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## **Distinct clonal anomalies involving in acute myeloid leukemia at diagnosis and after bone marrow transplantation**

Etienne Braekeleer, Nathalie Douet-Guilbert, Audrey Basinko, Marie-Josée Bris, Frédéric Morel, Christian Berthou, Claude Férec, Marc Braekeleer

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Response to Reviewers: ANSWERS TO THE REVIEWER

First major comment (association of 7q deletions and microdeletions of the RUNX1 gene): the discussion on this subject was limited to the association of 7q deletions and RUNX1 mutations (page 5).

Second major comment (other genes involved): we completed the paragraph by adding the 3 genes located in the interval between both BACs with a description of "our" candidate gene (page 5).

Third major comment (RUNX1 constitutively prone to breakage): indeed, as suggested by the reviewer, we think there was a pre-leukemic stem cell in which acquired RUNX1 alterations cooperated with pre-existing lesions to result in full blown acute leukemia. This was added in the last paragraph of the manuscript (page 6).

All three minor comments were taken care of.

**DISTINCT CLONAL ANOMALIES INVOLVING *RUNX1* IN ACUTE MYELOID  
LEUKEMIA AT DIAGNOSIS AND AFTER BONE MARROW  
TRANSPLANTATION.**

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**Running title:** Distinct *RUNX1* anomalies in AML

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## DISTINCT CLONAL ANOMALIES INVOLVING *RUNX1* IN ACUTE MYELOID LEUKEMIA AT DIAGNOSIS AND AFTER BONE MARROW TRANSPLANTATION.

Dear Editor,

The *RUNX* gene family includes three evolutionarily conserved genes (*RUNX1*, *RUNX2* and *RUNX3*) encoding transcription factors involved in cell lineage differentiation during development and various forms of cancer [1]. *RUNX1* gene (also known as *AML1 PEBP2  $\alpha$ B*, or *CBFA2*), located in chromosome 21q22.3, is crucial for the establishment of definite hematopoiesis and the generation of hematopoietic stem cells in the embryo. *RUNX1* acts as a key regulator of hematopoiesis through the regulation of various hematopoietic genes [1-3].

*RUNX1* impairment resulting from mutation, deletion, translocation or amplification is reported to contribute to the pathogenesis of acute leukemias [4]. We report here distinct clonal anomalies involving the *RUNX1* gene occurring in the course of the disease in a patient with acute myeloblastic leukemia (AML).

This 51-year-old man was first seen in February 2006 because of persistent rhinitis and recurrent epistaxis. Physical examination showed no adenopathy or hepatosplenomegaly. The peripheral blood count revealed a leukocytosis of  $8.6 \times 10^9/l$  with 37% blasts, 12% neutrophils, 25% monocytes and promonocytes, a hemoglobin level of 10.1g/l and a platelet count of  $105 \times 10^9/l$ . The bone marrow aspirate showed hypercellularity with 68% blasts positive for CD13, CD33, CD117, MPO and CD65. AML-M1 was diagnosed (FAB classification). The patient received induction chemotherapy according to the LAM2001 protocol (Idarubicin and Cytarabin) followed by re-induction because of chemoresistance at day 15. The patient also received central nervous system prophylaxis with intrathecal

1 injections. A complete remission was obtained. An allogenic bone marrow transplantation  
2 (BMT) was performed in August 2006. The patient relapsed in December 2006. Bone marrow  
3 aspirate revealed 54% blast cells whose morphology and immunophenotype (positive for  
4 CD13, CD33, CD34, CD117) appeared identical to the blasts at diagnosis. The patient  
5 received a second BMT in February 2007. A complete remission was obtained. An isolated  
6 cutaneous granulocytic sarcoma localized at the penis, with normal bone marrow morphology,  
7 was diagnosed in July 2008. Surgery followed by radiotherapy was applied. In November  
8 2008, a gingival granulocytic sarcoma and a medullar relapse, with 45% blasts, were  
9 observed. Induction chemotherapy failed to obtain a remission, the sole result being a  
10 stabilization of the leukocytosis.  
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27 R-banded karyotypes were established from sequential bone marrow aspirate samples that had  
28 been cultured for 24 hours synchronized by FUdR. Fluorescence *in situ* hybridization (FISH)  
29 analyses were performed using whole chromosome painting (WCP) of chromosomes 3, 7, 11,  
30 21, 22 and arm specific probes (PCP) 3p, 3q (QBIOgene®, Illkirch, France), telomeric probes  
31 of chromosome 3 (Abbott®, Rungis, France) and locus specific identifier (LSI) MLL,  
32 BCR/ABL1, TEL/AML1 (Abbott®) as well as bacterial artificial chromosome (BAC) clones  
33 RP11-13M6, RP11-198D7, RP11-434P20, RP11-91K9, RP11-245C23, RP11-12L14 located  
34 on the long arm of chromosome 3 (3q26.3) and BAC RP11-77G18 covering *AML1*.  
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48 At diagnosis, a sole abnormal clone was detected. Karyotype was interpreted as  
49 46,XY,der(7)t(7;11)(q31;q14)[7]/46,XY[15].ish der(7)(WCP7+,MLL+), resulting in partial  
50 monosomy 7q and partial trisomy 11q including trisomy *MLL* without rearrangement (Table  
51 1) (Figs 1-A & 2-A).  
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1 At 1<sup>st</sup> relapse following the 1<sup>st</sup> BMT (December 2006), conventional cytogenetics, completed  
2 by FISH studies, revealed a new abnormal clone showing *AML1* rearrangement:  
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4 46,XY,t(3;11;22;21;3)(p13;q14;q12;q22;q26)[15]/46,XY[5].ish t(3;11;22;21;3)(WCP3+,  
5 PCP3p+,PCP3q+;WCP11+,MLL+,WCP3+,PCP3p+;WCP22+,BCR+,WCP11+,MLL+;  
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7 WCP21+,AML1+,WCP22+;WCP3+,PCP3p+,PCP3q+,AML1+) (Fig 1-B). No *MLL*  
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9 amplification was detected by FISH. FISH with BAC clone (RP11-77G18) covering the  
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11 *RUNX1* gene showed one signal on der(21) and one signal on der(3) (Fig 2-B). Breakpoints  
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13 on 3q occurred between RP11-434P20 and RP11-245C23 with deletion of RP11-91K9. This  
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15 translocation disappeared; interphase FISH on cells from the penis granulocytic sarcoma (July  
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17 2008) showed three *RUNX1* signals, presumably signing re-emergence of the translocation  
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19 (Table 1).  
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29 FISH analyses using TEL/*AML1* probe were performed retrospectively at diagnosis and all  
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31 along the disease. They revealed an abnormal clone with *RUNX1* deletion (undetected by  
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33 banding techniques), distinct from the clone with *MLL* amplification, present at diagnosis  
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35 (Table 1) (Fig 2-A). With chemotherapy and BMT, this clone disappeared until July 2008  
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37 before being again observed in bone marrow cells 4 months before the 2<sup>nd</sup> medullary relapse  
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39 was evidenced (Table 1).  
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46 The *RUNX1* gene is known to be frequently deregulated in human leukemia through different  
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48 mechanisms [4]. In the case reported here, at the time of AML diagnosis, an overlooked clone  
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50 with *RUNX1* deletion was present. It coexisted with a partial monosomic 7q/trisomic 11q  
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52 clone, consistently observed in AML. Although hemizygous loss of *RUNX1* function is  
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54 suspected to play a key role in the development of myelodysplastic syndrome/acute  
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1 myeloblastic leukemia (MDS/AML) patients, it is not sufficient to develop overt leukemia  
2 and acquisition of additional genetic alterations is necessary [5, 6].  
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7 *RUNX1* mutations were found to be significantly associated with monosomy 7 or partial  
8 deletion of the long arm of chromosome 7 in studies of MDS/AML patients [6, 7]. *RUNX1*  
9 mutations were suggested to be the major factor to develop MDS/AML in conjunction with  
10 additional gene alterations belonging to the RTK-RAS signal transduction pathway [6].  
11 Interestingly, in our case, *RUNX1* deletion and 7q deletion did not occur in the same cells.  
12 However, an additional *RUNX1* mutation could not be excluded.  
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24 At first relapse, none of the clones present at diagnosis was detected but the *RUNX1* gene was  
25 rearranged in a complex 3;21 translocation involving five chromosomes. The t(3;21)(q26;q22)  
26 is a recurrent abnormality in therapy-related AML, occurring primarily after treatment with  
27 topoisomerase II inhibitors [8]. Three genes at 3q26, *EVII*, *MDS1* and *EAP* are known to be  
28 fused with *RUNX1* in the (3;21) translocation [9, 10]. In our case, the breakpoint at 3q26 is 8  
29 megabases distal to the *EVII-MDS1-EAP* cluster, arguing for the involvement of another  
30 gene. Three genes have been mapped in the interval between RP11-434P20 and RP11-  
31 245C23. These are *KCNMB2*, a calcium channel, *WIG1*, a wildtype p53-induced gene, and  
32 *TBLIXR1*, a transducin-beta-like 1 receptor 1 (<http://www.ncbi.nlm.nih.gov/mapview/>).  
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1 This report raises the question of whether the *RUNX1* gene was constitutively prone to  
2 breakage in the patient. Whether there was a pre-leukemic stem cell in which acquired  
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4 *RUNX1* alterations cooperated with pre-existing lesions to result in full blown acute leukemia  
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6 remains hypothetical.  
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**Table 1.** Conventional and molecular cytogenetic results obtained during the course of the disease.

Dates	Disease stage	Conventional cytogenetics	FISH
February 2006	Diagnosis	BM: 46,XY,der(7)t(7;11)(q31;q14)[7]/46,XY[15]	M: 3 MLL & 2 AML1 signals (32%) M: 2 MLL & 1 AML1 signal (8%) I: 2 MLL & 1 AML1 signal (14.5%)
March 2006	Re-induction	BM: 46,XY[22]	M: 3 MLL & 2 AML1 signals (2%) M: 2 MLL & 1 AML1 signal (12%) I: 3 MLL & 2 AML1 signals (1%) I: 2 MLL & 1 AML1 signal (10%)
July 2006	1 <sup>st</sup> complete remission	BM: ND	M: 2 MLL & 2 AML1 signals (100%) I: 2 MLL & 2 AML1 signals (100%)
October 2006	After 1 <sup>st</sup> BMT	BM: ND	M: 2 MLL & 2 AML1 signals (100%) I: 2 MLL & 2 AML1 signals (100%)
December 2006	1 <sup>st</sup> relapse	BM: 46,XY,t(3;11;22;21;3)(p13;q14;q12;q22;q26)[15]/46,XY[5]	M: 2 MLL & 3 AML1 signals (75%)
March 2007	After 2 <sup>nd</sup> BMT	BM: ND	M: 2 MLL & 2 AML1 signals (100%) I: 2 MLL & 2 AML1 signals (100%)
June 2007	2 <sup>nd</sup> complete remission	BM: 46,XY[21]	M: 2 MLL & 2 AML1 signals (100%) I: 2 MLL & 2 AML1 signals (100%)
October 2007	2 <sup>nd</sup> complete remission	BM: 46,XY[21]	M: 2 MLL & 2 AML1 signals (100%) I: 2 MLL & 2 AML1 signals (100%)
February 2008	2 <sup>nd</sup> complete remission	BM: 46,XY[21]	M: 2 MLL & 2 AML1 signals (100%) I: 2 MLL & 2 AML1 signals (100%)
July 2008	Granulocytic sarcoma	GS: no metaphases	I: 2 MLL & 3 AML1 signals (58%)
July 2008		BM: 46,XY[50]	M: 2 MLL & 2 AML1 signals (100%) I: 2 MLL & 1 AML1 signal (7%)
November 2008	2 <sup>nd</sup> medullary relapse	ND	ND

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BM: bone marrow  
GS: granulocytic sarcoma  
M: metaphase  
I: interphase  
ND: not done  
BMT: bone marrow transplantation

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2 **LEGENDS TO THE FIGURES**  
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7 **Figure 1.** Conventional cytogenetics  
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9 A. Partial R-banded karyotype at diagnosis showing der(7)t(7;11)(q31;q14)  
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11 B. R-banded karyotype at first relapse following the first BMT:  
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13 46,XY,t(3;11;22;21;3)(p13;q14;q12;q22;q26)  
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22 **Figure 2.** Fluorescence *in situ* hybridization  
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24 A. FISH at diagnosis with LSI MLL dual color and TEL/AML1 ES dual color  
25 (Green/Orange) revealing two distinct clones (left nucleus is trisomic for 11q and right  
26 nucleus is deleted for *RUNX1*).  
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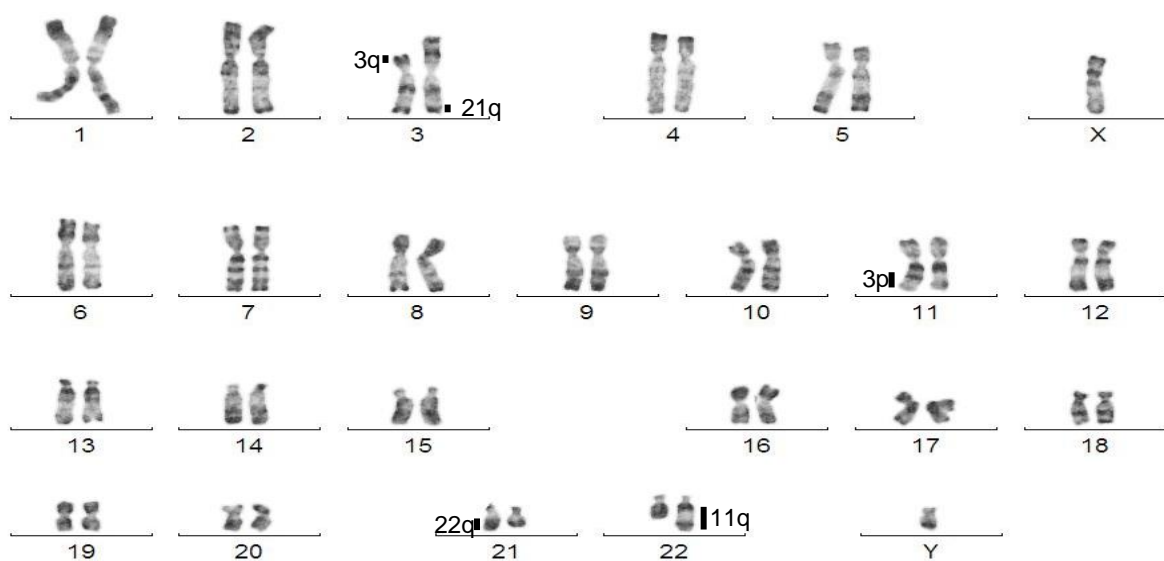
31 B. FISH at first relapse following the first BMT with BAC RP11-13M6 (orange) and BAC  
32 RP11-77G18 (green) showing disruption of *AML1* by t(3;21).  
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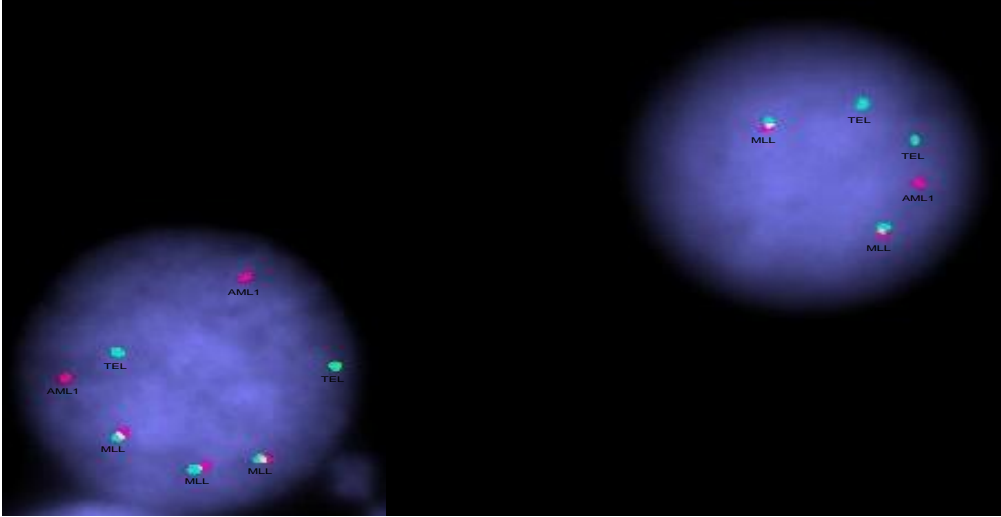


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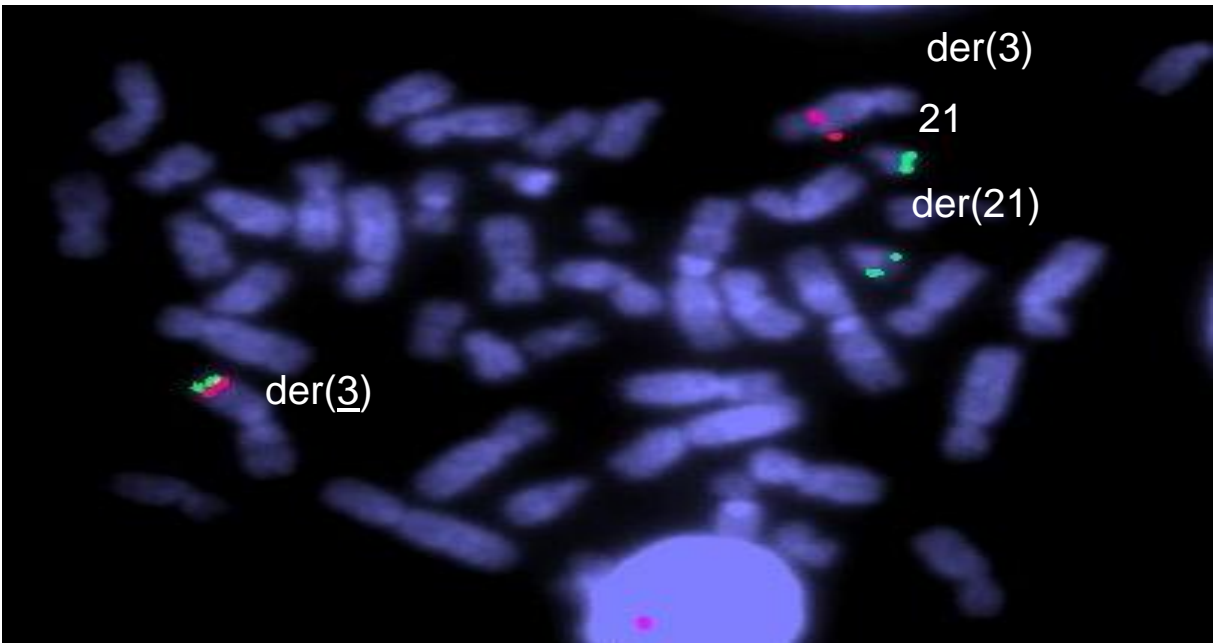


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**A**



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**\*Conflict of interest**

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