Absence of bcr/abl rearrangement in 41 patients with essential thrombocythemia

We report cytogenetic and fluorescence *in situ* hybridization (FISH) findings with the BCR/ABL probe in 41 consecutive newly diagnosed patients with essential thrombocythemia. All patients had a normal karyotype and none of them showed the BCR/ABL rearrangement as detectable by FISH.

Sir,

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (CMPD) characterized by extreme thrombocytosis and megakaryocytic hyperplasia, for which no primary cause can be found.[1,2] Strict diagnostic criteria have been proposed by the *Polycythemia Vera Study Group* (PVSG).[1] Karyotypic abnormalities are rare (occurring in approximately 5% of patients) and a specific chromosomal anomaly has not been established in this disorder.[3-5] We report cytogenetic and fluorescence *in situ* hybridization (FISH) findings using the BCR/ABL probe in a series of 41 patients newly diagnosed as having ET.

Bone marrow cells were obtained from a total of 41 patients who fulfilled the criteria of ET from the PVSG[1] between 1985 and 1998. All patients were studied at diagnosis prior to any treatment. Chromosome analyses were carried out in all cases (Table 1) with a 24-hour culture method. FISH studies on cultured bone marrow cells were performed in all patients using a dual-color locus specific BCR/ABL probe (VYSIS, Inc., Downers Grove, USA) following the proceedings supplied by the manufacturer. A minimum of 200 nuclei per case were analyzed by two different observers. Cut-off levels for the BCR/ABL rearrangement were defined by adding three standard deviations to the mean of the frequency of blood cells with BCR/ABL rearrangement FISH signals in ten normal specimens. We considered there to be BCR/ABL rearrangement when the number of cells with a BCR/ABL signal was ≥ 12%.

Mitoses were obtained from 40 out of 41 patients, and three of them showed polyploid metaphases (Table 1). No BCR-ABL rearrangement was found in any patient by FISH. The percentage of rearranged cells ranged between 1 and 10.3% (Table 1).

Table 1. Cytogenetic and *in situ* hybridization studies in 41 patients with essential thrombocythemia.

Conventional cytogenetics	
Number of patients	41
No mitosis	1
Normal karyotype	40
FISH results	
Number of patients	41
Number of cells/patient analyzed	200
Number of patients with BCR/ABL rearrangement	0

Previous papers observed that some ET patients showed the BCR/ABL rearrangement.[6,7] In our series, this critical point was analyzed using the FISH technique with the BCR/ABL probe, and no patient was positive for the BCR/ABL rearrangement. The Philadelphia chromosome, as detected by conventional cytogenetics, was also missing. Blickstein *et al.*[8] examined the BCR-ABL status by reverse transcriptase two-step nested polymerase chain reaction (PCR) in 25 Ph' negative ET patients, and observed that 12 were BCR-ABL positive and 13 were negative. Based on their findings, the investigators suggested the possibility of a new variant of ET. As a consequence, Marasca *et al*.[9] presented a series of 20 ET patients, but in only one patient was the BCR-ABL rearrangement detected by RT-PCR. This patient progressed to a blastic crisis 12 years after diagnosis. Recently, Hackwell *et al.*[10] presented the RT-PCR study of 18 consecutive newly diagnosed ET patients. None showed the BCR-ABL rearrangement.

It is unlikely that technical reasons could account for the discrepancy among these studies; perhaps these differences could be explained by the inclusion of patients not fulfilling the strict criteria necessary for a diagnosis of ET. Based on our data, we can not confirm the presence of BCR-ABL-positive ET cases but we agree with Blickstein *et al.*[8] that BCR/ABL status should be examined in all ET patients.

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Key words

Cytogenetics, in situ hybridization (ISH), essential thrombocythemia, Philadelphia chromosome.

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References

1. Murphy S, Iland H, Rosenthal D, Lazlo J. Essential thrombocythemia. An interim report from the Polycythemia Vera Study Group. Semin Hematol 1986; 23:177-82.
2. Stoll DB, Peterson P, Exten R, et al. Clinical presentation and natural history of patients with essential thrombocythemia and the Philadelphia chromosome. Am J Hematol 1988; 27:77-83.
3. Third International Workshop on Chromosomes in Leukemia 1980. Report on essential thrombocythe-

mia. Cancer Genet Cytogenet 1981; 4:138-42.
4. Knapp RH, Dewald GW, Pierre RV. Cytogenetic studies in 174 consecutive patients with preleukemic or myelodysplastic syndromes. Mayo Clin Proc 1985; 60: 507-16.
5. Mertens F, Johansson B, Heim S, Kristofferson U, Mitelman F. Karyotypic patterns in chronic myeloproliferative disorders: report on 74 cases and review of the literature. Leukemia 1991; 5:214-20.
6. Morris CM, Fitzgerald PH, Hollings PE, et al. Essential thrombocythaemia and the Philadelphia chromosome. Br J Haematol 1988; 70:13-9.
7. Corradini P, Palumbo AP, Battaglio S, Ponzio G, Boccadoro M, Pileri A. Analysis of the breakpoint cluster region in essential thrombocythemia. Haematologica 1990; 75:573-5.
8. Blickstein D, Aviram A, Luboshitz J, et al. BCR-ABL transcripts in bone marrow aspirates of Philadelphia-negative essential thrombocythemia patients: clinical presentation. Blood 1997; 90:2768-71.
9. Marasca R, Luppi M, Zucchini P, Longo G, Torelli G, Emilia G. Might essential thrombocythemia carry Ph anomaly? Blood 1998; 91:3084-5.
10. Hackwell S, Ross F, Cullis JO. Patients with essential thrombocythemia do not express BCR-ABL transcripts. Blood 1999; 93:2420-1.

Cladribine as monotherapy or combined with dexamethasone and idarubicin or mitoxantrone in previously treated patients with low-grade lymphoid malignancies

We compared efficiency and toxicity of cladribine (2-CdA) in monotherapy and in combination with dexamethasone and mitoxantrone (CMD) or idarubicin (CID) in pretreated patients with low grade NHL. We have shown that 2-CdA has significant activity in this disease but the addition of dexamethasone and anthracyclines may not be of advantage compared to 2-CdA alone.

Sir,

The long-term prognosis for patients with advanced relapsed or refractory low grade non-Hodgkin's lymphoma (LGNHL) remains poor. Therefore many new therapeutic approaches including new chemotherapy agents and new combinations regimens have been evaluated.

In this report we present our experience with the new purine analog 2-chlorodeoxyadenosine (2-CdA) used as monotherapy and combined with dexamethasone and mitoxantrone (CMD) or dexamethasone and idarubicin (CID) in patients with LGNHL.

This study was an open phase II single institution trial initiated in January 1996 and closed in April 1999. Criteria for entry into the study included a histologic diagnosis of LGNHL,[1] and advanced stage of refractory or relapsed disease. All patients had received at least three prior regimens of chemotherapy for NHL.

2-CdA was given at a dose 0.12 mg/kg in a 2-hour intravenous infusion, on days 1-5. In the CMD program mitoxantrone 10 mg/m^2 i.v., day 1 and dexamethasone 20 mg p.o., days 1-5, were added to 2-CdA. In the CID program idarubicin 12 mg/m^2 i.v., day 1 was given. The cycles were repeated at 4-week intervals in most cases.

Table 1. Patient characteristics.

	2-CdA	*CMD*	*CID*
Number of patients	36	17	5
Age (years)			
Median	63	61	60
Range	35-75	32-69	48-70
Sex (male/female)	23/13	10/7	2/3
Histology			
B-cell lymphocytic	20	9	1
Prolymphocytic	1	4	2
Lymphoplasmocytic	12	3	2
Centroblastic/centrocytic	3	1	0
Ann Arbor stage			
III	7	1	0
IV	29	16	5
B symptoms	28	18	5
Bone marrow involvement	19	9	3
Elevated LDH level	18	10	4
International Prognostic index			
3	1	0	0
4	30	13	4
5	5	3	1
Pre-treatment duration, years			
Median	4.5	3	4
Range	1-8	2-7	2-6
Prior course of chemotherapy			
Median	6.5	7	6
Range	5-10	5-11	3-8
Prior radiotherapy	21	9	2

Complete and partial responses (CR and PR) were defined as reported elsewhere.[2] Toxic effects were monitored and assessed according to the WHO criteria.[3]

Fifty-eight patients entered the study. Thirty-six of them were treated with 2-CdA alone, 17 received chemotherapy according to the CMD regimen and 5 according to the CID program. The characteristics of the patients are listed in Table 1.

Of the 36 patients treated with 2-CdA alone 13 (36.2%) responded. The median duration of CRs was 12 months (range from 7 to 48 months) and the median duration of PRs was 6 months. The CRs were observed after a median of 4 cycles and PRs after a median of 3 cycles of 2-CdA.

Of the 17 patients treated with CMD, 7 (41.2%) responded. The CRs were observed after 2 and 3 cycles of CMD and lasted 13 and 8+ months. PRs were observed after a median of 2 cycles of 2-CdA and their median duration was 7 months.

Among the 5 patients treated with CID we observed 1 CR and 1 PR which lasted, respectively, 6+ and 5 months. Both CR and PR were observed after 2 courses of CID. Table 2 summarizes the patients' responses.

Both 2-CdA and CMD (or CID) programs were well tolerated. The major toxicity was myelosuppression. Grade 3 or 4 thrombocytopenia occurred in 36.5% of patients treated with 2-CdA and 34% treated with