

Report of 34 patients with clonal chromosomal abnormalities in Philadelphia-negative cells during imatinib treatment of Philadelphia-positive chronic myeloid leukemia

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Imatinib mesylate (Gleevec®), an inhibitor of the BCR-ABL tyrosine kinase, was introduced recently into the therapy of chronic myeloid leukemia (CML). Several cases of emergence of clonal chromosomal abnormalities after therapy with imatinib have been reported, but their incidence, etiology and prognosis remain to be clarified. We report here a large series of 34 CML patients treated with imatinib who developed Philadelphia (Ph)-negative clones. Among 1001 patients with Ph-positive CML treated with imatinib, 34 (3.4%) developed clonal chromosomal abnormalities in Ph-negative cells. Three patients were treated with imatinib up-front. The most common cytogenetic abnormalities were trisomy 8 and monosomy 7 in twelve and seven patients, respectively. In 15 patients, fluorescent *in situ* hybridization with specific probes was performed in materials archived before the initiation of imatinib. The Ph-negative clone was related to previous therapy in three patients, and represented a minor pre-existing clone that expanded after the eradication of Ph-positive cells with imatinib in two others. However, in 11 patients, the new clonal chromosomal abnormalities were not detected and imatinib may have had a direct effect. No myelodysplasia was found in our cohort. With a median follow-up of 24 months, one patient showed CML acceleration and two relapsed.

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Introduction

Chronic myeloid leukemia (CML) is a clonal hematological disorder that develops in the stem-cell compartment. The hallmark of CML is the translocation t(9;22)(q34;q11), which is referred to as the Philadelphia (Ph) chromosome, present in about 95% of CML patients.¹ At the molecular level, the translocation results in a rearrangement between the *ABL* gene located on chromosome 9 and the *BCR* gene located on

chromosome 22 forming a hybrid gene. The *BCR/ABL* fusion gene codes a p210 protein kinase that plays a central role in the pathogenesis of CML.²

Imatinib mesylate (STI571), also known as Gleevec® (Novartis, Basel, Switzerland), is an ABL kinase inhibitor that induces growth arrest and apoptosis in BCR-ABL hematopoietic cells. This molecular inhibitor targeted treatment produces major or complete cytogenetic remissions (MCR or CCR) in 60% of CML patients, who are intolerant or refractory to prior interferon alpha (INF α) therapy and up to 87% in newly diagnosed CML patients.^{3,4} Eight cases of development of clonal cytogenetic abnormalities in Ph-negative metaphases have been reported following therapy with INF α ^{5–10} and 47 with imatinib.^{11–22} The incidence of such abnormalities and their clinical significance remain to be clarified. We report here the occurrence of clonal cytogenetic abnormalities in Ph-negative metaphases in 34 CML patients treated with imatinib, the largest cohort to our knowledge.

Patients and methods

Patients

A retrospective survey was performed on the results of cytogenetic studies in 1001 patients with Ph-positive CML from 15 different French institutions enrolled in Novartis Pharma trials and treated with imatinib (Gleevec® 400 or 600 mg/day) from September 2000 to October 2002. Of those, 34 patients have developed clonal chromosomal abnormalities in Ph-negative cells.

Conventional cytogenetics

Conventional cytogenetic was performed in each local cytogenetic laboratory after 24 to 72 h or overnight unstimulated culture of bone marrow. RHG banding was obtained using phosphate buffer and Giemsa staining.²³ Chromosome abnorm-

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alities were described according to the ISCN 1995 (International System for Human Cytogenetic Nomenclature). Cytogenetic responses were based on the analysis of at least 20 metaphases in most patients (from 30 to 60 metaphases in 12 patients; from 20 to 30 metaphases in 17 patients), and on less than 20 metaphases in five patients with a predominant Ph-positive clone. They were defined as completeCR (CCR): no Ph+ cells, majorCR <36% Ph-positive cells, minorCR 36–65% Ph-positive cells; minimalCR: 66–95% Ph-positive cells; and no cytogenetic response (NOR): 96–100% Ph-positive cells.³

Fluorescent in situ hybridization (FISH)

Centromeric probes were used for chromosome 7 (CEP 7) and chromosome 8 (CEP 8) (Vysis, Downers Grove, IL, USA or Q-Biogen, Illkirch, France), whole chromosome painting was performed for chromosomes 2 and 3 (WCP 2 and WCP 3) (Q-Biogen, Illkirch, France) and t(9;22)(q34;q11) was detected with specific probes (LSI ES BCR-ABL) (Vysis, Downers Grove, IL, USA). Hybridization was performed as recommended by the manufacturer's protocol. Slides were mounted and counterstained with antifade DAPI (Vectashield), visualized using a fluorescent microscope and analyzed with a FISH Analysis software. In all, 10–50 metaphases were analyzed and 100–500 interphase cells were scored.

Morphologic review

May–Grünwald–Giemsa (MGG)-stained bone marrow aspirate smears were reviewed by three morphologists (VL, MI and GF). Marrow cellularity, proportion of blasts and myelodysplastic morphology were examined with caution. According to the WHO proposals, a dysplasia less than 10% of myeloid lineage was not considered as significant.

Results

Study population

In total, 34 patients with clonal chromosomal abnormalities in Ph-negative cells were studied. There were 19 male and 15 female patients. At the time of imatinib treatment, the median age was 56 years (ranging from 24 to 79 years), 29 patients were in chronic phase and five in an accelerated phase. The median duration of disease was 31 months (ranging from 0 to 108 months). Three patients were treated up-front with imatinib (nos. 12, 22 and 33). Three patients have been previously treated with hydroxyurea (HU) alone, 28 with INF α including 12 in combination with cytarabine (Ara-C). The median follow-up duration after imatinib therapy was 24 months (ranging from 11 to 36 months). Patient's characteristics are shown in Table 1.

Table 1 Characteristics of patients

Patients	Age (years)	Sex	Treatment (Tt) prior imatinib	Best response prior imatinib	Disease duration prior imatinib (months)	Disease phase at imatinib Tt
1	46	F	INF Ara-C	MinimalCR (86%)	48	CP
2	33	M	HU, INF	MinimalCR (88%)	13	CP
3	52	F	INF, HU	MinimalCR (87%)	108	AP
4	61	M	INF, HU	MajorCR (7%)	51	CP
5	60	M	INF, HU, Ara-C	MinimalCR (68%)	30	CP
6	67	M	HU 1 month		1	AP
7	62	F	HU, INF, Ara-C	CCR	32	CP
8	67	F	INF–Ara-C, HU	NOR	27	CP
9	67	F	HU, INF–Ara-C	NOR	36	CP
10	55	M	INF	MajorCR (20%)	48	CP
11	62	M	IFN	MinimalCR (90%)	45	CP
12	52	F	None		At diagnosis	CP
13	24	F	INF–Ara-C	NOR	16	CP
14	46	F	INF–Ara-C	MajorCR (27%)	24	CP
15	49	M	INF	NOR	22	CP
16	52	F	INF–Ara-C, HU	NOR	94	AP
17	43	F	HU, INF	?	24	CP
18	52	M	HU	NOR	5	CP
19	43	M	INF–Ara-C	NOR	4	AP
20	47	M	INF–Ara-C	MinimalCR (70%)	48	AP
21	68	M	INF	CCR	80	CP
22	77	M	None		At diagnosis	CP
23	38	M	HU, INF	NOR	3	CP
24	55	M	HU	ND	2	CP
25	41	F	HU, INF	MinimalCR (90%)	60	CP
26	60	M	INF	MinimalCR (75%)	25	CP
27	73	F	INF, HU	NOR	42	CP
28	79	F	INF	MinimalCR (84%)	16	CP
29	71	M	INF, HU	MajorCR (30%)	43	CP
30	50	F	HU, INF–Ara-C	NOR(97%)	30	CP
31	66	M	HU, INF	MinimalCR (76%)	36	CP
32	56	M	HU, INF–Ara-C	MinimalCR (80%)	23	CP
33	76	M	None		At diagnosis	CP
34	47	F	INF	MinimalCR (90%)	11	CP

F = female; M = male; Ara-C = cytarabine; HU = hydroxyurea; INF = interferon; CCR = complete cytogenetic response; MajorCR = major cytogenetic response; MinimalCR = minimal cytogenetic response; NOR = no cytogenetic response; (%) = % of Ph-positive cells; AP = accelerated phase; CP = chronic phase; ND = no determined.

SPOTLIGHT

Cytogenetic analysis

At diagnosis, 32 patients showed a classic t(9;22) Ph chromosome translocation, loss of chromosome Y, and an interstitial deletion of the short arm of chromosome 7 was superimposed onto the Ph chromosome in patients 11 and 34, respectively.

Prior to imatinib initiation, the Ph was present in 75–100% of the metaphases in 26 cases, in 7, 30 and 33% in three cases and absent in one case. During imatinib treatment, clonal chromosomal abnormalities in the Ph-negative cells were observed in 34 patients after a median of 10 months of therapy (3–24 months). The occurrence of the abnormal clone in relation to time is shown in Figure 2. No significant difference was observed in patients previously treated with chemotherapy (HU or Ara-C) compared to patients only exposed to INF α (data not shown). Numerical chromosomal abnormalities were noted 29 times in 26 cases (trisomy 8, $n=12$; monosomy 7, $n=7$; loss of chromosome Y, $n=5$; gain of chromosomal marker, $n=3$, gain of chromosome Y, $n=1$ and trisomy 15, $n=1$). In nine cases, structural chromosomal abnormalities occurred with reciprocal balanced translocations (t(7;22)(q22;q13), t(2;3)(q21;p14), t(3;11)(q27;q13), t(1;6)(p32;p24–25)) or five deletions (del(20)(q11) in two cases, del(7)(p13p21), del(7)(q21q36) and del(7)(q11)). Three patients (nos. 2, 22 and 34) showed two unrelated clones.

FISH analysis

In three patients (nos. 1, 3 and 23) with trisomy 8, FISH was performed using simultaneous CEP 8 and LSI ES BCR-ABL probes and no cryptic BCR-ABL rearrangement was detected in cells carrying trisomy 8 (Figure 1). In 15 patients (nos. 1, 3, 9, 11, 12, 13, 19, 20, 21, 23, 24, 25, 27, 33 and 34), FISH with specific probes was made in archived material prior to the imatinib treatment. The secondary clonal abnormality was detected in four patients (nos. 20, 21, 24 and 33). Results of karyotypic and FISH analyses are shown in Table 2.

Cytological examination

The morphology of bone marrow specimens obtained from 28 patients with clonal abnormalities was reviewed. The cellularity was markedly decreased in 14 cases, slightly decreased in seven and normal or increased in seven cases. Dysmyelopoiesis was absent or not significant in 22 cases and not evaluable in four cases because of hypocellularity. In two cases (nos. 18 and 31), cytological abnormalities of the myeloid lineage were observed. In patient 18, megaloblastic changes were obvious but pre-existed before imatinib treatment. Both dyserythropoiesis and dysgranulopoiesis changes in less than 10% of the elements was noted in patient 31 who had been previously treated with HU and IFN. In summary, the morphological review did not allow a definitive diagnosis of myelodysplastic syndrome in any case.

Response to therapy and follow-up

A cytogenetic response improvement under imatinib therapy was obtained in all 34 patients. A total of 24 patients (71%) achieved an MCR including 17 patients in CCR (50%). Five patients were in minorCR and five in minimalCR. An MCR was achieved in all patients with the loss of chromosome Y (including four CCRs), although it was observed in only 45%

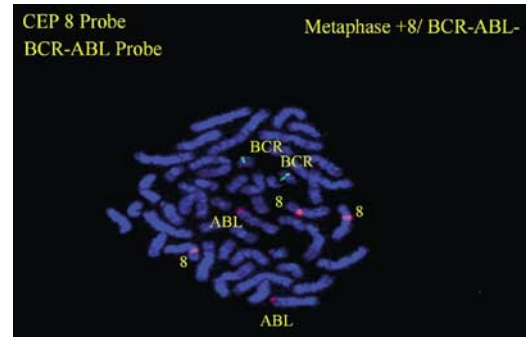


Figure 1 FISH using simultaneous CEP 8 and LSI ES BCR-ABL probes. No cryptic BCR-ABL rearrangement was detected in cells carrying trisomy 8

of patients harboring trisomy 8 or abnormalities on long arm of chromosome 7 ($P=0.005$, Fisher's exact test). Among the 17 patients in CCR when clonal Ph-negative cells appeared, 15 have been monitored for the BCR-ABL transcript and only one (no. 18) was in molecular remission. After a median follow-up of 24 months, one patient progressed to accelerated phase (no. 3) and two patients relapsed (nos. 11 and 12).

Discussion

Among 1001 patients with Ph-positive CML treated with imatinib, 34 patients developed clonal chromosomal abnormalities in Ph-negative cells 10 months (range 3–24 months) after imatinib therapy was initiated. The occurrence of new clones in relation to time is illustrated with a Kaplan–Meier plot in Figure 2. The incidence of such abnormalities is 3.4% in this retrospective cohort, the largest reported to our knowledge. In smaller series reported previously, the incidence of chromosomal abnormalities in Ph-negative cells ranged from 2%^{11,12} to 15.2%.¹⁶ However, the exact incidence is difficult to evaluate because complete karyotyping of Ph-negative metaphases during follow-up might not have been performed systematically. Clonal chromosomal abnormalities were mainly numerical chromosomal abnormalities (74%), principally trisomy 8 (34%) and monosomy 7 (20%). These numerical aberrations are frequent and recurrent in myeloid diseases, as are structural abnormalities such as interstitial deletion of long arm of chromosome 7, del(7q), deletion of long arm of chromosome 20, del(20q) and the loss of chromosome Y found in 2 (6%), 2 (6%) and 5 (14%) of our patients, respectively. Monosomy 7 and del(7q) are unfavorable prognostic chromosome aberrations in myelodysplasia and are often associated with therapy-related myelodysplasia or acute myeloid leukemia (tMDS or tAML). In our series, in 28 patients, no myelodysplastic features were evidenced despite a thorough morphological review. These results are in concordance with the study of Medina *et al*,²² who have recently reported 21 cases of Ph-negative clonal evolution without features of myelodysplasia. Conversely, O'Dwyer *et al* have described mild dysplastic abnormalities in all patients under imatinib whether they developed clonal changes in Ph-negative cells or not. Their imatinib-treated patients also displayed various degrees of megaloblastic changes, which we also observed in our cohort.²⁰

For two of the chromosomal abnormalities observed in the Ph-negative cells, $-Y$ and $+8$, we cannot rule out the hypothesis of an age-dependent process and of a constitutional mosaicism, respectively. Loss of chromosome Y is common in

Table 2 Results of karyotypic and FISH analysis

Patients	Karyotype at the time of imatinib	Karyotype with clonal abnormality in Ph-cells with imatinib	Delay for clonally Ph-cells emergence (mth) Cytogenetic response	Myelodysplasia
1	46,XX,t(9;22) [30] IP-FISH C8: 0.8% Cut-off: 2%	46,XX,t(9;22) [25] 46,XX [1] 47,XX,+8 [2] .ish9q34(ABLx2)22q11.2(BCRx2)	17 MinimalCR (89%)	No Hypoplastic
2	46,XY,t(9;22)[15] 46,XY [2]	46,XY,t(9;22) [6] 48,XY,t(9;22),+8,+14 [2] 46,XY [13]/ 47,XY,+Y [5] 46,XY,-22,+mar [2]	6 MajorCR (28%)	No Hypoplastic
3	46,XX,t(9;22) [29] 45,XX,t(9;22),-21 [4] IP-FISH C8: 2% Cut-off: 2%	46,XX,t(9;22) [13] 47,XX,+8 [14] .ish9q34(ABLx2)22q11.2(BCRx2)	8 MinorCR (48%)	No Hypoplastic
4	46,XY,t(9;22)[2] 46,XY [28]	46,XY [28] 47,XY,+15 [2]	3 CCR	No
5	46,XY,t(9;22) [30]	46,XY,t(9;22) [4] 46,XY [18] 46,XY,del(7)(q21q36) [8]	6 MajorCR (13%)	No Hypoplastic
6	46,XY,t(9;22) [23]	46,XY [28] 46,XY,t(3;11)(q27;q13) [2]	3 CCR	No
7	46,XX,t(9;22)[10] 46,XX [20]	46,XX [29] 47,XX,+mar [2]	3 CCR	No
8	46,XX,t(9;22) [30]	46,XX,t(9;22) [21]/46,XX [1] 45,XX,-7 [3]	9 MinimalCR (84%)	No
9	46,XX,t(9;22) [30] IP-FISH C8: 0%	46,XX,t(9;22) [1]/46,XX [16]/ 47,XX,+8 [14]	9 MajorCR (3%)	Not evaluable Hypoplastic
10	46,XY,t(9;22)[21] 46,XY [4]	46,XY [45] 46,XY,del(20)(q11) [5]	6 CCR	No
11	45,X,-Y,t(9;22) [50] FISH C7: 0% IP and MP	45,X,-Y,t(9;22)[10] 45,XY,-7 [10]	10 MinorCR (50%)	No Hypoplastic
12	46,XX,t(9;22) [20] IP-FISH C8: 0%	46,XX [8] 47,XX,+8 [32]	19 CCR	Not evaluable
13	46,XX,t(9;22) [4] IP-FISH C7: 0%	46,XX,t(9;22) [3] 46,XX [2] 45,XX,-7 [11]	6 MajorCR (19%)	Not evaluable
14	46,XX,t(9;22) [20]	46,XX,t(9;22) [12] 47,XX,+8 [8]	12 MinorCR (60%)	Not evaluable
15	46,XY,t(9;22) [20]	46,XY,t(9;22) [15] 45,XY,-7 [5]	12 MinimalCR (75%)	Not evaluable
16	46,XX,t(9;22) [20]	46,XX,t(9;22) [28] 45,XY,-7 [3]	15 MinimalCR (90%)	No
17	46,XX,t(9;22) [20]	46,XX,t(9;22) [5] 47,XX,+8 [28]	18 MajorCR (15%)	No Hypoplastic
18	46,XY,t(9;22) [20]	46,XY [18] 46,XY,t(7;22)(q22;q13) [6]	6 CCR	Megaloblastic change
19	46,XY,t(9;22) [9] 45,X,-Y,t(9;22) [20] FISH C8: 0%IP and MP	46,XY [24] 47,XY,+8 [8]	6 CCR	DysG.
20	46,XY,t(9;22) [60] IP-FISH C7:7% Cut-off: 5%	46,XY [25] 45,XY,-7 [5] 46,XY,r(7) [10]	9 CCR	No
21	46,XY [28] MP FISH WCP2 and 3: 14%	46,XY [26] 46,XY,t(2;3)(q21;p14) [4] .ish t(2;3)(WCP2+,3+)	6 CCR	No Hypoplastic
22	46,XY,t(9;22) [20]	46,XY [15] 46,XY,del(20)(q11)[11] 45,X,-Y[5]	4 CCR	Not evaluable
23	46,XY,t(9;22) [20] FISH C8: 0% IP and MP	46,XY, t(9 ;22) [8] 46,XY [5] 47,XY,+8 [5]/48,XY,+8,+m [2] .ish9q34(ABLx2)22q11.2(BCRx2)	10 MinorCR (40%)	No
24	46,XY,t(9;22) [20] FISH XY: 0% MP and 2.7% IP Cutoff: 2%	46,XY [14] 45,X,-Y [6]	6 CCR	No Hypoplastic

Table 2 Continued

Patients	Karyotype at the time of imatinib	Karyotype with clonal abnormality in Ph-negative cells with imatinib	Delay for clonally Ph-negative cells emergence (mth) Cytogenetic response	Myelodysplasia
25	46,XX,t(9;22)[52] 46,XX [6] FISH C8: 0% IP	46,XX, t(9;22) [5] 46,XX [12] 47,XY,+8 [3]	12 MajorCR (25%)	No Hypoplastic
26	46,XY,t(9;22) [15] 46,XY [5]	46,XY [14] 45,X,-Y [6]	10 CCR	No Hypoplastic
27	46,XX,t(9;22) [20] FISH C8: 1% IP Cutoff: 2%	46,XX,t(9;22) [8] 46,XX [4] 47,XX,+8 [5]	16 MinorCR (47%)	No
28	46,XX,t(9;22) [16] 46,XX [3]	46,XX [23] 46,XX,del(7)(p13p21) [7]	8 CCR	No Hypoplastic
29	46,XY,t(9;22) [9] 46,XY [21]	46,XY [28] 45,X,-Y [4]	14 CCR	No Hypoplastic
30	46,XX,t(9;22) [29] 46,XX [1]	46,XX [10] 47,XX,+8 [10]	24 CCR	Not evaluable
31	46,XY,t(9;22) [13] 46,XY [4]	46,XY [12] 45,XY,-7,der(17)ins(7;17)(?;p?) [12]	8 CCR	DysG.
32	46,XX,t(9;22) [22]	46,XY [39] 46,XY,t(1;6)(p32;p24-25) [4]	17 CCR	No
33	46,XY,t(9;22) [15] FISH SY: 1.6% IP Cut-off: 1%	46,XY,t(9;22)[1] 46,XY [24] 45,X,-Y [5]	3 MajorCR (3%)	Hypoplastic
34	46,XX,t(9;22) [11] 46,XX,t(9;22),del(7)(p11p13) [7] 46,XX [2] FISH C8: 0% IP	46,XX,t(9;22) [2] 46,XX,t(9;22),del(7)(p11p13) [11] 46,XX [4] 47,XX,+8 [1]	11 MinimalCR (72%)	No Hypoplastic
		46,XX,t(9;22),del(7)(p11p13)[4] 46,XX [8] 47,XX,+8 [5] 46,XX,del(7)(q11) [3]	17 MajorCR (20%)	

IP = interphase; MP = metaphase; FISH = fluorescent *in situ* hybridization; C8 = chromosome 8 centromeric probe; C7 = chromosome 7 centromeric probe; WCP = whole chromosome painting; CCR = complete cytogenetic response; MajorCR = major cytogenetic response; MinorCR = minor cytogenetic response; MinimalCR = minimal cytogenetic response; mth = months; NOR = no cytogenetic response (%) = % of Ph-positive cells; DysG = dysgranulopoiesis; DysE = dyserythropoiesis.

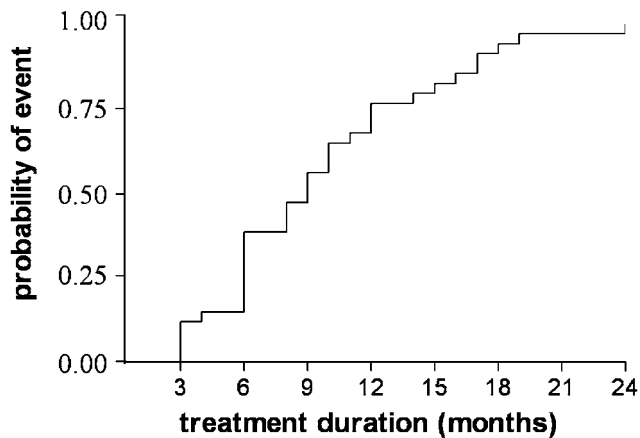


Figure 2 Kaplan-Meier plot showing the occurrence of new clones in relation to time in the studied. Population ($n=34$).

men and has been related to age. Wiktor *et al*²⁴ have reported that a minimal value of 75% metaphases with chromosome Y loss, even in older males, can be considered as a disease-associated clonal population. We found the loss of chromosome Y in five patients 55, 60, 71, 76 and 77 years old. Their karyotype showed a loss of Y in 30, 30, 13, 17 and 16%

metaphases, respectively. These isolated losses of Y might therefore reflect an age-dependent process. In case 34, this process cannot account for the fast expansion of the clone -Y (17% at 3 months and 53% at 6 months) that occurred during Gleevec[®] exposure. Trisomy 8 may derive from a constitutional clone as postulated by Maserati *et al*,²⁵ who have evidenced a constitutional trisomy 8 mosaicism in 15–20% of myelodysplastic syndromes with trisomy 8. Furthermore, the accurate incidence of this mosaicism is difficult to assess because constitutional trisomy 8 may be associated with a near-normal phenotype and a normal Intellectual Quotient.²⁶ In our series, trisomy 8 was an acquired abnormality in all patients with material that could be analyzed by FISH for mosaicism.

With regard to the emergence of clonal abnormalities in the Ph-negative cell population in CML patients, previous reports have focused attention on the potentially deleterious role of imatinib. The sample size of the present study allowed us to better determine the proper contribution of imatinib to this phenomenon.

In a first group of patients, chromosomal abnormalities already existed before imatinib therapy. We retrospectively performed FISH analysis in 15 samples archived before imatinib treatment to define whether these chromosomal abnormalities were present previously. They were detected in four patients (nos. 20, 21, 24 and 33). Patients 20 and 21 were treated by INF

for 4 and 5 years, respectively, and the percentage of cells carrying clonal abnormalities decreased with time. In these cases, the emergence of clonal changes in Ph-negative cells is probably related to INF therapy. In patients 24 and 33, the loss of chromosome Y was detected in 2.7 and 1.6% of interphasic cells in samples archived before imatinib. In these two patients, the Ph-negative clone with -Y pre-existed before any treatment and represented a minor clone that expanded after the eradication of Ph-positive cells under imatinib treatment. In fact, in patient 24, loss of Y was detected in 30% of cells and the cytogenetic response was complete. In patient 33, loss of Y was detected in 17 and 53% of cells after, respectively, 3 and 6 months of imatinib therapy and the cytogenetic response was major. As a majority of reported CML patients had a history of exposure to chemotherapy, it is tempting to assume that previous treatments were responsible for the emergence of these Ph-negative clones after the selective suppression of the predominant Ph-positive clone with imatinib. This explanation was also suggested by Bumm *et al*,¹⁵ but their series included only CML patients with a previous exposure.

In a second group of 11 patients, the clonal chromosomal abnormalities characterizing the Ph-negative cells were not detected in the samples archived before the initiation of imatinib. Two of them were treated with imatinib up-front (nos. 12 and 22) and another (no. 6) had received HU for only 1 month before imatinib therapy. Four similar cases have been reported recently, three untreated and one treated by HU for only 5 weeks.^{17,21,22} Taken as a whole (current series and literature), clonal changes in Ph-negative cells have so far been described in six patients treated with imatinib up-front and in two others who had received only HU. These findings strongly suggest a causative role of imatinib. In case 35, the emergence of a second stemline with 7q-, concomitant to the clonal expansion of the first stemline with trisomy 8 (7-25%) further argues in favor of a direct role of imatinib. Indeed, as suggested by Bumm *et al*,¹⁵ imatinib is an ABL kinase inhibitor and its permanent inhibition can produce genetic damage, since it cannot interact with proteins involved in the DNA repair.²⁷⁻³³

At the time of emergence of clonal abnormalities, all patients had achieved a degree of cytogenetic response: 17 patients were in CCR, seven in majorCR, five in minorCR and five in minimalCR. The 71% of the MCRs observed in this report were similar to the percentages previously reported in pretreated patients. Patients with the loss of chromosome Y fared better than the others, since all of them achieved a MCR. Only one of the 34 patients (no. 3) progressed to an accelerate phase, and two have relapsed (nos. 11 and 12) 24 and 34 months after imatinib therapy, respectively. After a median follow-up of 24 months, no progression of CML was observed in the 31 other patients.

In conclusion, our observations support the hypothesis that imatinib may have favored the emergence of clonal chromosomal abnormalities in 11 cases. Cytogenetic abnormalities in the Ph-negative clone were not associated with myelodysplasia. These changes did not impair the cytogenetic response to imatinib, and imatinib therapy was not interrupted in any of these responding patients. Furthermore, the current findings suggest that patients on imatinib should be regularly monitored by cytology and conventional karyotype even in the case of CCR. All metaphases, including the Ph-negative ones should be fully karyotyped. With the aim of determining the genuine frequency of clonal abnormalities occurring in Ph-negative cells, their correlation with morphologic features and their impact on a long-term follow-up, the France Intergroupe pour la Leucémie Myeloïde Chronique (FILMC) group is currently

establishing a French registry of patients treated with imatinib including cytology, cytogenetics and clinical data.

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