional Review Board of the Department of Cellular Biotechnologies and Hematology, University "La Sapienza" of Rome. After diagnosis, leukemic cells were collected, isolated by density-gradient centrifugation over Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) and cryopreserved in liquid nitrogen at our Institution in Rome. All samples contained more than 90% leukemic cells. The immunophenotypic, cytogenetic and molecular features of all cases were extensively and uniformly characterized.[11] In addition, samples were evaluated for cell cycle distribution by a flow cytometric acridine-orange assay, as previously described.[12] Follow-up data were collected at our Institution.

RNA extraction and oligonucleotide preparation

For oligonucleotide array analysis, total RNA was extracted using the TRIzol reagent (Gibco, Grand Island, NY, USA) and further purified using the SV total RNA isolation system (Promega, Madison, WI, USA), with minor modifications. HGU95aV2 gene chips (Affymetrix, Santa Clara, CA, USA) were used to determine gene expression profiles. The detailed protocol for sample preparation and microarray processing is available on the manufacturer's website (*http://www.affymetrix.com/support/technical/manual/expression_manual.affx*).

Data analysis and statistical methods

The mean *ABL1* expression in the 37 B-lineage ALL BCR/ABL$^+$ patients previously analyzed was used as the cut-off for defining high or low levels of expression in T-ALL patients. The choice of this cut-off derives from the fact that BCR/ABL$^+$ patients do indeed overexpress *ABL1* and therefore these cases represent an appropriate biological control for the definition of patients with high *ABL1* expression. As previously described,[13-15] Affymetrix U95Av2 gene expression data were processed and analyzed with dChip (*www.dchip.org*),[16] which uses an invariant set normalization method; model based expressions were computed for each array and probe set using the PM-MM model. Unsupervised and supervised clustering were used as described by Eisen *et al.*,[17] and the distance between two genes was computed as 1 minus the correlation between the standardized expression values across samples. Non-specific filtering criteria for unsupervised clustering were defined as follows: (i) the gene expression level was required to be higher than 100 in >5% of the samples; (ii) the ratio of the standard deviation to the mean expression across all samples was required to be between 0.5 and 10. To identify genes differentially expressed between *ABL1* high expressing vs low expressing samples, genes were required to have an average expression ≥100 in at least one group, a fold change difference ≥2 and a p-value of 0.05. Furthermore, to strengthen the robustness of the signature identified, the false discovery rate (FDR) was calculated over 5000 permutations. A sample correlation matrix was performed using the dChip program.

RT-PCR analysis

For the detection of *NUP214-ABL1* the following primers were tested: 5' NUP23: 5'-GTATTTTCCTGTTCTCTCACC-3'; 5' NUP29: 5'-CAAAGCAACGCTCCTGCTTT-3', 5' NUP31: 5'-TCTCATCCTATCTTGCTTCCT-3', 5' NUP32: 5'-TCTGTGTTCTGAGAAGCAGGT-3', 5' NUP34: 5'-ATCATGAGTGTCGTGTGTATT-3' combined with 3': ABLa3B: 5'-GTTTGGGCTTCACACCATTCC-3'. The primer sequences for *EML1-ABL1* are described elsewhere.[6] The following PCR conditions were used: denaturation at 94° C for 2 min; 35 cycles of denaturation (94°C) for

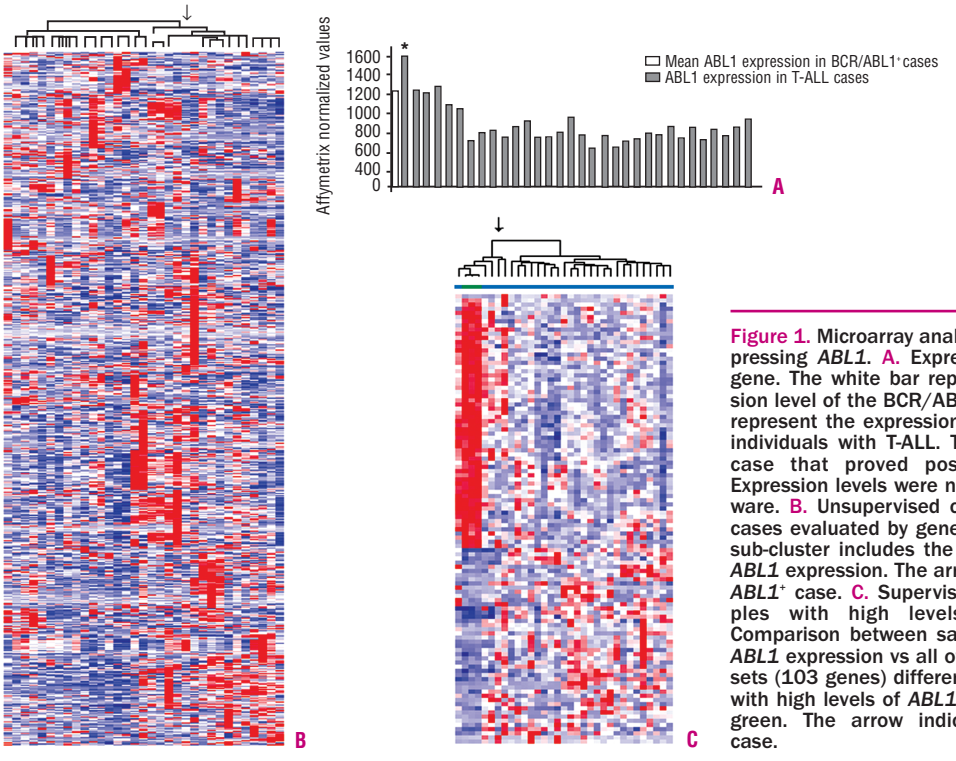

Figure 1. Microarray analysis of T-ALL cases overexpressing *ABL1*. A. Expression levels of the ABL1 gene. The white bar represents the mean expression level of the BCR/ABL$^+$ cases (n=37); gray bars represent the expression levels of *ABL1* in the 33 individuals with T-ALL. The asterisk indicates the case that proved positive for *NUP214-ABL1*. Expression levels were normalized with dChip software. B. Unsupervised clustering of the 33 T-ALL cases evaluated by gene expression profiling. One sub-cluster includes the cases with high levels of *ABL1* expression. The arrow indicates the *NUP214-ABL1*$^+$ case. C. Supervised clustering of the samples with high levels of *ABL1* expression. Comparison between samples with high levels of *ABL1* expression vs all others identified 108 probe sets (103 genes) differentially expressed. Samples with high levels of *ABL1* expression are labeled in green. The arrow indicates the *NUP214-ABL1*$^+$ case.

60 seconds, annealing (60°C) for 60 seconds, extension (72°C) for 60 seconds, and a final cycle of 10 min at 72°C. All reactions were carried out in a 25 μL volume containing 2.5 μL of cDNA sample, 10 pmol of each primer, 10 mM dNTP, 2.5 U AmpliTaq DNA Polymerase with 25 mM $MgCl_2$ and 10X buffer (Applied Biosystems, Foster City, CA, USA) using the PCR Gene Amp PCR System 9700 (Applied Biosystems). PCR positive products were sequenced using dye terminator chemistry (Big Dye Kit v3.1; Applied Biosystems) in an automatic ABI PRISM 3100 AVANT DNA sequencer.

Comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH)

CGH was performed on samples from the four patients who had high levels of *ABL1*, according to the method of Kallioniemi and colleagues.[18, 19] Target metaphases were obtained from the peripheral blood of a normal male after culture for 72 hours with phytohemagglutinin. CGH analysis was carried out using a fluorescence microscope (Provis, Olympus, Hamburg, Germany) equipped with a cooled CCD camera (Sensys, Photometrics, Tucson, AZ, USA) run by SmartCapture software (Vysis, Stuttgart, Germany). Chromosomal regions were considered overrepresented when the corresponding green:red ratio exceeded 1.18 and underrepresented when the ratio was less than 0.83. FISH analysis was performed as previously described.[20] The chromosome 9q34 region was evaluated using the BCR/ABL extra signal probe (Vysis Inc., Downers Grove, IL, USA), BAC 544A12 for the *NUP214* gene and BAC 83J21 for the 3'end of the *ABL1* gene (kindly provided by Prof. Rocchi, DAPEG, University of Bari, Italy). Five to seven abnormal metaphases were analyzed in each experiment.

Quantitative polymerase chain reaction (Q-PCR) analysis

Q-PCR analysis was performed using the ABI PRISM 7700 Sequence Detector (Applied Biosystems) and the SYBR green I dye (Applied Biosystems) method.[21] For each sample, run in triplicate, C_T values for *GAPDH* were determined for normalization purposes and the ΔC_T (ΔC_T) between *GAPDH* and *ABL1* were calculated.

The following primers were used: 5' GAPDH: 5'-CCACCCATGGCAAATTCC-3', 3' GAPDH: 5'-GATGGGATTTCCATTGATGACA-3'; 5' ABL: 5'-CCTTTTCGTTGCACTGTATGATTT-3'; 3' ABL: 5'-CCTAAGACCCGGAGCTTTT-3'.

Results

Microarray analysis of T-ALL patients with high ABL1 expression

Within our series of patients evaluated by microarray analysis, the mean level of *ABL1* expression in the B-lineage ALL BCR/ABL$^+$ cases after dChip normalization was 1202.71±408.49 SD: this value was used as a cut-point to define T-ALL patients with high *ABL1*

Table 1. Annotation analysis of the highly expressed genes in the T-ALL patients overexpressing *ABL1*. Functional annotation was performed using the DAVID database, based on gene ontology. The list below reports annotations with a *p* value <0.001.

Category	*Term*	*Count*	*%*	*P*
GOTERM_BP_ALL	Biopolymer metabolism	24	42.1	5×10^{-6}
GOTERM_BP_ALL	DNA metabolism	11	19.3	4×10^{-5}
GOTERM_BP_ALL	Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	24	42.1	1.9×10^{-4}
GOTERM_CC_ALL	Intracellular	38	66.7	2.1×10^{-4}
GOTERM_CC_ALL	Nucleus	25	43.9	2.4×10^{-4}
GOTERM_BP_ALL	Cellular physiological process	45	78.9	5.5×10^{-4}
GOTERM_CC_ALL	Intracellular membrane-bound organelle	30	52.6	6.9×10^{-4}
GOTERM_CC_ALL	Membrane-bound organelle	30	52.6	7.0×10^{-4}
GOTERM_BP_ALL	Macromolecule metabolism	26	45.6	2.1×10^{-3}
GOTERM_BP_ALL	Cellular process	48	84.2	2.6×10^{-3}
GOTERM_BP_ALL	Cellular metabolism	36	63.2	2.7×10^{-3}
GOTERM_CC_ALL	Intracellular organelle	32	56.1	2.9×10^{-3}
GOTERM_CC_ALL	Organelle	32	56.1	2.9×10^{-3}
GOTERM_BP_ALL	Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	16	28.1	4.7×10^{-3}
GOTERM_MF_ALL	Protein serine/threonine kinase activity	6	10.5	6.2×10^{-3}
GOTERM_BP_ALL	Biopolymer modification	13	22.8	6.7×10^{-3}
GOTERM_BP_ALL	Regulation of cellular metabolism	16	28.1	7.9×10^{-3}
GOTERM_BP_ALL	Primary metabolism	34	59.6	7.9×10^{-3}
GOTERM_MF_ALL	Nucleotide binding	13	22.8	8.1×10^{-3}
GOTERM_BP_ALL	Regulation of gene expression, epigenetic	3	5.3	8.8×10^{-3}
GOTERM_BP_ALL	Response to DNA damage	5	8.8	8.9×10^{-3}
GOTERM_MF_ALL	Protein binding	21	36.8	9.3×10^{-3}

expression. Using this cut-point, three T-ALL cases were found to have high levels of *ABL1* expression and a fourth case showed levels even higher than the mean level detected in the BCR/ABL$^+$ group (Figure 1A); after ordered-ranking, these cases fell in the 90th percentile. None of these cases harbored the BCR/ABL rearrangement.

Q-PCR was performed in the 33 T-ALL patients and confirmed the high levels of *ABL1* expression in these four patients. Pearson's correlation coefficient between oligonucleotide arrays and Q-PCR data was -0.72.

Unsupervised analysis of the 33 T-ALL patients identified 979 genes and recognized three major clusters. One of these clusters contained four cases described above, of which three had high levels of *ABL1* expression and the fourth case, although having high levels of *ABL1* expression, did not reach the established cut-point. These results suggest a similar mechanism of transformation for these latter samples (Figure 1B). Of note, the single case that showed the highest levels of *ABL1* did not cluster with the other three cases, suggesting a distinct genetic event.

In order to evaluate the degree of similarity between these patients, we also performed a sample correlation matrix, based on the genes selected by the unsupervised clustering: this approach highlighted a tight grouping of these three samples (*data not shown*), again reflecting a similar pattern of expression.

Next, we performed a supervised analysis comparing the three samples with high *ABL1* expression levels vs all the other samples. This approach resulted in the identification of 108 probe sets, corresponding to 103 genes, with a 90th percentile FDR of 57%. As shown in Figure 1C, also this analysis highlighted a strong similarity between the three cases with high levels of *ABL1* expression, but not with the patient having the highest levels of ABL1 expression.

Among the genes selected, 58 were more highly

Table 2. Conventional cytogenetics, CGH, RT-PCR and FISH results of the four cases with high levels of ***ABL1*** expression.

UPN	*Response to therapy*	*Conventional cytogenetics*	*CGH analysis*	*RT-PCR**	*FISH#*
12008	Refractory	11 metaphases: 46,XY 15 metaphases:46,XY, del6q	Loss 6q11-q16	Positive	Positive
19008	Refractory	15 metaphases: 46,XY	Gain 1q31.3-qter; Loss 5q14.3-q23, 16q22-qter	Negative	Negative
56007	CCR	NE	Loss 10q25.2-qter	Negative	Negative
43006	Refractory	20 metaphases: 46,XY 47,XY,del4(p14) + marker	Loss 4p14-pter	Negative	Negative

**RT-PCR evaluated for the NUP214-ABL rearrangement; #FISH evaluated for the ABL1 amplification and NUP214-ABL1 rearrangement; CCR: continuous complete remission.*

expressed in the samples displaying high *ABL1* expression. Functional annotation analysis, performed using DAVID (*http://david.abcc.ncifcrf.gov*), showed enrichment of genes that are involved in DNA metabolism (*TOPBP1, EZH2, BAF53, RFC4 TRRAP, ATRX*). Several of the other genes identified are involved in the regulation of transcription, as well as in DNA repair (*TOPBP1, ATRX*). Moreover, among the genes known to be involved in leukemic transformation we identified *SIL* and *DEK*. The complete list of genes is reported in Table 1 (*supplementary data, online only*) and the functional annotation analysis in Table 1, printed edition.

RT-PCR analysis for the presence of NUP214-ABL and EML1-ABL1

In order to check for the possible presence of the *NUP214-ABL1* fusion gene in the cases with high *ABL1* expression, RT-PCR was performed in all 33 T-ALL samples evaluated by oligonucleotide arrays, as well as prospectively in another 17 T-ALL cases. As shown in Figure 2, among the four samples with high levels of *ABL1* expression, only the patient (UPN 12008) with *ABL1* levels higher than those found in the BCR/ABL$^+$ group showed the presence of *NUP214-ABL1*, whereas the remaining cases overexpressing *ABL1* tested negative for the *NUP214-ABL1* fusion gene. Further analysis of this single case identified the breakpoint region on the exon 32 of *NUP214*. All samples were also tested for *EML1-ABL1*: none of them carried this rearrangement (*data not shown*).

CGH and FISH analysis

Following these results, we evaluated the four cases with high *ABL1* expression also by CGH and FISH analysis. As summarized in Table 2, it was not possible to identify an amplification of the 9q34 region by CGH in any of them. However, all the samples showed genetic imbalances. Variable losses were found in all cases, involving chromosomes 4p, 5q, 6q, 10q and 16q. Gains at chromosome 1q were found in only one case. FISH analysis (Figure 3A) showed the presence of extra signals (from two to ten) only in the case that was positive by RT-PCR. Amplifications were not found in two cases, while material was not available for the last case.

To further confirm that the patient harbored the *NUP214-ABL1* rearrangement and not amplification of *ABL1*, we used BAC 544A12 for the *NUP214* gene and BAC 83J21 for the 3' end of the *ABL1* gene: as shown in Figure 3B, this approach confirmed the presence of the *NUP214-ABL1* rearrangement.

RT-PCR evaluation of the presence of NUP214-ABL1 in newly diagnosed patients

Next, we used RT-PCR to look for *NUP214-ABL1* in 17 newly diagnosed T-ALL cases: none of these cases showed the presence of the rearrangement. Similarly, *EML1-ABL1* rearrangements were not detected.

Q-PCR was performed on these 17 consecutive patients, on the samples previously analyzed by oligonucleotide arrays and, as positive controls, on the K562 cell line and five samples from five patients with B-lineage ALL who harbored the BCR-ABL rearrangement. The ΔC_T was 0.64 for K562 cells, 2.55 for the B-lineage ALL BCR-ABL$^+$ patients (average expression), 1.64 for the *NUP214-ABL1*$^+$ case, 2.59 and 2.9 for the two other cases (RNA was not available for one) with high levels of *ABL1* expression, whereas the ΔC_T mean expression for the additional samples was 5.07 with no case showing ΔC_T values lower than 3.

Collectively, our results show that the incidence of *NUP214-ABL1* in T-ALL was 2% and that of *ABL1* overexpression in T-ALL was 8% in our series.

Clinico-biological characteristics of patients overexpressing ABL1

Of the four patients with high levels of *ABL1* expression evaluated by oligonucleotide arrays, three were males and one was female; their median age was 24 years (range: 16-41) and their median white blood cell count (WBC) was 42×10^9/L (range: 23.9-143). Overall, there were no statistical differences between the *ABL1* overexpressing samples and the remaining cases. Conventional cytogenetic analysis showed a normal

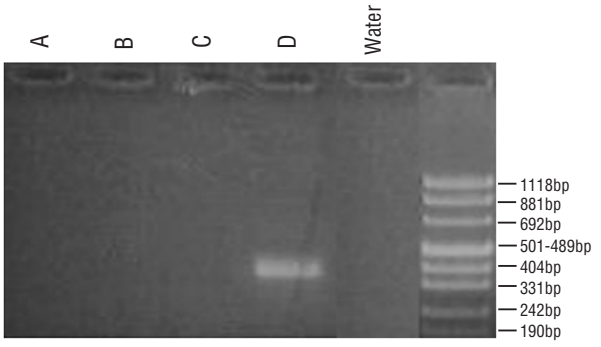

Figure 2. RT-PCR results for the four patients with high levels of *ABL1* expression. Only the patient (labeled as D) with the highest levels of *ABL1* expression proved positive for *NUP214-ABL1*.

karyotype in one case and the presence of a del(4)(p14) in another case, while the third case was not evaluable; the case (UPN 12008) that harbored the *NUP214-ABL1* rearrangement had a concurrent del(6q).

Immunophenotypic analysis of the three *ABL1*-overexpressing cases revealed a pre-T ALL profile in two and a cortical T-ALL in one case, while the *NUP214-ABL1*$^+$ case (UPN 12008) had a mature T-ALL.[22]

Cell cycle analysis of the three samples showed a decreased level of apoptosis and increased proliferation. In fact, the mean percentage of apoptotic cells was 2.9 compared to 7.1 in samples not-overexpressing *ABL1;* the mean percentage of cells in S-phase and the RNA-G1 index were 7.38 *vs* 8.23 (p=ns) and 16.2 *vs* 19.37 (p=0.06), respectively.

More importantly, from a clinical standpoint, of the four patients with high levels of *ABL1* expression, including the *NUP214-ABL1*$^+$ case, three were refractory to induction chemotherapy and only one is in continuous complete remission 55 months after having achieved complete remission.

Lack of association with other putative oncogenes

In the three cases overexpressing *ABL1*, we identified *SIL* and *DEK* among the highly expressed genes. We used RT-PCR to test whether these cases carried the *SIL-TAL1* or *DEK-CAN* aberrations, associated with T-lineage ALL[23] and acute myeloid leukemia, respectively.[24-26] Despite the documented high expression of these two genes by array analysis, these patients did not express *SIL-TAL1* and/or *DEK-CAN* fusion genes. Similarly, and at variance from previous reports,[5, 7-9] none of these three cases, nor the single patient carrying *NUP214-ABL1*, had p15/p16 deletions.

Finally, *HOX11* and *HOX11L2* were analyzed by oligonucleotide arrays or FISH analysis. *HOX11* expression was increased in two of the three cases and was not increased in the *NUP214-ABL1*$^+$ patient. FISH analysis of *HOX11L2* excluded the presence of the t(5;14), associated with *HOX11L2* expression, in all four cases (*data not shown*).

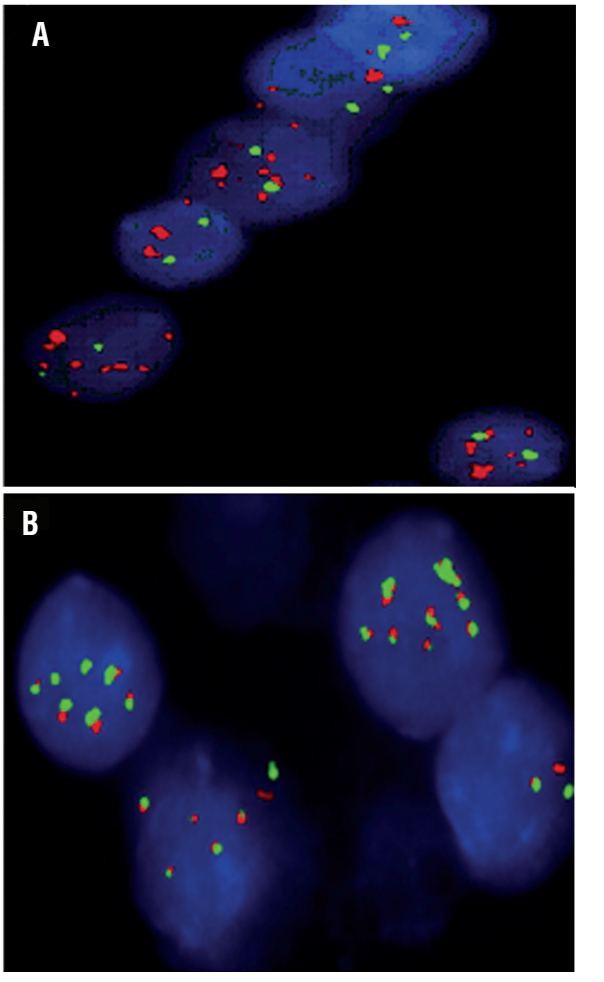

Figure 3. FISH analysis of the *NUP214-ABL1*$^+$ case. A. Detection of extra *ABL1* signals. FISH analysis of the case with the NUP214-ABL1 protein. *ABL1* signals range from 2-10. Green fluorescence: BCR; red fluorescence: *ABL1*. B. Detection of the *NUP214-ABL1* rearrangement. The *NUP214-ABL1* rearrangement was detected using the BAC 544A12 for the *NUP214* gene and BAC 83J21 for the 3'end of *ABL1*. Green fluorescence: BAC 83J21; red fluorescence: BAC 544A12.

Discussion

In this study, we used oligonucleotide array data to identify novel mechanisms of transformation in adult T-ALL. Recently, in fact, new rearrangements involving *ABL1* and *ABL1* amplifications have been described in T-ALL: *NUP214-ABL1*[5] derives from the formation of episomes that eventually lead to the fusion of *NUP214* and *ABL1*, both localized on chromosome 9q34, and *EML-ABL1*[6] generated by a t(9;14)(q34;q32); *ABL1* amplifications have been reported to occur in roughly 5% of T-ALL cases, being less frequent in pediatric cohorts.[7-9] Based on these recent reports, we were interested in identifying patients with such abnormalities using gene expression profiling. Within 33 adults with T-ALL, three cases had high levels of *ABL1*, comparable

to those found in BCR/ABL$^+$ B-lineage ALL, and one case had *ABL1* levels higher than those found in BCR/ABL$^+$ B-lineage ALL. Unsupervised clustering of the whole T-ALL population identified one sub-cluster containing the three cases that had *ABL1* overexpression: this finding is not trivial, since unsupervised analysis is based on the use of non-specific filters to select genes and is therefore not biased by superimposed biological information. Furthermore, supervised analysis of these three samples revealed a unique signature and a strong degree of resemblance between each other (correlation between samples ≥0.89, max=1). These cases were characterized by a high expression of a large set of genes. Among these, we observed an overrepresentation of genes involved in DNA replication and chromatin remodeling (*TOPBP1, RFC4, REV3L*), regulation of transcription (*BAF53, EZH2, ATRX*) and, to a lesser extent, DNA repair, suggesting that overexpression of *ABL1* may lead to an impairment in the chromatin structure and ultimately to a disruption of transcription.

It is of interest that a large set of these genes show a high degree of correlation between each other, further supporting the possibility that there may be an impairment involving not a single gene, but a whole pathway. As an example, *TOPBP1* becomes phosphorylated in response to DNA damage[27] and, more importantly, it has recently been shown to act as a repressor of *ABL1*, with an inverse correlation between the two protein products:[28] the fact that patients with high levels of *ABL1* expression concomitantly express high levels of *TOPBP1* suggests disruption in this feedback loop in leukemic cells. In a similar fashion, *ATRX* interacts with *EZH2*,[29] which also forms a complex with *EED*, a gene that has methylating functions;[30] again, *BAF53* forms a complex with *TRRAP*, involved in the control of *C-MYC* oncogenic activity.[31]

From a clinico-biological standpoint, these samples had cytogenetic imbalances, although no genetic event was common to all three: p15/p16 deletions and *HOX11L2* were not detected in these patients, while *HOX11* expression (evaluated by oligonucleotide arrays) was increased in two of the three cases. Cell cycle analysis showed an increase in the rate of proliferation and a decrease in the rate of apoptosis. Interestingly, these cases had a poor outcome, two out of three being refractory to induction chemotherapy.

Further characterization by array CGH may help to discriminate whether such cases have frank overexpression, or, as in other reports, they carry an amplification.

In unsupervised clustering, the fourth patient overexpressing *ABL1* and showing the highest levels of this tyrosine kinase did not cluster together with the other samples, suggesting that in this sample a different mechanism of leukemogenesis may have occurred: indeed, the presence of the *NUP214-ABL1* rearrangement was detected by RT-PCR and subsequently confirmed by FISH analysis. In line with this finding, Q-PCR showed high levels of *ABL1* expression. Identification of a gene signature associated with *NUP214-ABL1* was not feasible for statistical reasons.

In contrast to previous reports,[5,7-9] p15 and/or p16 deletions, as well as *HOX11* and *HOX11L2* overexpression, were not observed in this patient. Genetic characterization revealed a 6q deletion: this is of interest, considering that this deletion appears to be associated most frequently with a T-cell phenotype.[32] Furthermore, it has been suggested that the *NUP214-ABL1* fusion may not be sufficient to induce leukemia,[5] but that this requires additional genetic events: thus, in this patient the 6q deletion may have represented the secondary event and, in turn, *NUP214-ABL1* may have conferred greater aggressiveness to the leukemic cells.

From a clinical standpoint, this patient had hyperleukocytosis (WBC of 143×10^9/L) and physical examination showed liver, spleen and mediastinal enlargement. The patient was refractory to induction chemotherapy: similar findings have been previously described for such patients.[5,9,10] However, further evaluations are required to reach a firm conclusion on the likely outcome of these patients, whose prognosis may also be influenced by the presence of additional genetic abnormalities.

Our data are promising and may influence the treatment of patients with these features, since such patients may benefit from more intensive treatment strategies, possibly using combination therapies based on an association of standard polychemotherapeutic protocols with tyrosine kinase inhibitors, such as imatinib and/or II generation inhibitors, such as dasatinib.

In conclusion, the results of this study indicate that gene expression profiling can be used to identify T-ALL patients carrying a rearrangement involving *ABL1*. Overall, within our series of adult T-ALL we found that *ABL1* was overexpressed in 8% of cases. Since the outcome of these patients appears to be unfavorable in the majority of the reported series,[5,7–9,33,34] with few exceptions,[35] prospective screening based on a Q-PCR approach needs to be carried out in order to identify early such patients, who, ultimately, may benefit from a different, targeted therapeutic strategy.

Authors' Contributions

SC performed research, analyzed data and the wrote paper; ST performed research; EMG performed research; CA analyzed data; CM performed research; LE, RM, MM, MRR: performed reseach; AV: analyzed data; JR contributed analytical tools; CM provided analytical tools and critically revised the manuscript; AG designed research and critically revised the manuscript; RF designed research and critically revised the manuscript.

Conflict of Interest

The authors reported no potential conflicts of interest.

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