temperature, but within the group of patients with malaria no correlation was observed with body temperature.

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Key words: plasmodium falciparum malaria, leukocyte alkaline phosphatase, PMN activation.

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Acute Myeloid Leukemia

Molecular monitoring to identify a threshold of CBFβ/MYH11 transcript below which continuous complete remission of acute myeloid leukemia inv(16) is likely

Many patients with the inv(16) positive acute myeloid leukemia (AML) achieve complete remission (CR). Using real-time reverse transcriptase polymerase chain reaction (RT-PCR), we previously proposed critical CBFβ-MYH11 transcript copy number thresholds to predict relapse or cure. We now update the molecular follow-up of our patients, also presenting the therapeutic management of these patients.

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We extend the molecular follow-up of 17 patients with inv(16) acute myeloid leukemia (AML) for whom cytogenetic and molecular follow-up data are available, dividing the group into those who relapsed and those who have not. Having monitored the expression of CBFβ-MYH11 during the follow-up we establish a value below which continuous complete remission is likely.

Although the outcome of AML is predominantly related to age,[1] it is of prime importance to distinguish subgroups of patients with different risks of disease. Cytogenetic analysis at diagnosis is generally considered the single most valuable prognostic factor in AML;[1,3] in particular, patients with t(8;21), t(15;17), or inv(16) were found to have a relatively favorable prognosis.[2,3] In the case of acute promyelocytic leukemia, molecular monitoring of minimal residual disease (MRD) status by qualitative or, preferably, quantitative real time PCR (RQ-PCR) analysis of the PML-RAR transcript can provide a suitable tool for predicting relapse and offering indications for allogeneic transplantation.[3] In the case of inv(16) positive AML the prognostic significance of the different levels of CBFβ-MYH11 transcript copy number in monitoring MRD remains to be defined.[4]

To provide further information on the threshold of CBFβ-MYH11 transcript copy number above which relapse occurs and below which continuous complete remission (CR) is likely, in the present work we update the follow-up analysis of our series of patients[5,6] with inv(16)-positive AML patients treated with different therapies (one cycle of induction therapy and one or two cycles of consolidation therapy, autologous bone marrow transplantation in 13 patients and allogeneic bone marrow transplantation in 3 patients). The clinical data are summarized in Table 1.

All 17 patients underwent molecular and cytogenetic analysis during the follow-up. Samples of bone marrow (n=201) and peripheral blood (n=6) were collected for routine care of the patients. All RQ-PCR experiments were performed at least in triplicate. Since January 2000, we also applied the same RQ-PCR conditions and protocol for the CBFβ/MYH11 fusion transcript as those currently recommended by the *European Concerted Action (EAC) of standardization of fusion transcripts in AML patients* (i.e. CBFβ-MYH11 per 10^4 copies of transcript copy numbers normalized to the ABL housekeeping gene). Comparisons of the CBFβ-MYH11/ABL ratios at diagnosis, relapse and during treatment and CR were performed by the Kruskal-Wallis test. The χ^2 test was used for binary variables. All analyses were performed using the SPSS software package (SPSS Inc., Chicago, IL, USA).

Fifteen patients are currently alive (88%) either in first CR (n=12, 70.5%) or second CR (n=3, 17.6%). The response rate after induction therapy was 100%. Four patients of the seventeen (29%) had a relapse but three (11%) went on to achieve a second CR lasting >36 months. The median overall survival (OS) was 62 months (range 37-140) with median disease free survival (DFS) in 1st or 2nd CR being 56 months (range 36-109).

We retrospectively analyzed the CBFβ-MYH11/ABL ratios of peri-/post-treatment samples from 17 patients using RQ-PCR. The 17 patients were divided into non-relapsing and relapsing subgroups. All ratios <12 (minimum, 0) obtained during or after treatment (n=101) belonged to patients who have continued to remain in long-term CR without relapsing. By contrast, all ratios >25 (maximum, 710) obtained during or after treatment belonged to patients who went on

Table 1. Clinical data of patients with inv(16)(p13q22).

UPN	*Sex/Age*	*WBC at diagnosis*	*FAB cytotype*	*Karyotype*	*Type of transcript*	*Therapy*	*OS/DFS (months)*	*Clinical outcome after CT/Clinical Status*
1	F/51	2,4	M1	46,XX(3)/46,XX, inv(16)(p13q22)(7)	A	ICE/NOVIA/FLAG/ASCT	101/100	CR/AW
2	M/34	65,4	M2	46,XY(1)/46,XY,del(7), inv(16)(p13q22)(13)	A	ICE/NOVIA/ ASCT	96/95	CR/AW
3	M/38	–	M4Eo	46,XY(2)/46,XY, inv(16)(p13q22)(13)	A	ICE/NOVIA/SCT	94/93	CR/AW
4	F/51	–	M4Eo breast cancer	46,XX(1)/46,XX, inv(16)(p13q22)(21)	A	ICE/NOVIA/FLANG/ FLANG/SCT	38/37	CR/breast cancer-died
5	F/58	4	M4Eo	46,XX(2)/46,XX, inv(16)(p13q22)(28)	A	ICE/NOVIA/ASCT/ FLANG/FLANG	85/56	2nd CR/AW
6	M/60	79,3	M4Eo	46,XY(2)/46,XY, inv(16)(p13q22)(19)	A	ICE/NOVIA/ FLANG/ASCT-PBSC	81/79	CR/AW
7	M/37	8,3	M4Eo	46,XY(3)/46,XY, inv(16)(p13q22)(16)	A	ICE/NOVIA /SCT	80/79	CR/AW
8	M/57	2,3	M4Eo	47,XY,inv(16)(p13q22), +22(12)/ 47,XY,t(9;19)(q22q13), inv(16)(p13q22)(8)	A	ICE/NOVIA/ ASCT	74/72	CR/AW
9	M/57	79	M2	46,XY(1)/46,XY, inv(16)(p13q22)(14)	A	MEC6/MEC4/ASCT/ FLANG/FLANG	141/109	2nd CR/AW
10	M/35	130	M4Eo	46,XY(1)/47,XY, inv(16)(p13q22),+9(14)	A	ICE/FLANG/FLANG/ ASCT/MEC4/BMT	62/46	2nd CR/AW
11	F/60	22,4	M2	46,XX(1)/47,XX, inv(16)(p13q22),+8(30)	D	ICE/FLANG/FLANG	60/56	CR/AW
12	M/49	42	M4Eo	46,XY(1)/47,XY, inv(16)(p13q22),+8(28)	A	ICE/FLANG/FLANG/ASCT	55/54	CR/AW
13	M/49	56	M4Eo	46,XY(2)/46,XY, inv(16)(p13q22)(23)	A	ICE/FLAN/FLAN/ASCT	52/51	CR/AW
14	F/27	–	M4Eo	46,XX,inv(16)(p13q22), +X(25)	A	ICE/FLAN/FLAN/ASCT	39/36	CR/relapse-died
15	F/26	38	M4Eo	46,XX,inv(16) (p13q22)(30)	E	ICE/FLAN/FLAN/ASCT	49/47	CR/AW
16	M/17	–	M4Eo(GS)	46,XY(1)/46,XY, inv(16)(p13q22)(28)	A	ICE/FLAN/FLAN/ASCT	48/47	CR/AW
17	M/16	132	M4Eo	46,XY, inv(16) (p13q22)(30)	C	ICE/FLAN/FLAN/ASCT	37/36	CR/AW
Median (range)	49 (16-60)	42 (2,3-132)					62(37-141)/ 56(36-109)	

OS: overall survival; DFS: disease-free survival; CR: complete remission; AW= alive and well.

to relapse. The difference between these two groups was highly significant (Kruskal-Wallis test, $p<0.0001$). Six assays fell within an intermediate *gray* zone of ratios between 12–25, which was not clearly associated with either relapse or long-term CR. Of these, 3 referred to two patients (#5 and 14) who went on to relapse, and 3 to three patients (# 4, 7 and 11) who have remained in long-term CR. Our extended follow-up has now shown that the ratios of the latter three patients fell and remained below the threshold (12 copies) associated with long-term CR. By contrast, the ratios of the other two patients returned above the upper threshold (25 copies) prior to relapse. Taken together, these findings confirm the concept that prolonged monitoring of these patients by RQ-PCR allows different risks of relapse to be distinguished.[6] These data fit with others reported in the literature[4,7-10] which also found that low CBFβ-MYH11 fusion transcript thresholds predicted continuous long-term CR, even if with slightly different values. These slight discrepancies could be related to the different sampling times and the different therapeutic programs employed. Therefore, it

would be interesting to monitor patients on completion of their therapeutic program at identical time points and using the same sampling modality.

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Myeloproliferative Disorders

Hypereosinophilic syndrome and cyclic oscillations in blood cell counts. A clonal disorder of hematopoiesis originating in a pluripotent stem cell

We studied a patient with hypereosinophilic syndrome (HES) who had myeloproliferative features, was unresponsive to imatinib mesylate, and showed cyclic oscillations in blood cell counts. No rearrangement in *PDGFRA*, *PDGFRB* and *ETV6* genes was detected. Clonal analysis of hematopoiesis consistently showed skewed X-chromosome inactivation patterns in both granulocytes and T-lymphocytes, indicating a clonal myeloproliferative disorder originating in a pluripotent stem cell.

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The term hypereosinophilic syndrome (HES) is used to define conditions characterized by elevated eosinophil counts (persistently greater than 1.5×10^9/L), variable damage to end organs such as the heart, lungs, skin, joints and nervous system, and no ascertainable cause for the eosinophilia.[1] Abnormal clones of T cells producing interleukin-5 were found in some patients, suggesting that HES can be a clonal T-cell disorder.[2] Recent reports,[3,4] however, indicate that in most instances HES is a myeloproliferative disease caused by constitutive activation of tyrosine kinases. These observations explain the remarkable efficacy of imatinib mesylate, at least in a portion of HES patients. Myeloproliferative disorders typically result from clonal expansion of mutated hematopoietic stem cells and clonal analysis of hematopoiesis using X-chromosome inactivation patterns has shown that the same clinical phenotype may arise from clonal proliferation of different hematopoietic progenitors.[5]

A 41-year old woman presented in 1995 with episodes of pruritus, pulmonary symptoms including wheezing, dyspnea, and cough, fatigue and facial edema. These manifestations worsened in the following years and the patient ultimately required prednisone and inhaled β_2-agonists to control her symptoms. In October 2002, a complete blood count showed: hemoglobin 13.0 g/dL, white cell count 17.3×10^9/L (eosinophils 30%), and platelets 442×10^9/L. A bone marrow biopsy showed hypercellularity (>70%) with granulocytic hyperplasia and marked eosinophilia. Cytogenetic analysis was normal and polymerase chain reaction analysis was negative for the *BCR-ABL* rearrangement. Serum eosinophil cationic protein was markedly elevated (246 μg/L, normal range 0-15 μg/L). Chest X-ray and an echocardiogram were normal; stool examination for ova and parasites was negative, and rheumatologic investigations were unremarkable. A diagnosis of idiopathic hypereosinophilic syndrome was made. In February 2003, due to worsening clinical signs, treatment with hydroxyurea, 1 g per day, was started. Skin and lung symptoms persisted, so a trial of imatinib, 100 mg daily, was initiated in April 2003. Despite increasing the dose to 400 mg daily, there was no significant improvement in the patient's hypereosinophilia. Hydroxyurea, 2 g per day, was started again, but this woman benefited most from prednisone: her current treatment (January 2004) consists of prednisone, 12.5 mg/day, and hydroxyurea, 500 mg/day.

As shown in Figure 1A, during 2003 marked periodic oscillations were observed in both WBC and platelet count, irrespective of the treatment given (hydroxyurea or imatinib mesylate). In particular, the platelet count fluctuated between 136 and 817×10^9/L. When we examined eosinophil and lymphocyte trends, it was apparent that these cell types also fluc-