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Investigation of minimal residual disease in adult Ph1 positive acute lymphoblastic leukemia by a combination of cell sorting and fluorescence *in situ* hybridization: a preliminary study on 6 cases

We studied minimal residual disease in 6 patients with Philadelphia positive acute lymphoblastic leukemia (Ph1 ALL) in remission by using a combination of cell sorting (CD34+) and FISH. The detection of residual leukemic cells in Ph1 ALL by using this method is much more sensitive than conventional morphology and cytogenetic analyses.

Sir,

In Ph1 ALL, conventional cytogenetic methods are useful for diagnosing the disease but not sensitive enough to detect low numbers of residual leukemic cells.[1-3] Flourescence *in situ* (FISH) analysis makes possible to score a large number of cells but in practice does not seem to be more sensitive than conventional cytogenetics for minimal residual disease (MRD) analysis. Reverse trasncription-polymerase chain reaction (RT-PCR) analysis of bcr-abl fusion trancripts is useful in the evaluation of MRD after treatment, particularly after allogenic bone marrow transplantation (allo BMT). However, after induction chemotherapy, most of the patients remain PCR positive in the bone marrow.[4-6] In this study, we sequentially analyzed the presence of MRD in 6 patients with Ph1 ALL during clinical remission, using a combination of cell sorting and FISH.

All patients studied had CD34 positive common B-ALL and Ph1 chromosome by cytogenetic analysis at diagnosis. By RT-PCR, 3 had p190BCR-ABL (#1, #5 and 6), 3 had p210BCR-ABL (#2 to 4). Patients #1 to 3 were studied only after chemotherapy (CT), patient #4 after CT and allo-BMT, and patients 5 and 6 after allo-BMT. For each sample, cytogenetic and FISH analyses were performed in parallel. All BM samples had <5% blasts and 0.5 to 1.5 % total CD34 positive cells and were Ph1 negative (50 metaphases analyzed). The isolation of CD34+ progenitor cells was performed using the MACS cell isolation kit (Tebu, Perray en Yvelines, France). For each sample, the purity after separation was higher than 95%. FISH was performed using a classical BCR-ABL probe mixture (Vysis, Woodcreek, IL, USA). The positivity threshold for both the major and minor breakpoints was considered to be 4% (mean±2 SD).

After induction CT, 6 to 9% and 10 to 38% BCR-ABL positive CD34+ cells were detected in blood and BM samples respectively, in patients #1 to 4, confirming that in adult Ph1 ALL, induction CT generally only achieves partial leukemic reduction. Patients #1 and #2 remained positive after the first consolidation course, had an increase in the percentage of positive cells after the second consolidation course and relapsed 5 and 6 weeks later, respectively. This also confirmed that chemotherapy alone is generally unable to eradicate the leukemic clone in Ph1 ALL.

Bone marrow and blood samples from patients #5 and #6 were analyzed 1.5, 3, 6 and 1.5, 3, 9 months respectively after transplantation and remained negative. Both patients died in CR (*to authors: CR = complete remission? cytogenetic response?*), 7 and 12 months respectively after confirming the more favorable results obtained by allo-BMT in Ph1 ALL. Finally Patient #4, who underwent allo BMT, had negative findings in blood and BM, 6 weeks after the procedure (Table 1) but relapsed 6 months later. Possible reasons for these discordant results included the fact that the sample may not have been representative due to non-homogeneous distribution of leukemia in the BM,[7] a possible change in phenotype (ie loss of CD34) at the time of relapse,[8] or the relatively prolonged interval between samples.

The sensitivity of the combination of CD34 selection and FISH we used, in the range of 10^{-3}, is lower than RT-PCR (10^{-4} to 10^{-6}). Such lower sensitivity could be interesting for example for detecting patients at high risk of relapse. Our results also show the lower sensitivity of the combination of CD34 selection and FISH in blood as compared to BM. In Ph1-ALL, similar results have been obtained using RT-PCR.[9]

Thus, in our hands, detection of MRD by cell sorting and FISH is a relatively simple and sensitive tech-

Table 1. Percentage of BCR-ABL CD34+ cells in 6 cases of adult Ph1 positive ALL

Pt. no.	*After induction CHT*		*After first consolidation CHT*		*After first consolidation CHT*		*CR duration (months)*
	blood	*BM*	*blood*	*BM*	*blood*	*BM*	
Pts.treated with CHT only							
1	8	34	5	41	39	38	Relapse: 3
2	9	38	3	5	18	39	Relapse: 3
3	8	10	NE	NE	NE	NE	Relpase: 2

Pt. no.	*After induction CHT*		*6 weeks after allo BMT*		*3 months after allo BMT*		*6 months after allo BMT*		*9 months after allo BMT*		*CR duration (months)*
	blood	*BM*	*blood*	*BM*	*blood*	*BM*	*blood*	*BM*	*blood*	*BM*	
Allografted pts.											
4	6	36	1	3	NE	NE	NE	NE	NE	NE	Relapse: 8
5	NE	NE	2	3	1.5	3	2	1	NE	NE	CR: 7+
6	NE	NE	1	2	2	2	NE	NE	2	3	CR: 12+

Pts.: patients; CHT: chemotherapy; BM: bone marrow; BMT: bone marrow transplantation; CR: complete remission; NE: not evaluable.

nique for the follow-up of Ph1 ALL patients, which may help predict clinical outcome. The value of this simple methodology should be tested in a larger trial on MRD in Ph1 ALL.

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Key words

Acute lymphoblastic leukemia, Philadelphia chromosome, FISH, minimal residual disease.

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Transformation of severe aplastic anemia into myelodysplastic syndrome with monosomy 7: monoclonal origin detected by HUMARA gene analysis during the aplastic anemia phase

A patient with aplastic anemia showed a monoclonal pattern on the HUMARA gene before the development of a myelodysplastic syndrome (MDS). The present case strongly suggests that if patients with aplastic anemia reveal monoclonality of the HUMARA gene, they should be considered to have a borderline disorder between aplastic anemia and MDS.

Sir,

Aplastic anemia is a hematopoietic disease of stem cells that causes pancytopenia and hypocellular bone marrow. Treatment with immunosuppressive agents, cytokines, and allogeneic bone marrow transplantation improves long-term survival.[1] However, long-term survivors with aplastic anemia have a high risk of developing solid tumors, acute myeloid leukemia, and myelodysplastic syndrome (MDS).[2] Recently, some patients with aplastic anemia have been reported to show clonal hematopoiesis,[3] but it is not clear whether this truly represents the clonal growth of progenitor cells, as occurs in MDS. Here, we describe an elderly woman with severe aplastic anemia and monoclonality of the human androgen receptor (HUMARA) gene who developed MDS with monosomy 7 after treatment with granulocyte-colony stimulating factor (G-CSF) and cyclosporin A.

A 77-year-old woman presented in February 1997 with purpura of the limbs. Hematological findings were as follows: hemoglobin 66 g/L, reticulocytes 6 $\times 10^9$/L, platelets 13×10^9/L, leukocytes 1.7×10^9/L, neutrophils 0.4×10^9/L. Bone marrow aspiration and biopsy revealed marked hypocellularity without proliferation of blasts and trilineage dysplastic changes. Cytogenetic analysis of bone marrow cells revealed a normal female karyotype (Table 1), and monosomy 7 was not detected by fluorescence *in situ* hybridization (FISH) using the CEP7 (D7Z1) probe (Vysis, IL, USA). She was diagnosed as having aplastic anemia, and was started on treatment with cyclosporin A and G-CSF. However, analysis of the HUMARA gene showed a monoclonal pattern in mononuclear cells obtained from the peripheral blood and bone marrow. In December 1998, bone marrow aspiration showed a hypocellular marrow with psuedo-Pelger neutrophils and megaloblastic changes. Cytogenetic analysis of her bone marrow revealed 17 abnormal mitoses [45,XX,-7,t(12,21)(q23;q22)] and only 3 normal mitoses (46,XX). The monosomy 7 was detected in 60.1% of interphase bone marrow cells by FISH. Therefore, transformation to hypoplastic MDS (refractory anemia) was diagnosed. G-CSF therapy was abandoned in January 1999, when she had been administered a total dose of 140 μg/kg over 3 months. She has now developed refractory anaemia with excess of blasts without cytotoxic agents.

In most cases of aplastic anemia, MDS may occur in the presence of monosomy 7 after long-term immunosuppressive and G-CSF therapy.[4] The cumu-