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SHORT REPORT

Deletion of the 5′-ABL region: a recurrent anomaly detected by fluorescence *in situ* hybridization in about 10% of Philadelphia-positive chronic myeloid leukaemia patients

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Summary. Inclusion of the *BCR–ABL* ES probe in routine cytogenetics led to the identification of a subgroup of Philadelphia positive (Ph+) chronic myeloid leukaemia patients characterized by a 5′-*ABL* deletion. This anomaly was observed in 5/51 cases (9·8%). Cytological and clinical data suggest that the 5′-*ABL* deletion may be associated with dysplastic features of polymorphonuclear cells and metamyelocytes and a short chronic phase duration.

Keywords: chronic myeloid leukaemia, Philadelphia chromosome, BCR–ABL positive, 5′-ABL deletion, FISH.

Bone marrow cells of 90–95% of chronic myeloid leukaemia (CML) patients harbour the Philadelphia chromosome, which results from the translocation t(9;22)(q34;q11) (Rowley, 1973). At the molecular level, the translocation juxtaposes 3′ DNA sequences of the *ABL* oncogene mapping on 9q34.1 with 5′ DNA sequences of the *BCR* gene located on 22q11, giving rise to a chimaeric *BCR–ABL* fusion gene on the derivative 22 (Melo, 1996).

New fluorescence *in situ* hybridization (FISH) strategies are now available that are highly sensitive to detect the *BCR–ABL* fusion gene on interphase nuclei (Bentz *et al*, 1994; Sinclair *et al*, 1997; Buno *et al*, 1998). In this context, the LSI *BCR–ABL* ES (extra signal) dual-colour translocation probe was introduced by Vysis (Downer's Grove, IL, USA) in 1998. In this novel system, the *ABL* probe (650 kb) spans both sides of the breakpoint on 9q so that Ph+ cells show (i) co-localization of the *BCR* and *ABL* probes and (ii) the presence of a third independent fluorescent signal for *ABL* (split signal remaining on the derivative 9). Using this new system, we observed a deletion of the 5′-*ABL* region in 5/51 Ph+ patients. We report here the clinical and cytogenetic findings for these five patients.

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MATERIALS AND METHODS

Fifty-one consecutive patients were included in the study: 31 men and 20 women, aged 26–84 years (median 54·5 years). All patients were studied at diagnosis, before any therapy. The diagnosis of CML was based on typical peripheral blood findings and the presence of the Philadelphia chromosome in marrow cells. The patients were re-evaluated with the new LSI *BCR–ABL* ES probe. Five hundred nuclei/patients were analysed.

RESULTS

Although the expected signals for the *BCR–ABL* ES probe were observed in Ph+ cells from 46/51 cases, the remaining five patients (9·8%) showed an atypical hybridization pattern that resulted from the loss of the split *ABL* signal normally present on the derivative 9, which is consistent with a deletion of the 5′-*ABL* region (Fig 1A and B). The clinical and cytogenetic data for these five patients are summarized in Table I. Interestingly, they presented with similar cytological features, including (i) small and monolobulated megakaryocytes (4/5 patients); (ii) polymorphonuclear cells and metamyelocytes with clumped chromatin (4/5 patients); (iii) Pelger-Huët or Pelger-Huët-like anomalies (3/5 patients) ($P < 0·025$); and (iv) dysgranulopoiesis

Table I. Clinical and cytogenetic data of patients with both the translocation and the 5′-ABL deletion.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	Male	Male	Female	Male	Female
Age (years)	44	51	84	53	38
Haematocrit (%)	42	41·2	32·8	34·8	34·5
Platelets (10^9/l)	516	341	617	214	368
White blood cell count (10^9/l)	188	78·8	151	343	168
Blasts (%)	2·5	2	1	0·6	1·6
Splenomegaly (cm)	4	3	–	9	23
Sokal index	0·81	0·82	1·17	0·97	1·46
Ph chromosome (% metaphase)	100	100	100	100	100
FISH					
Expected pattern	0·35	0·25	0·2	0·3	0·3
Co-localization pattern	93·6	86·2	94·5	97·5	95·4
Disease stage	Chronic phase	Chronic phase	Chronic phase	Chronic phase	Chronic phase
Treatment	Hydrea, IFN-α	Myleran, IFN-α	Hydrea	Hydrea	ARA-C, IFN-α, Hydrea
Small monolobulated megakaryocytes	–	+	+	+	+
Clumped chromatin in neutrophils	+	+	+	+	–
Pelger- Huët anomaly	–	+	+	+	–
Hypogranular/agranular neutrophils	–	–	+	+	+
BMT	+	+	–	+	+
Delay from diagnosis (months)	22	29	–	8	13
Duration of chronic phase (months)	–	16	9	–	–
Survival (months)	48*	45	10	22*	14*
Cause of death	–	Infectious disease	AML	–	–

*Still alive. IFN-α, α-interferon. ARA-C, cytarabine.

including hypogranular and agranular elements (3/5 patients) (Table I). Two patients presented with the four cytological features ($P < 0{\cdot}001$).

Patients 1, 4 and 5 underwent bone marrow transplantation (BMT) in chronic phase (Table I). Patient 2 developed an acceleration phase (AP) and a blast crisis (BC) 16 and 25 months, respectively, after diagnosis. An extra Ph chromosome was observed during acceleration and blast crisis in, respectively, 4% and 6% of the metaphases. He underwent BMT 29 months after diagnosis but died from neural infection. Patient 3 presented with acute myeloid leukaemia and 65% circulating blasts 9 months after diagnosis. No cytogenetic analysis was performed at that time. She died 3 months later.

DISCUSSION

Screening of CML patients with the *BCR–ABL* ES probe (Vysis) led to the identification of a subgroup of patients with a 5′-*ABL* deletion, including the arginosuccinate synthetase gene. In our series, 5/51 patients (9·8%) showed both the *BCR–ABL* rearrangement and the 5′-*ABL* deletion. Similar observations have been reported by Dewald *et al* (1998, 1999) with the D-FISH probe from Oncor. They observed atypical hybridization patterns consistent with loss of 3′-*BCR*, loss of 5′-*ABL* or loss of both segments in 28/141 (19%) Ph+ CML patients (Dewald *et al*, 1999). More particularly, the 5′-*ABL* segment was lost in 17/141 (12%) patients.

However, it remains to be determined whether the 5′-*ABL* deletion is to be considered as a secondary chromosomal change, heralding the evolution towards an acceleration phase (AP) or blast crisis (BC), or whether the deletion characterizes a subset of CML patients at diagnosis. In the light of our results, we cannot favour either of these two hypotheses. On the one hand, the FISH data indicated that the translocation and the deletion appeared in a one-step event as we were unable to detect a subclone with the expected hybridization pattern. On the other hand, patients with both the translocation and the submicroscopic deletion seemed to have particular cytological features associated with a short chronic phase duration. In our series, the mean duration of chronic phase for the two patients that developed an AP and/or BC was 12·5 months (9–16 months). This interval is markedly shorter than the mean duration of chronic phase (30–60 months) reported in the literature (Sawyers, 1999). The possible prognostic significance of the 5′-*ABL* deletion was also noted in the study of Dewald *et al* (1998), in which atypical hybridization patterns were observed in patients with masked Ph (five patients) or with additional chromosomal changes characteristic of the AP or BC phases (two patients). However, the observation of an extra Ph during AP and BC in one of our patient indicates that the 5′-*ABL* deletion is probably not directly involved in acceleration or blast crisis. Nevertheless, the deletion could favour the emergence of additional chromosomal anomalies, the mechanism of which remains to be elucidated.

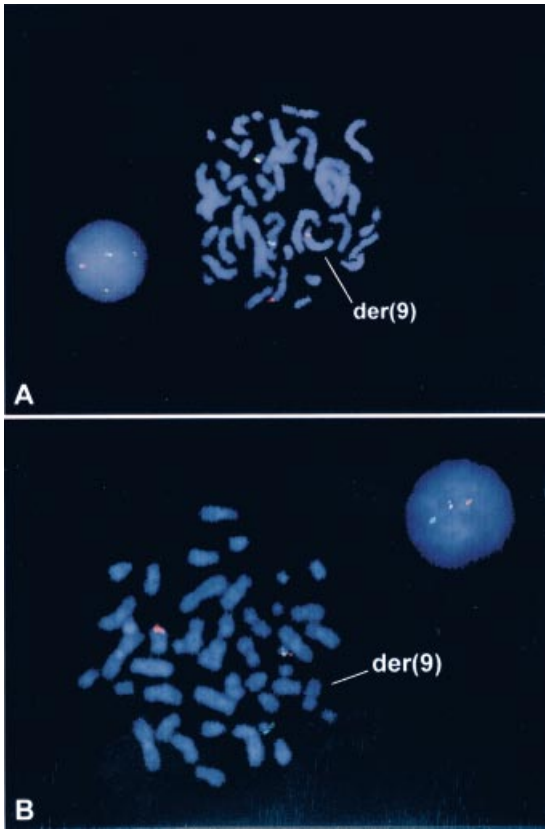

Fig 1. (A) In Ph+ nuclei and metaphases, the expected hybridization pattern consists of one green (BCR on chromosome 22), two orange (normal and split ABL on normal and derivative 9) and one yellow (fused BCR/split ABL on derivative 22) signals. (B) The split ABL signal on derivative 9 (arrow) is missing in patients with the atypical hybridization pattern.

In conclusion, the use of the new commercially available probes (*BCR–ABL* ES and D-FISH) could enable the subclassification of CML patients according to the presence of 5′-*ABL* and/or 3′-*BCR* deletions. Our results indicate that about 10% of CML patients have the 5′-*ABL* deletion, particular cytological features and a possible short chronic phase duration. However, our series is small and collection of large cohorts of patients is needed to assess these clinical findings.

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NOTE ADDED IN PROOF

During submission of this manuscript, Sinclair *et al* published a cohort of 16 patients with deletions of the 5′-*ABL* region. They showed that these deletions identify a subgroup of CML patients that have a poor prognosis compared with classical Ph+ CML patients [Sinclair, P.B., Nacheva, E.P., Leversha, M., Telford, N., Chang, J., Reid, A., Bench, A., Champion, K., Huntly, B. & Green, A.R. (2000). Large deletions at the (9;22) breakpoint are common and may identify a poor-prognosis subgroup of patients with chronic myeloid leukaemia. *Blood*, **95**, 738–744].

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