Identification of a novel e8/a4 BCR/ABL fusion transcript in a case of a transformed Sézary syndrome

This report deals with a case of Sézary syndrome, a rare peripheral T-cell lymphoproliferative disorder, in which cytogenetic analysis performed during the disease transformation revealed the presence of a t(9;22)(q34;q11.2) translocation. Molecular analyses identified a new transcript, an e8a4 *BCR-ABL* fusion mRNA which could be responsible for the disease transformation.

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Sézary syndrome (SS), characterized by a pruritic exfoliative erythroderma, lymphadenopathies and atypical peripheral blood T lymphocytes,[1] is considered an advanced stage of mycosis fungoides (MF). Transformation to a large cell lymphoma has been reported to occur in 8-55% of cases[2] usually with complex karyotypes.[3] Though a single case has been reported,[4] a Philadelphia chromosome (Ph^+) is unlikely to be found in cutaneous peripheral T-lineage lymphoproliferative disorders. A 74 year-old female with an MF diagnosed in June 1998 developed a SS in December 1998, with the appearance of lymph nodes and a lymphocyte count of 2 G/L. She presented 15% atypical small cells with no myeloproliferative features. PCR analysis, according to the report of BIOMED-2,[5] demonstrated a clonal rearrangement of T-cell receptor genes (TCRG) without clonal rearrangement of B-cell receptor genes. In 1999, the patient developed generalized lymphadenopathies, splenomegaly, had elevated LDH level (4508UI) and a leukocyte count of 263G/L with massive infiltration (93%) of tumoral cells, indicating a transformation to a large T-cell lymphoma.

Flow cytometry analysis of peripheral blood confirmed the T surface phenotype ($CD3^+$, $CD4^+$) of the tumoral population. No residual CD19 or CD20 positive cells could be identified.

The disease rapidly progressed and the patient died in October 1999. Chromosome studies, supplemented by fluorescence *in situ* hybridization (FISH) using the LSI BCR/ABL ES dual color translocation probe (Vysis Downer's Grove, IL, USA) and by M-FISH experiments, were performed on tumoral cells when the patient was referred to our department for the treatment of T-cell lymphoma in August 1999. Complex abnormalities were identified with the following karyotype: 45,X,der(X)t(X;1)(p22;p$\hat{}$35),del(1)(p34),der(1)t(1;10)(q2$\hat{}$4;q2$\hat{}$5),der(2)t(2;15)(q37;q22),der(4)ins(4;8)(q11;q12~22),der(6)t(6;9)(q11;p21),+8,-9,t(9;22)(q34;q11.2).isht(9;22)(ABL+, BCR;ABL+,BCR+),der(10)t(1;10)(q25;q21)t(10;10)(p14;p1$\hat{}$2),der(11)del(11)(p11)del(11)(q11),-13,der(15)t(8;15)(q2$\hat{}$3;q21),der(19)t(6;9;19)(q13~21; q34; p13).ish der(19)(ABL+) [15]. Interestingly, the patient presented structural changes involving chromosomes 1p, 2q, 6q and 19p, recurrently reported in SS.[3] FISH analysis demonstrated the typical M-BCR pattern in 52% of interphase nuclei (Figure 1A). Reverse transcription (RT) and quantitative real-time polymerase chain reaction (RQ-PCR) were performed on the same sample according to Standardised EAC (European Against Cancer) protocols previously reported[6] and expressed as *BCR-ABL/ABL* ratios according to the international scale now required.[7] The transcripts detected in the patient using the M-primers were quantified at the *BCR-ABL/ABL* rate of 0.8% which was not in keeping with the cytogenetic results positive in 52% of interphase nuclei. The direct sequencing of these PCR products showed an *e15a2* fusion transcript with no preservation of the open reading frame (ORF). A larger PCR analysis using a forward primer in the *BCR* gene (ENF402[6]) and a reverse primer (5'GCCCTCGTACACCTCCCCGTA3') in the *ABL* exon a4 revealed an atypical *e8a4* after direct sequencing (Figure 1B). However, unlike the *e8a2* transcripts previously described,[8-10] the direct junction between *BCR* exon e8 and *ABL* exon a4 led to the preservation of the ORF encompassing the whole oncogenic domains of the ABL tyrosine kinase. The quantification of the *e8a4* was carried out using RQ-PCR with an e8 forward primer from *BCR* gene,[10] the same reverse primer and a new probe (FAM 5'TCTACGTCTCCTCCGAGAGC3'TAMRA) in *ABL* exon a4. With a serial 10-fold dilution series for *e8a4* cDNA from the patient's sample, the assay was found to be linear over at

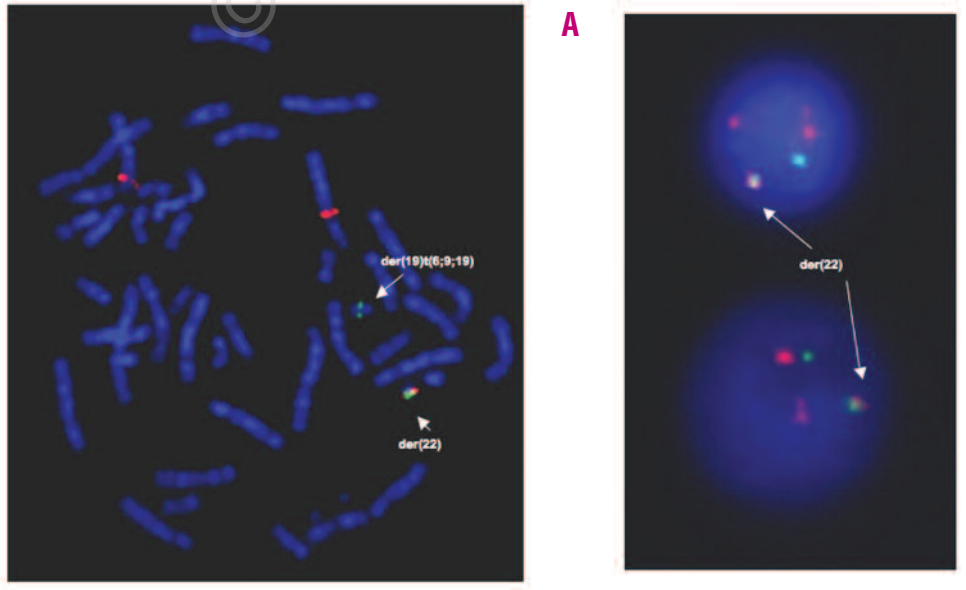

Figure 1A. FISH analysis using the LSI BCR/ABL ES dual color translocation probe, on metaphase spread (left) and on interphase nuclei (right). As a result of the BRC/ABL fusion, an orange/green fused signal (arrow) is located on the derivative chromosome 22.

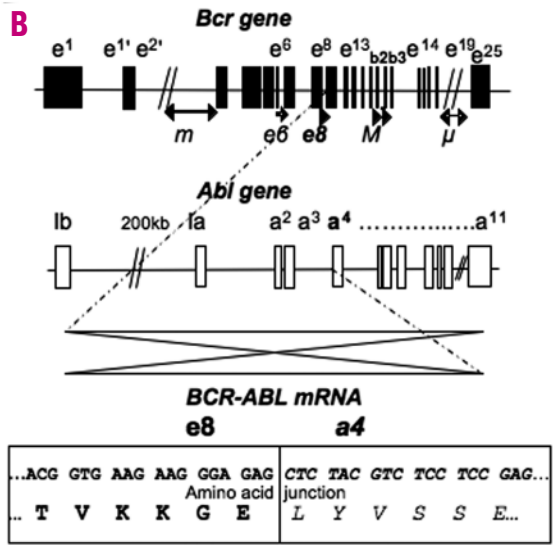

Figure 1B. Partial maps of BCR and ABL genes. Exons are indicated by boxes. Breakpoints from BCR gene are shown by black arrows. The nucleotide sequence and the amino-acid junction between exon e8 from BCR gene and exon a4 from ABL gene were indicated in bold an italic types respectively.

least 4 orders of magnitude (slope, -3.33; intercept, 45,3). The number of *e8a4* copies in the non diluted sample was estimated to be 13,600 for 10,000 copies of *ABL* which agreed with data obtained by cytogenetic analysis and confirmed that the *e8a4* must be the oncogenic fusion transcript. The detection of minority *e15a2* spliceoforms suggests that the breakpoint could be located downstream of the Major breakpoint. This genomic rearrangement could then produce multiple spliceoforms without ORF and then undergo nonsense-mediated mRNA decay as previously described. Unfortunately, no retrospective studies of the rearrangement could be performed at diagnosis because of lack of available frozen material.

Since this observation occurred in 1999, before the imatinib era, the clinical outcome for patients bearing this unusual *e8a4* transcript could not be predicted. It has to be underlined that the RQ-PCR method designed here is able to detect all known *BCR-ABL* variants and could represent as an interesting molecular strategy for patients with Ph^+ cytogenetic but negative molecular analyses. Indeed, the previous case of Ph^+ lymphoma did not show *BCR-ABL* rearrangement at molecular level.[4] Therefore, it is possible that this case may correspond to this rare *e8a4* transcript. Further studies of unusual BCR-ABL junctions and their associated diseases are required to gain further insight into their pathologic consequences and their sensitivity to new tyrosine kinase inhibitors.

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