An inv(16)(p13q22) positive acute myeloid leukaemia relapsing as acute precursor B-cell lymphoblastic leukaemia

We describe a case of a 38-year old male with inv(16)(p13q22) positive acute myeloid leukaemia (AML) with eosinophilia, relapsing after a molecular remission of almost three years. Remarkably, the leukaemia at relapse was identified as a precursor-B-cell acute lymphoblastic leukaemia (B-ALL) by cytology and immunophenotyping, but was inv(16)(p13q22) positive as revealed by interphase FISH, FICTION analysis, and real-time quantitative PCR. Analysis of immunoglobulin and T-cell receptor genes showed a bi-allelic DH2-JH rearrangement at relapse, but not at diagnosis. These findings indicate a myeloid to lymphoid lineage switch from an inv(16)(p13q22) positive leukaemia and show that *IGH* gene rearrangments can occur in the presence of *CBFB-MYH11* fusion transcripts.

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Lineage switch is a rare event in acute leukaemia at relapse, and usually concerns a therapy-related switch from ALL to AML late in the disease course.[1] Conversion of AML to ALL has only been reported in five cases, with a rather short time from diagnosis to conversion and without stable genotypic aberrations.[2] Here, we report clinical and laboratory features of an adult patient with inv(16)(p13q22) positive AML with eosinophilia, who relapsed with inv(16)(p13q22) positive precursor-B-ALL.

Case report

A 38-year old man was diagnosed with AML-M4Eo in February 1998 (Figure 1A). Flow cytometric analysis showed $CD13^{+}CD33^{+}MPO^{+}$ myeloblasts co-expressing CD19 (Figure 1B) and with partial expression of TdT. Cytogenetic analysis showed 46,XY,inv(16)(p13q22), and real-time quantitative PCR (RQ-PCR) confirmed a *CBFB-MYH11* type A fusion transcript.[3] With intensive chemotherapy according to an AML treatment protocol, he achieved molecular remission (Figure 1C). After a molecular BM relapse in August 1999, he underwent an allogeneic stem cell transplantation (SCT) and remained in molecular remission for almost three years (Figure 1C). In August 2002, RQ-PCR became positive again for the *CBFB-MYH11* type A fusion transcript (Figure 1C), with a BM relapse four months later (Figure 1A). Remarkably, immunophenotypic analysis now revealed a $CD10^{+}CD19^{+}$ precursor-B-ALL with 10% leukaemic B-lymphoblasts (Table 1), and without leukaemic myeloblasts (Figure 1B). Cytogenetic analysis was not done, but FISH analysis with the CBFB-probe showed a split signal in 15% of 200 nuclei (Figure 1D). Additional FICTION analysis identified CD10 positive mononuclear cells displaying a split signal (Figure 1E).[4] High levels of *CBFB-MYH11* type A fusion transcripts were found by RQ-PCR (Figure 1C), while *MLL-AF4* and *BCR-ABL* were not present. Chimerism studies by short tandem repeat analysis identified a 100% recipient DNA profile.[5] PCR-heteroduplex analyses of *IGH, IGK-Kde*, *TCRG, TCRB,* and *TCRD* gene rearrangements, was performed on diagnostic and relapse samples.[6-7] No Ig/TCR gene rearrangements were found at diagnosis, but bi-allelic DH2-JH rearrangements were found at relapse (Table 1). Two patient specific DH2-JH RQ-PCR assays were developed and reached a sensitivity of $10^{-4}/10^{-5}$.[8] RQ-PCR on the diagnostic AML showed no specific DH2-JH products. Re-induction therapy and donor lymphocyte infusions were given, but in May 2003 the patient relapsed again with inv(16)(p13q22) positive B-ALL. He deceased in July 2003 because of multiple organ failure within two weeks after a second allogeneic SCT.

Conversions from AML to ALL are extremely rare, with only three paediatric and two adult cases reported.[2] In these cases, the interval between diagnosis and conversion was rather short (range: 10-13 months), in contrast to our patient where the interval between AML and ALL was 59 months. Moreover, the clonal relationship between diagnosis and relapse was not reported in these published patients. The present patient is exceptional because he developed a precursor-B-ALL that was clonally related to his previous AML, as demonstrated most convincingly by FICTION analysis. To our knowledge, this is the first patient with CBFB-MYH11-positive precursor-B-ALL. It has to be assumed that the malignant

Figure 1. Morphological, immunophenotypical and molecular features at presentation and relapse. (A) May Grünwald Giemsa staining. At diagnosis, the BM contained 68% blasts and 15% aberrant eosinophils. The relapse BM showed 49% blast cells and 0.5% eosinophils. (B) Flow cytometric analysis of BM. Upper plots: diagnostic sample showing $CD13^{+}CD33^{+}CD34^{+}CD19^{+}$ myeloblasts. Analyses were performed on a FACScan flow cytometer (Becton Dickinson) by double labelling and blast cells were gated by their FSC-SSC characteristics. Lower plots: triple labelling of the relapse sample showing $CD13^{-}CD33^{-}CD34^{+}CD19^{+}$ B-lymphoblasts. Blast cells were analysed on a FACSCalibur (Becton Dickinson) and gated by their CD45-SSC characteristics. (C) RQ-PCR analysis of CBFB-MYH11 transcripts. RQ-PCR was performed as described previously.[3] Primer-probe sets were described elsewhere.[3] The normalized copy numbers of the CBFB-MYH11 type A fusion transcripts are shown as a function of time: ◇ *CBFB-MYH11* fusion transcripts in PB, n *CBFB-MYH11* fusiontranscripts in BM. MR: molecular relapse. HR: haematological-morphological relapse. SCT: stem cell transplantation. (D) FISH analysis on a relapse BM smear using the LSI CBFB dual color break apart probe for the region 16q22, as described by the manufacturer (Vysis Abbott Laboratories, IL, USA). 200 interphase nuclei were scored. On the normal chromosome 16, the green and red signal are colocalized yielding a yellow signal. Arrows show split signals indicative of inv(16)(p13q22). (E) FICTION analysis (fluorescence immunophenotyping and interphase cytogenetics as a tool for the investigation of neoplasms) was done as described elsewere.[4] A follow-up bone marrow smear of May 2003 with 7% residual leukaemic B-lymphoblast was analyzed using anti-CD10 (clone:W8E7, Becton Dickinson) and the LSI CBFB dual color break apart probe for the region 16q22. CD10 positive (intense blue staining) mononuclear cells (lymphoblasts) show split signals (arrows) indicative of inv(16)(p13q22). CD10 negative cells (a neutrophil band form is shown) have normal hybridisation signals. Split signals were detected exclusively in the CD10 positive cells.

Table 1. **Analyses of peripheral blood and bone marrow performed at diagnosis of aCML and during disease progression.**

	Diagnosis	*Relapse (59 months)*
Cytomorphology	AML-M4Eo (FAB)	Acute leukaemia
Percentage of blasts in BM	68 (67% on ANC)	49 (36% on ANC)
Percentage of eosinophils in BM	15	0.5
Cytochemistry		
Peroxidase	positive	negative
Immunophenotype		
Percentage of blast cells on ANC	51	10
	(% positivity within the gated blast cell population)	
CD34	70	92
CD117	48	0
TdT	48	84
HLA-DR	98	ND
CD13	58	14
CD15	61	4
CD33	87	0
CD65	85	ND
MPO	80	0
CD10	0	87 (strong)
CD19	80	87
CD20	0	25
CD24	1	64
CD2	0	ND
Immunogenotype		
Karyotype	46,XY,inv(16)(p13q22)	ND
RT-PCR	*CBFB-MYH11*, type A +	*CBFB-MYH11*, type A + MLL-AF4 - BCR-ABL -
FISH (probe: CBFB)	ND	split signal in 15%
IGH gene	no rearrangement	DH2.2-JH6b (-2/12/-9)
DH2.15-JH5b (-8/5/-4)		
IGK gene	no rearrangement	no rearrangement
TCRD, TCRG, TCRB gene	no rearrangement	no rearrangement
DNA profile	100% receptor	100% receptor

ANC: all nucleated cells, ND: not done.

clone withstood chemotherapy and allogeneic transplantation, but stayed under the detection limit (10^{-5}) of our current RQ-PCR assay during almost three years.

Sixteen cases of donor cell leukaemia (DCL) after transplantation for leukaemia have been reported, including one case with a t(9;22)(q34;q11) in recipient and donor leukaemia.[9] A longer interval between transplantation and relapse seems to favour the possibility that relapse originates from donor cells. In our case, molecular studies excluded the possibility of DCL.[10]

The AML cells present at diagnosis were CD19 positive, a marker frequently correlated with the presence of t(8;21)(q22;q22). Previous studies showed a significant association between positivity for B-cell markers and the presence of Ig/TCR rearrangements in AML.[6] However, no Ig/TCR gene rearrangements were found in the AML at diagnosis, whereas bi-allelic incomplete IGH gene rearrangements were observed in the precursor-B-ALL at relapse. Probably, activation of recombination activating gene products and TdT was induced when the leukaemic cells switched from myeloid to B-cell lineage, resulting in the IGH gene rearrangements. The presence of *CBFB-MYH11* fusion transcripts apparently did not block these rearrangements. However, the sole presence of incomplete IGH gene rearrangements in the precursor-B-ALL reflects a very immature immunogenotype and is found < 1% of primary precursor-B-ALL, suggesting that the original myeloid origin and/or the presence of *CBFB-MYH11* fusion transcripts might affect the Ig/TCR gene rearrangement process.[11]

The presented patient is exceptional because he developed a clonally related precursor-B-ALL after AML. Apparently, smouldering malignant cells can be present during many years and these cells might have the potential of changing their immunophenotype. Alternatively, the *CBFB-MYH11* aberration might have occurred as a first hit, with separate second hits resulting in the AML-M4Eo at first presentation and the precursor-B-ALL at second presentation. However, the precise mechanisms to fully explain our findings await further insights.

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