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Slow relapse in acute myeloid leukemia (AML) with inv(16) or t(16;16)

AML with inv(16)(p13q22) or t(16;16)(p13;q22), resulting in *CBFβ-MYH11* fusion transcript detectable by RT-PCR and RQ-PCR, is associated with an overall good prognosis, relapses however still occurring in 30 to 35 % of patients, and with higher frequency in older patients.[1,2] Molecular relapse generally precedes haematological relapse in AML with balanced translocations such as t(15;17) or t(8;21), but generally by only a few weeks or a few months.[3-9] Fewer data are available in AML with inv(16)/t(16;16). In 4 of the 5 relapses we observed in AML with inv(16)/t(16;16), the interval between molecular and hematological relapse was prolonged.

Between 2005 and 2009, nine AML patients with inv(16) or t(16;16), with a median age of 60 years (range, 21-75) who had reached CR using French cooperative AML trials (anthracycline–cytarabine induction chemotherapy followed by consolidation chemotherapy with high dose cytarabine, or intermediate dose cytarabine in elderly patients) at our center were prospectively monitored for minimal residual disease (MRD) based on *CBFβ-MYH11* fusion transcripts levels in bone marrow samples.

RQ-PCR was performed on bone marrow cells according to the Europe Against Cancer (EAC) Program recommendations for *CBFβ-MYH11* fusion transcripts (type A, D or E), using Taqman® technology, on an ABI PRISM 7000 (Applied Biosystems).[10] Quantification of CBFb-MYH11 fusion transcripts was normalized to the housekeeping ABL gene. Results were expressed by the ratio *CBFβ-MYH11* copy number/ABL copy number x 100 (%).[10] Median follow-up after CR achievement was 18 months (range, 3 - 33) and median number of MRD analyses per patient was 6 (range 1 - 9). Molecular relapse was defined as a 10-fold or greater increase in *CBFβ-MYH11* transcript level compared to the lowest level achieved.

Five of the 9 patients relapsed, after 11 to 23 months, in the bone marrow (no extramedullary relapse was seen). In one of them, the interval between molecular relapse and haematological relapse was short (1 month).

Table 1. Main characteristics of the 4 relapsing patients analyzed.

	Pt n. 1	Pt n. 2	Pt n. 3	Pt n. 4
Age (years)	58	60	64	67
Sex	M	M	M	F
BM blasts %	46	12	63	31
PB blasts %	64	34	60	11
WBC count (x10⁹/L)	25,9	71,7	21,0	65,6
Karyotype	Inv(16)	t(16;16)	Inv(16)	Inv(16)
Interval between first CR and molecular relapse (months)	9	19	3	20
Interval between molecular and hematological relapse (months)	10	6	7	8

BM: bone marrow; PB: peripheral blood; WBC: white blood cells; Pt: patient.

The 4 other haematological relapses occurred slowly, and were preceded in all cases by molecular relapse detected in bone marrow samples, by 10 (patient n. 1), 6 (patient n. 2), 7 (patient n. 3) and 8 (patient n°4) months, respectively. Baseline characteristics of those 4 patients are shown in Table 1. Patients n. 2 and n. 3 had c-KIT mutation in exon 8 and c-KIT D816V mutation, respectively (versus none of the patients who did not relapse) and patient n°3 had N-RAS mutation, while no patient had *FLT3-ITD* or *FLT3-835/I836* mutation. All 4 patients had achieved at least 3 log reduction of the fusion transcript level, after induction therapy in 3 of them, and after the first consolidation course in patient n. 1 (Figure 1). During the period of isolated molecular relapse, blood counts and marrow aspirates remained normal in patients n. 3 and 4, while cytopenias reappeared in patient n. 1 and abnormal marrow eosinophils in patient n. 2. A second CR was achieved in the 4 patients with chemotherapy, combined to Gemtuzumab in 3 cases. Three of them were subsequently allografted, and all 4 patients were alive, 2 to 11 months after hematological relapse. In acute promyelocytic leukaemia (APL), the median interval between molecular and haematological

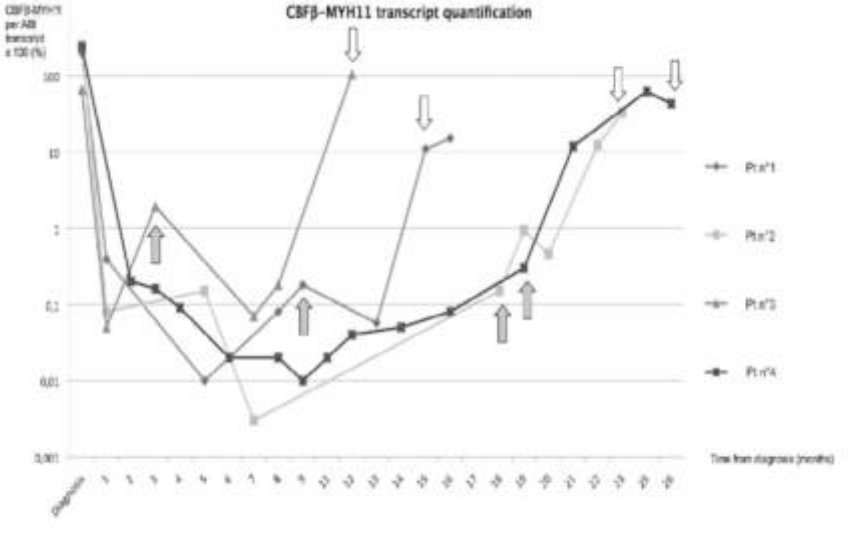

Figure 1. Minimal residual disease (MRD) sequentially measured by RQ-PCR in bone marrow samples in the 4 relapsing patients. MRD was expressed in CBFB-MYH11 copies per /100 ABL gene copies. Dots on curves represent MRD examinations. Full arrows indicate molecular relapse and empty arrows indicate haematological relapse. Follow-up is in months.

relapse was 3 to 4 months in published litterature[3-5] while in AML with t(8;21) it was generally less than 6 months[6-8] and at a median of 3 months in our experience.[9]

In AML with inv(16)/t(16;16), few studies are available: Schnittger *et al*. reported 6 relapses of *CBFβ-MYH11* AML with an interval between molecular and haematological relapse ranging from 1 to 5 months,[8] similar to what they observed in AML with *PML-RAR* and *AML1-ETO*. By contrast, Stentoft et al. reported, in 4 relapsing inv(16)/t(16;16) AML, an interval between molecular and haematological relapse of approximately 1 year, with a slow molecular progression rate of about 1 log per 100 days.[8] In our patients n. 2, 3 and 4, the increase in fusion transcript levels had comparable kinetics, while in our patient n. 1, it was even slightly slower, with a molecular progression rate of about 1 log per 130 days.

Thus, AML with inv(16)/t(16;16) AML may frequently relapse more slowly than other types of AML with balanced translocations. This interval between molecular and haematological relapse may justify frequent MRD monitoring in those patients, and therapeutic intervention before overt relapse.

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References

1. Marcucci G, Mrózek K, Ruppert AS, Maharry K, Kolitz JE, Moore JO, et al. Prognostic factors and outcome of core binding factor acute myeloid leukemia patients with t(8;21) differ from those of patients with inv(16): a Cancer and Leukemia Group B study. J Clin Oncol 23:5705-17.
2. Delaunay J, Vey N, Leblanc T, Fenaux P, Rigal-Huguet F, Witz F, et al. Prognosis of inv(16)/t(16;16) acute myeloid leukemia (AML): a survey of 110 cases from the French AML Intergroup. Blood 2003;102:462-9.
3. Diverio D, Rossi V, Avvisati G, De Santis S, Pistilli A, Pane F, et al. Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/RARα fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter "AIDA" trial. GIMEMA-AIEOP Multicenter "AIDA" Trial. Blood 1998; 92:784-9.
4. Cassinat B, de Botton S, Kelaidi C, Ades L, Zassadowski F, Guillemot I, et al. When can real-time quantitative RT-PCR effectively define molecular relapse in acute promyelocytic leukemia patients? (Results of the French Belgian Swiss APL Group). Leuk Res 2009 (in press).
5. Gallagher RE, Yeap BY, Bi W, Livak KJ, Beaubier N, Rao S, et al. Quantitative real-time RT-PCR analysis of PML-RAR mRNA in acute promyelocytic leukemia: assessment of prognostic significance in adult patients from intergroup protocol 0129. Blood 2003;101:2521-8.
6. Schnittger S, Weisser M, Schoch C, Hiddemann W, Haferlach T, Kern W. New score predicting for prognosis in PML-RARA+, AML1-ETO+, or CBFBMYH11+ acute myeloid leukemia based on quantification of fusion transcripts. Blood 2003;102:2746-55.
7. Tobal K, Newton J, Macheta M, Chang J, Morgenstern G, Evans PA, Morgan G, Lucas GS, Liu Yin JA. Molecular quantification of minimal residual disease in acute myeloid leukemia with t(8;21) can identifiy patients in durable remission and predict clinical relapse. Blood 2000;95:815-9.
8. Stentoft J, Hokland P, Ostergaard M, Hasle H, Nyvold CG. Minimal residual core binding factor AMLs by real time quantitative PCR initial response to chemotherapy predicts event free survival and close monitoring of peripheral blood unravels the kinetics of relapse. Leuk Res 2006;30:389-95.
9. Leroy H, de Botton S, Grardel-Duflos N, Darre S, Leleu X, Roumier C et al. Prognostic value of real-time quantitative PCR (RQ-PCR) in AML with t(8;21). Leukemia 2005;19:367-72.
10. Gabert J, Beillard E, van der Velden VHJ, Bi W, Grimwade D, Pallisgaard N, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – A Europe Against Cancer Program. Leukemia 2003; 17:2318-57.