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Acute Myeloid Leukemia

Patterns of AML1-ETO transcript expression in patients with acute myeloid leukemia and t(8;21) in complete hematologic remission

We used real-time reverse transcriptase polymerase chain reaction (RQ-PCR) to investigate the expression patterns of AML1-ETO fusion transcript during the follow-up of seven patients with acute myeloid leukemia (AML) associated with t(8;21)(q22;q22). In contrast to previous reports of persistent qualitative RT-PCR positivity even during long-term complete remission, our data suggest that a high proportion of t(8;21)-associated AML patients may achieve RQ-PCR negativity after successful treatment. Our data also suggest the possible existence of at least two patterns of transcript expression after successful chemotherapy: 1) early RQ-PCR negativity (sometimes followed by transient positivity); 2) gradual achievement of RQ-PCR negativity.

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The t(8;21)(q22;q22) translocation is found in about 10-15% of patients with acute myeloid leukemia (AML) and is frequently the only cytogenetic abnormality present. It is typically associated with FAB M2 morphology and a relatively good prognosis.¹ The t(8;21) creates an AML1-ETO fusion gene on the derivative chromosome 8. The AML1 gene encodes for a transcription factor essential for normal hematopoiesis, whereas the function of ETO is still unknown.^{2,3} Qualitative reverse transcriptase polymerase chain reaction (RT-PCR) detection of residual t(8;21)-positivity has produced contradictory results^{4,5} and does not distinguish patients in complete remission (CR) who are cured from those destined to relapse.^{6,7} We used real time RT-PCR (RQ-PCR) to investigate expression patterns of AML1-ETO fusion transcript in 68 bone marrow samples taken during the follow-up of seven patients with t(8;21)-associated AML who were routinely addressed to molecular (qualitative RT-PCR) analysis at our Institute from May 1993 onwards (Table 1). Of these, six were treated at the "L. & A. Seragnoli" Institute, while the remaining patient (#6) was referred to us for molecular monitoring during and after treatment at the Institute of Hematology of the University of Taranto, Italy. All patients provided written informed consent to their participation in the study.

Table 1. Clinical and karyotype characteristics of patients affected by AML with t(8;21)(q22;q22).

Patient No.	Sex/Age	FAB	Therapy	Clinical outcome	Karyotype
1	F/31	M2	ICE/NOVIA/BMT	CR/AW	46,XX,t(8;21)(q22;q22)(18)
2	M/47	M1	ICE/NOVIA/ABMT	CR/AW	46,XX,t(8;21)(q22;q22)(12)
3	F/41	M2	ICE/NOVIA/ABMT/FLAN/BMT	2 nd CR/AW	46,XX,t(8;21)(q22;q22)(16)
4	F/29	M4Eo	ICE/NOVIA/ABMT-PBSC	CR/AW	46,XX,t(8;21)(q22;q22)(8)/ 46,XX,t(8;21)(q22;q22),der(16), t(3;16)(q21;q22)(13) 46,XX,t(8;21)(q22;q22),del(9)(q22)(4)
5	F/22	M4	ICE/NOVIA/ABMT-PBSC	CR/AW	46,XX,t(8;21)(q22;q22)(20)
6	M/47	M4	FLAI/MEC4	CR/AW	46,XX,t(8;21)(q22;q22)(12)
7	M/22	M2	FLAI/MEC4	CR/AW	46,XX,t(8;21)(q22;q22)(20)

Median 31
(range) (22-47)

CR: complete remission; AW: alive and well.

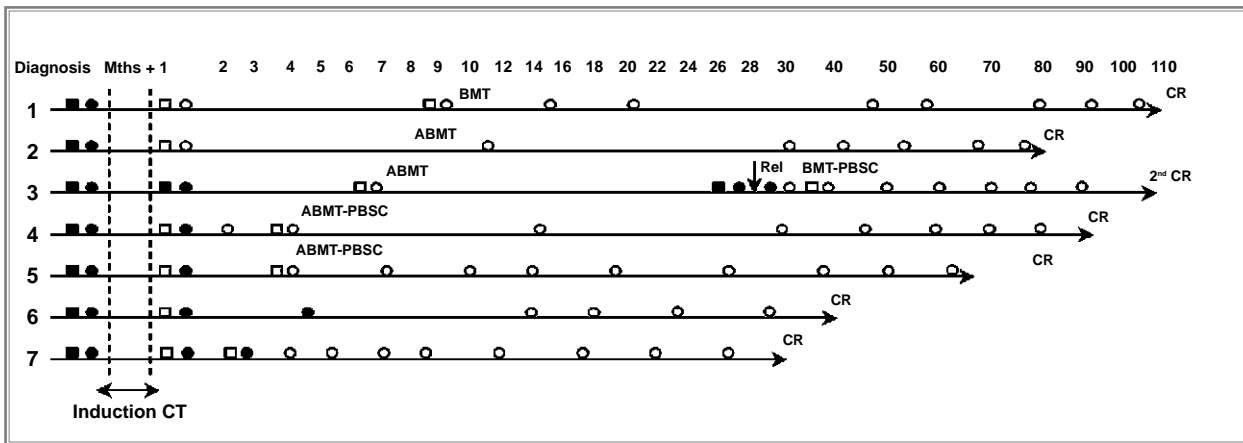


Figure 1. Schematic representation of karyotypic and qualitative RT-PCR follow up of seven AML patients with t(8;21)(q22;q22). Karyotypic analysis negative (□) or positive (■) for t(8;21); molecular analysis negative (○) or positive (●) for AML1-ETO. ABMT, autologous bone marrow transplantation; BMT, allogeneic bone marrow transplantation; ABMT-PBSC, autologous transplantation with peripheral blood stem cells, BMT-PBSC, allogeneic transplantation with peripheral blood stem cells.

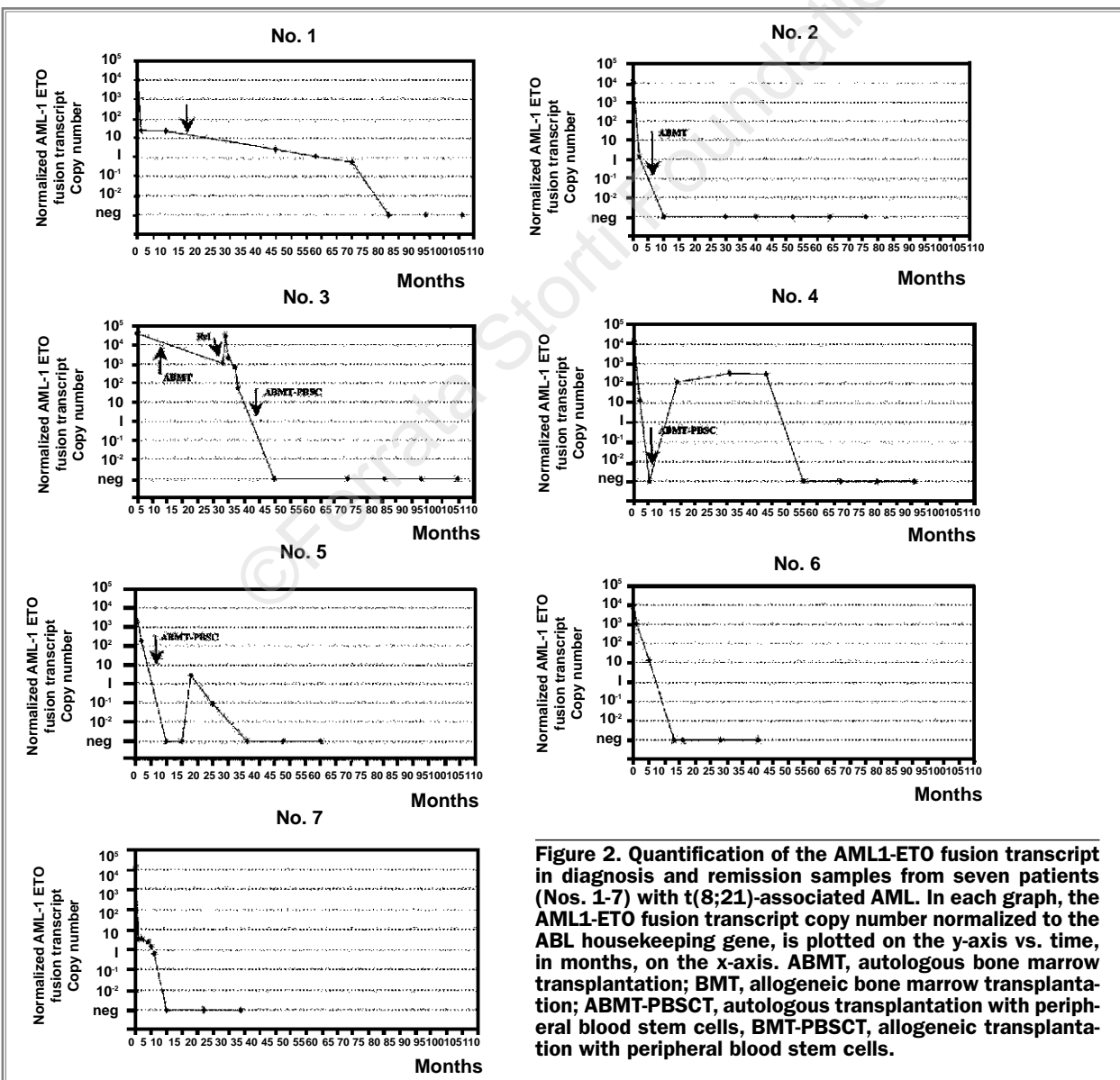


Figure 2. Quantification of the AML1-ETO fusion transcript in diagnosis and remission samples from seven patients (Nos. 1-7) with t(8;21)-associated AML. In each graph, the AML1-ETO fusion transcript copy number normalized to the ABL housekeeping gene, is plotted on the y-axis vs. time, in months, on the x-axis. ABMT, autologous bone marrow transplantation; BMT, allogeneic bone marrow transplantation; ABMT-PBSC, autologous transplantation with peripheral blood stem cells, BMT-PBSC, allogeneic transplantation with peripheral blood stem cells.

Total cellular RNA extraction and cDNA synthesis were performed as previously described.⁸ Qualitative RT-PCR was routinely performed at diagnosis and during follow-up, according to the BIOMED-1 Concerted Action protocol.⁹ RQ-PCR was retrospectively performed as described elsewhere.^{8,10,11} Using ABL as the control gene, normalized fusion transcript levels were reported as (AML1-ETO copy number/ABL copy number)×10⁴. The χ^2 test was used for statistical analysis with computations performed by the SPSS software package (SPSS Inc., Chicago, IL, USA).

At a median follow-up of 76 months (range 34–106 months), six patients (88%) are in first CR, and one patient is in second CR after relapsing at 28 months. All 7 patients showed t(8;21) (q22;q22) at diagnosis, and one patient also had additional karyotypic abnormalities (Table 1). The cytogenetic results are summarized in Figure 1. Two patients (29%) (#1 and 2) achieved qualitative RT-PCR negativity after induction chemotherapy, while the remaining five (#3–7) became negative after the first consolidation chemotherapy (Figure 1). Analysis by RQ-PCR was positive in 32 of the 68 samples. Significantly, 18 of these 32 samples were negative by RT-PCR suggesting that RQ-PCR is the more sensitive technique ($p < 0.002$). Figure 2 shows AML1-ETO expression patterns according to RQ-PCR analysis. In patient #3, who relapsed 28 months after diagnosis, the normalized AML1-ETO transcript copy number at relapse was similar to that found at diagnosis (30,213 and 40,230, respectively); after allogeneic bone marrow transplantation (BMT) with peripheral blood stem cells, the patient achieved RQ-PCR negativity (17 months after relapse). All the six patients in first CR (#1, 2 and 4–7) with a follow-up of at least 34 months showed a 1- to 5-log reduction in normalized AML1-ETO transcript levels immediately after induction chemotherapy. Subsequent patterns varied. Five patients (#2 and 4–7) rapidly achieved RQ-PCR negativity, but in two of them (#4 and 5) a subsequent transient return to positivity was recorded. Patient #1 achieved RQ-PCR negativity at 82 months from diagnosis after a progressive drop in transcript burden following allogeneic BMT. The remaining patient (#7) gradually achieved RQ-PCR negativity following an initial 3-log drop after induction chemotherapy.

These data suggest that a high proportion of patients with t(8;21)-associated AML may achieve RQ-PCR negativity after treatment. Our data also suggest the possible existence of at least two patterns of transcript expression after successful chemotherapy: 1) early RQ-PCR negativity (sometimes followed by transient positivity); 2) gradual achievement of RQ-PCR negativity. Furthermore, our results clearly show that RQ-PCR is more sensitive and informative than qualitative PCR as a means of monitoring the behavior of the disease cell population during remission.

Our findings raise the question as to whether patients with t(8;21) who achieve RQ-PCR negativity may have intrinsically manageable levels of residual disease. A longer follow-up on a larger number of patients will be necessary to test this hypothesis. In any case, our findings support the concept that once a patient with t(8;21)-associated AML has achieved RQ-PCR negativity, he or she may have a good chance of residual disease remaining under control for at least several years without the need for further maintenance therapy.

Silvia Buonamici,* Emanuela Ottaviani,* Giuseppe Visani,*
Francesca Bonifazi,* Mauro Fiacchini,* Michele Bacarani,*
Giovanni Martinelli*

*Institute of Hematology and Medical Oncology "L. and A. Seràgnoli", University of Bologna, Italy; °University of Illinois at Chicago, Chicago, IL, USA; °Department of Hematology, H San Salvatore, Pesaro, Italy

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Correspondence: Dr. Giovanni Martinelli, Molecular Biology Unit, Institute of Haematology and Medical Oncology "Seràgnoli", University of Bologna, via Massarenti 9, 40138 Bologna, Italy. Phone: international +39.051.6364075. Fax: international +39.051.6364037. E-mail: gmartino@kaiser.alma.unibo.it

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