Chronic myeloid leukemia in chronic phase responding to imatinib: the occurrence of additional cytogenetic abnormalities predicts disease progression

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Background and Objectives. The acquisition of additional cytogenetic changes (clonal evolution, CE) during treatment of chronic myeloid leukemia (CML) with imatinib mesylate is currently regarded as an index of increasing resistance to imatinib. Therefore, to investigate whether CE as an isolated event increases the risk of disease progression during imatinib treatment, we compared the outcome of patients with CML in chronic phase (CML-CP) who developed CE whilst in complete hematologic remission with the outcome of comparable patients in complete hematologic remission who showed no evidence of CE.

Design and Methods. We serially studied cytogenetic findings in 102 patients receiving the Abl-tyrosine kinase inhibitor, imatinib mesylate, as sole agent to treat CML-CP and who had no evidence of CE before initiation of imatinib treatment.

Results. CE was identified during treatment with imatinib in 15 patients, 10 of whom were in complete hematologic remission. In most cases these changes occurred exclusively in the Ph^+ population but in three patients additional changes occurred in a co-existing Ph-negative population. Patients with *de novo* CE in the absence of any other sign of disease progression had a significantly higher incidence of progression by 18 months than did non-CE patients (progression-free survival 34.3% (CI 10.5-69.8%) vs. 94.1% (CI 80.6-98.4%), $p<0.0001$).

Interpretation and Conclusions. Based on this relatively small series of patients, we conclude that acquisition of clonal evolution increases the risk of subsequent disease progression also in CML patients in complete hematologic remission on imatinib.

Key words: chronic myeloid leukemia, imatinib mesylate, clonal evolution.

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Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder characterized in almost every patient by the presence in leukemia cells of a 22q- (Philadelphia, Ph) chromosome and a BCR-ABL fusion gene that results from a reciprocal translocation involving chromosomes 9 and 22, t(9;22)(q34;q11). The BCR-ABL fusion gene encodes an oncoprotein with greatly enhanced tyrosine kinase activity, which produces a CML-like disease in mice[1] and is believed to underlie the chronic phase of CML in humans.

The emergence of *non-random* karyotypic abnormalities in addition to t(9;22) (clonal evolution, CE) is well described in CML patients treated with hydroxyurea or interferon-α (IFNα) where it is regarded as indicating disease progression.[2] However, some patients who develop CE on IFNα may still remain in hematologically defined chronic phase for long periods, and sometimes the new cytogenetic abnormality disappears spontaneously. The commonest changes include trisomy 8, abnormalities of chromosome 17q and trisomy 19; less frequent abnormalities are monosomies of chromosomes 7, 17 or Y, trisomies of chromosomes 17 and 21 and the translocation t(3;21)(q26;q22).[3]

The recent introduction of the Abl tyrosine kinase inhibitor, imatinib mesylate, has increased the number of options for treating Ph^+ leukemias.[4] The drug is active in CML patients judged to be intolerant of or refractory to IFNα[4,5] and induces complete cytogenetic remissions in at least 70% of previously untreated CML patients.[6] It is regarded by some as the best single agent for treating CML in chronic phase, although survival data are not yet available.

The incidence and the clinical relevance of CE in patients treated with imatinib has not, as yet, been investigated. Moreover, it would be important to identify any cytogenetic or molecular events associated with resistance to imatinib that might predict disease progression[7-15] because it would permit treatment to be altered appropriately at the earliest opportunity.[16] This study was, therefore, undertaken to document the incidence of CE in patients treated with imatinib and to ascertain whether CE predicts disease progression.

Design and Methods

Patients' characteristics

Between January 2000 and April 2002, 145 adult patients intolerant of IFNα or in whom this therapy had failed were treated at Hammersmith Hospital in London

Table 1. Pre-treatment characteristics of the study population of 102 CP CML patients.

Variable	*No.*
Age (yr) (median, range)	52 (17-76)
Gender, n. (%)	
Male	48 (47)
Female	54 (53)
Time from diagnosis (yr) (median, range)	3.3 (0.3-18)
Autologous transplant, n. (%)	11 (10.7)
Sokal score	
Low risk, n. (%)	25 (24.7)
Intermediate risk n. (%)	35 (34.6)
High risk n. (%)	41 (40.7)
Spleen >5cm, n. (%)	15 (14.7)
Ph^+ metaphases, n. (%)	
1-34%	2 (2)
35-64%	2 (2)
65-94%	4 (3.9)
95-100%	93 (92.1)
Duration of interferon therapy (yr.) (median, range)	1.6 (0.3-13)
Interferon response	
Hematologic resistance, n. (%)	23 (22.5)
Cytogenetic resistance, n. (%)	55 (54)
Intolerant, n. (%)	24 (23.5)
Peripheral blood (median, range)	
Leukocytes ($\times10^9$/L)	9 (2-88.9)
Hemoglobin (g/dL)	12.4 (7.1-17.6)
Platelets ($\times10^9$/L)	263 (114-1970)
Basophils (%)	0 (0-12)
Blasts (%)	0 (0-12)
Bone marrow (median, range)	
Blasts (%)	3 (0-13)
Blasts > 5% (%)	11 (10.6)
Blasts + Promyelocytes (%)	11 (1-29)
Basophils (%)	1 (0-9)

with imatinib for CP CML in the context of several clinical trials. Informed consent to treatment in the respective trial had been obtained from each patient before enrollment. Fifteen patients from this series were enrolled in a multi-center phase II trial; results from this trial have been recently reported.[5] Of the 145 patients, 102 had no sign of CE when starting imatinib. This population was the subject of our analysis (Table 1). Chronic phase was defined as fulfilling all the following criteria: (a) peripheral or marrow blasts less than 15%, (b) peripheral or marrow blasts + promyelocytes less than 30%, (c) peripheral or marrow basophils less than 20%, and (d) platelets equal or greater to 100×10^9/L.[2] Accelerated phase was defined by the presence of one or more of the follows features: (a) peripheral or marrow blasts, 15 to 29%, (b) peripheral or marrow blasts + promyelocytes, equal to or greater than 30%, (c) peripheral or marrow basophils, equal to or greater than 20%, or (d) platelets less than 100 $\times10^9$/L unrelated to therapy.[2] The occurrence of additional karyotypic abnormalities was not considered a criterion for acceleration. Blastic phase was defined by either $\geq$ 30% blasts in peripheral blood or bone marrow or presence of extramedullary blastic disease. The Sokal prognostic score at diagnosis was calculated as described elsewhere.[17] CE was defined as the presence of cytogenetic abnormalities other than Ph chromosome, variant Ph or duplication of Ph, loss of Y chromosome or constitutional chromosomal aberrations.

Eligibility criteria

Failure of treatment with IFNα was defined by hematologic or cytogenetic criteria. Hematologic failure was defined as failure to achieve complete hematologic remission despite six months of treatment or relapse after a complete hematologic remission (increase in leukocytes to at least 20 $\times10^9$/L). Complete hematologic remission was defined as a leukocyte count less than 10×10^9/L with normal differential, platelet count less than 450×10^9/L, absence of extramedullary involvement, $\leq$ 5% blasts in marrow, and no liver or spleen enlargement. Cytogenetic failure was defined by the finding of a marrow aspirate with at least 65% Ph^+ metaphases despite one year of treatment, or by an increase in percentage of Ph^+ metaphases to at least 65% or by an increase of at least 30%. Intolerance of IFNα was defined by the presence of grade 3 or higher non-hematologic toxicity[18] persisting for more than one month with IFNα treatment at a dose of 25 million units or more per week.

Treatment with imatinib

In general all patients received imatinib at a daily oral dose of 400 mg daily; no concomitant chemotherapy was administered other than short courses of hydroxyurea or anagrelide when deemed necessary. Imatinib dosage was adjusted depending on tolerance and response; doses were reduced in the presence of grade III-IV thrombocytopenia or grade III-IV neutropenia. Wherever possible the dose was maintained above 300 mg/day. Doses were increased by 200 mg up to a maximum of 400 mg twice daily in case of failure to achieve or loss of complete hematologic remission or loss of complete cytogenetic response.

Follow-up

Patients were assessed for response to treatment by weekly physical examination, full blood count

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and biochemistry for the first 6 weeks and at 6-week intervals thereafter; bone marrow morphology and cytogenetics were assessed every 3 months. Cytogenetic studies were performed with standard methods using Giemsa banding (G-banding). Thirty metaphases were examined when possible. Progression-free survival was defined as absence of progression into accelerated phase or blastic phase disease according to the criteria defined above. Cytogenetic responses were classified in accordance with standard UK Medical Research Council practice as complete (0% Ph^+ metaphases), major (1-34% Ph^+ metaphases), partial (35-65% Ph^+), minor (66-95% Ph^+), and no response (95-100% Ph^+). The median duration of follow-up was 353 days (range 91-842); 95% of the patients were followed for at least 180 days.

BCR-ABL detection by fluorescence in situ hybridization (FISH)

Patients developing CE were retrospectively screened for deletions adjacent to the ABL-BCR gene fusion on the derivative chromosome 9 using the BCR/ABL dual color, dual fusion probe (Vysis UK). The assay was performed according to manufacturer's recommendations. The probe is a mixture of a BCR probe labeled with SpectrumGreen and an ABL probe labeled with SpectrumOrange. The ABL probe has a target region of approximately 650kb extending from a region centromeric to the argininosuccinate synthetase gene (ASS) and telomeric to the last ABL exon. The BCR probe spans a region of about 1.5Mb, extending 900kb telomeric to BCR.

Statistical methods

Probabilities of survival, progression-free survival and CE were estimated by the method of Kaplan and Meier. The influence of possible prognostic variables on progression-free survival and CE was assessed by the log-rank test. Variables with a *p* value <0.1 were entered into a proportional hazards regression analysis. In the multivariate analysis of progression-free survival, CE was entered as a time-dependent co-variable. *p*-values are two-sided and confidence intervals refer to 95% boundaries. All the data were collected and analyzed by the authors.

Results

Incidence of CE

Of the 102 patients studied, 10 developed additional karyotypic abnormalities in Ph-positive cells whilst in complete hematologic response (isolated CE) at a median time of 146 days (Figure 1). Pre-treatment and follow-up characteristics of these patients are shown in Table 2. Five other patients developed CE at the same time as documentation of disease acceleration. Among the patients with isolated CE, frequently seen clonal changes were

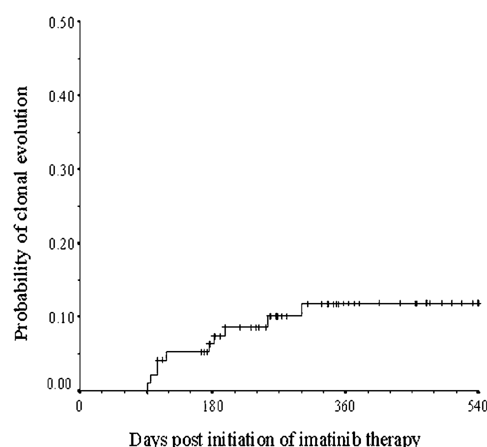

Figure 1. Probability of isolated CE during treatment with imatinib. Ten out of 102 chronic phase patients developed isolated CE at a median interval of 146 days from the start of imatinib treatment.

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Table 2. Pre-treatment and follow-up characteristics of the 10 CML patients developing CE while in complete hematologic remission.

Patient	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Age (yrs)	69	71	43	48	49	48	17	61	57	64
Sokal score	low	int	int	high	low	int	high	high	high	int
BCR-ABL	b3a2		b2a2	b2a2			b3a2 + b2a2	b3a2	b3a2	b3a2
Time diagnosis to imatinib (yrs)	2.9	3.4	5	1.6	1.7	11.3	2.6	13.7	6.6	8
Pre-imatinib variables										
Disease phase	CP	CP	CP	CP	CP	CP	CP	CP	CP	CP
Ph-chromosome positive	100	100	100	100	100	100	100	100	100	100
Variables after 3 months										
Disease status	CHR#	CHR	CHR	CHR	CHR	CHR	CHR	CHR	CHR	CHR
Ph^+ metaphases (%)	70	100	100	100	80	100	100	100	100	100
Neutrophils <1 ($\times 10^9$/L)*	yes	yes	yes	yes	no	yes	yes	yes	no	no
Variables at CE										
Time from imatinib to CE (days)	254	94	175	182	196	105	92	117	85	310
Ph^+ metaphases (%)	35	100	100	76	21	100	100	100	100	100
Time from CE to progression (days)	NA	263	62	102	NA	140	NA	NA	NA	99

*#CHR (complete hematologic response); *one or more episodes of neutropenia between day 45 and day 90.*

Table 3. Cytogenetic analysis of the 10 patients showing isolated CE during imatinib treatment.

Patient n.	*Cytogenetic analysis*
1	46,XX,t(9;22)(q34;q11.2) [11]/ 47,XX,+8 [15]/46,XX,del(5)(q13q22) [2]/46, XX [3]
2	46,XY,t(9;22)(q34;q11.2) [18]/45,XY,idem,der(14;17)(q10;q10) [2]
3	46,XX,t(9;22)(q34;q11.2) [6]/46,idem,add(17)(p13) [26]
4	46,XX,t(9;22;10)(q34;q11.2;q22) [10]/46,idem,inv(3)(q21q26) [3]/46,idem, inv(3)(q21q26),del(5)(q15),-21,+mar [10]/46,XX [7]
5	46,XX,t(9;22)(q34;q11.2) [7]/47,XX,+8 [2]/46,XX [23]
6	46,XX,t(9;22)(q34;q11.2) [15]/46,idem,t(12;17)(q24;q21) [9]
7	46,XX,t(9;13;22)(q34;q14;q11.2) [13]/46,idem,t(11;17)(q22;p1) [7]
8	46,XX,t(9;22)(q34;q11.2) [28]/46,idem,i(7)(q10) [4]
9	46,XX,t(9;22)(q34;q11.2) [27]/46,idem,add(13)(q3) [3]
10	46,XX,t(9;22)(q34;q11.2) [60]/46, idem,t(7;12)(q35;q13) [3]

abnormalities in chromosome 17, deletion 5 and trisomy 8 (Table 3). Interestingly, when more than one additional abnormality was present, this usually occurred in the same cell clone. Of note, three patients with persisting Ph$^+$ cells developed chromosomal abnormalities in Ph$^-$ cells, as described below.

Influence of CE on progression-free survival

For the study population of 102 patients, the probabilities of survival and progression-free survival at 18 months were 91% (CI 78-96%) and 72% (CI 55-84%), respectively. Median survival and progression-free survival times were not reached. To address the question of whether CE in the absence of any other sign of disease progression (isolated CE) was predictive of progression to advanced phase disease, we selected the 60 patients who had achieved complete hematologic remission after 3 months of imatinib treatment. Potential prognostic disease and treatment-related variables were also examined (Table 4). This analysis showed that at 18 months patients with isolated CE had a progression-free survival of 34.3% (CI: 10.5-69.8%) whilst the non-CE patients had a progression-free survival of 94.1% (CI 80.6-98.4%, p<0.0001, Figure 2). Other variables found to be significant in predicting for disease progression in the univariate analysis were a previous autograft, high pre-imatinib marrow blast percentage, lack of partial cytogenetic response at three months of imatinib therapy, and one or more grade III neutropenic episodes between day 45 and day 90 of imatinib treatment. Of note, the presence of deletion in the derivative chromosome 9, recently recognized as an important predictor of poor outcome[19] was investigated retrospectively in patients developing isolated CE and was found in

Table 4. Probabilities of progression-free survival at 18 months for 60 patients achieving complete hematologic remission on imatinib.

	N.	*Probability of progression-free survival (%)*	*p*
Age (yrs)			0.56
≤ 55	37	83.5	
> 55	23	83.3	
Sokal score			0.40
Low	15	100	
Intermediate	20	84.0	
High	25	76.6	
Time from diagnosis			0.75
≤ 2 years	18	86.6	
>2 years	42	81.5	
Autograft			
Yes	7	68.6	0.03
No	53	85.9	
Pre-imatinib disease variables			
Hb (g/dL)			0.89
≤ 10	6	75.7	
>10	54	84.8	
Platelets (×10^9/L)			0.45
≤ 400	48	86.8	
> 400	12	66.7	
LDH (IU/L)			0.43
≤ 400	24	90.6	
> 400	29	75.1	
Peripheral blasts (%)			0.28
< 1	56	90.3	
≥ 1	4	75.0	
Peripheral basophils (%)			0.10
< 1	56	94.2	
≥ 1	4	75.0	
Marrow blasts (%)			0.037
≤ 5	45	90.4	
> 5	9	68.5	
Marrow promyelocytes (%)			0.34
≤ 8	29	92.5	
> 8	25	76.5	
Marrow basophils (%)			0.97
< 1	32	85.1	
≥ 1	22	84.5	
Ph$^+$ metaphases (%)			0.47
< 95	6	100	
95-100	54	82.3	
3-month imatinib variables			
Neutrophils (×10^9/L)			0.010
< 1	24	63.5	
≥ 1	36	94.1	
Platelets (×10^9/L)			0.22
< 100	8	62.5	
≥ 100	52	86.4	
Ph-positive metaphases (%)			0.021
100-65	30	71.6	
0-64	28	100	
Dose intensity days 0-90			0.57
< 300 mg/day	15	90.9	
> 300 mg/day	45	80.8	
Isolated CE			< 0.0001
no	50	94.1	
yes	10	34.3	

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Table 5. Probabilities of CE at 18 months after starting of imatinib therapy.

	N.	Probability of CE (%)	p
Age (yrs)			0.92
≤ 55	58	9.5	
> 55	44	16.3	
Sokal score			0.17
Low	25	0	
Intermediate	35	12.4	
High	41	19.1	
Time from diagnosis			0.66
≤ 2 years	26	9.9	
>2 year	76	12.6	
Autograft			0.49
Yes	11	14.2	
No	91	13.0	
Pre-imatinib disease variables			
Hb (g/dL)			0.61
≤ 10	15	20.5	
>10	87	10.7	
Platelets (×10⁹/L)			0.63
≤ 400	77	11.5	
> 400	25	12.5	
LDH (IU/L)			0.78
≤ 400	37	9.5	
> 400	54	11.6	
Peripheral blasts (%)			0.82
< 1	88	9.4	
≥ 1	11	10	
Peripheral basophils (%)			0.50
< 1	70	10	
≥ 1	29	15.5	
Marrow blasts (%)			0.17
≤ 5	70	8.0	
> 5	21	17.5	
Marrow promyelocytes (%)			0.34
≤ 8	45	7.6	
> 8	46	11.8	
Marrow basophils (%)			0.58
< 1	54	9.3	
≥ 1	37	11.8	
Ph⁺ metaphases (%)			0.34
< 95	8	0	
≥ 95	93	12.9	
*Variables after 3 months of imatinib**			
Neutrophils (×10⁹/L)			0.0003
< 1	30	35.0	
≥ 1	66	3.9	
Platelets (×10⁹/L)			0.47
< 100	12	22.0	
≥ 100	84	10.8	
Ph⁺ metaphases (%)			0.0084
100-65	56	20.5	
0-64	37	0	
Dose intensity days 0-90			0.074
< 300 mg/day	27	21.2	
> 300 mg/day	69	8.2	

**Six patients progressed within three months from starting imatinib treatment and were therefore excluded from the analysis.*

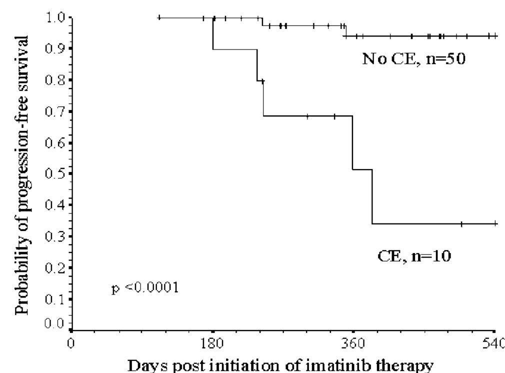

Figure 2. Progression-free survival in patients developing CE on imatinib while in complete hematologic remission compared to in patients without CE (No CE). Ten patients with isolated CE while in complete hematologic remission were compared to 50 patients in complete hematologic remission on imatinib treatment but with no CE. Progression-free survival at 18 months for patients with CE was 34.3 % (CI 10.5-69.8%), whilst that for patients with no CE was 94.1% (CI 80.6-98.4%).

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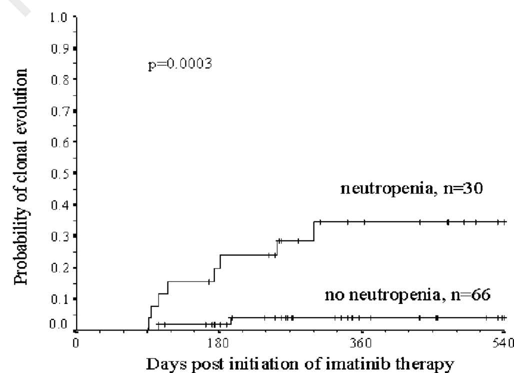

Figure 3. Probability of isolated CE during treatment with imatinib based on neutropenia. The probability of CE was 35% for patients developing one or more grade III neutropenic episodes between days 45 and 90 and 4% for patients without neutropenia.

only one (#9). In the multivariate analysis only *de novo* CE (RR 14.8, CI 2.8-76.6) was predictive of disease progression.

Predictive factors for the occurrence of CE

Having demonstrated the prognostic role of CE, we then wished to examine our ability to identify patients likely to acquire additional clonal abnormalities from their diagnostic, pre-treatment with

imatinib and/or 3-month post-imatinib treatment variables (Table 5). The univariate analysis revealed that one or more grade III neutropenic episodes between days 45 and 90 and a lack of a partial cytogenetic response at three months were associated with an increased risk of CE. However, only neutropenia was significant in the multivariate analysis (RR= 9.9, CI= 2.1-46.8) (Figure 3).

CE on imatinib occurs also in Ph$^-$ cells

Among the 145 CP patients who received imatinib at the Hammersmith Hospital during the last 28 months, three (2%) developed additional abnormalities in Ph$^-$ cells assessed by G-banding and FISH. The first patient (#1 in Tables 2 and 3) was diagnosed as having CP CML in February 1997. She was treated initially with IFNα and started imatinib three years later because intolerant of IFNα. By 6 months she had achieved a major cytogenetic response and at 9 months developed a trisomy 8 in Ph negative cells. At 14 months, a del(5)(q13q22) was also identified in Ph negative cells. At 25 months follow-up, the patient was in complete hematologic remission but both clonal abnormalities were still present. The second patient (#5 in Tables 2 and 3) was diagnosed with CP CML in January 1999 and treated initially with IFNα; she was classified as cytogenetically resistant and switched to imatinib in October 2000. By 6 months she had achieved a major cytogenetic response; at 14 months from starting treatment she developed a trisomy 8 in Ph negative cells while maintaining her major cytogenetic response. At 18 months follow-up the patient was still in complete hematologic remission. The third patient was diagnosed with CP CML in January 2000 and started imatinib in October 2000 as hematologically refractory to IFNα. At that time an additional t(12;16) was present in Ph$^+$ cells, so this patient was not included in our series of CE occurring during imatinib treatment. In July 2001 this patient became 90% Ph negative although in February 2002 monosomy 7 appeared in Ph$^-$ cells while in complete hematologic remission. FISH analysis in all three patients confirmed that cells classified as Ph-negative cells by G-banding also lacked a BCR-ABL fusion gene. None of these three patients had ever received any conventional cytotoxic drug as part of their anti-leukemia treatment. The significance of this finding remains unclear.

Discussion

Cytogenetic CE in IFNα-treated patients is usually an indication of disease progression.[2,20] However, some patients who develop CE on IFNα may continue hematologically to be in chronic phase for long periods of time, sometimes with spontaneous disappearance of the new karyotypic abnormality. However, abnormalities involving chromosome 17 are especially ominous and patients in whom the additional cytogenetic abnormalities predominate may fare especially badly.[21] The extent to which cytogenetic abnormalities occur and may affect prognosis in patients treated with imatinib has not yet been studied. We report here that CE can indeed occur also during treatment with imatinib and the incidence, 15% in this series, is comparable to that seen during treatment with IFNα.[22] As with IFNα-treated patients, the commonest findings were abnormalities of chromosome 17 and trisomy 8.

To address the question of whether CE in the absence of any other sign of disease progression predicted progression to advanced phase, we selected CP patients who had achieved complete hematologic remission after 3 months of imatinib treatment and analyzed the impact of CE on progression-free survival. We found that among patients achieving complete hematologic remission, the finding of CE was the only independent factor that predicted disease progression. We then asked whether the occurrence of isolated CE could be predicted before or soon after beginning imatinib treatment. We found that the occurrence of one or more neutropenic episodes between days 45 and 90 of imatinib treatment predicted for occurrence of CE. These studies suggest an ongoing requirement for regular cytogenetic studies even in the presence of good hematologic control. If our finding that the acquisition of additional chromosomal abnormalities while receiving imatinib is associated with an increased susceptibility to disease progression is confirmed, then their occurrence may be an indication for the initiation of alternative therapies.

Our observation on the correlation between neutropenia and the appearance of CE is consistent with current notions of stem cell kinetics[23] in CML, the capacity of imatinib to selectively to suppress Ph$^+$ hematopoiesis and the fact that CE may reflect additional genomic-related events resulting in a BCR-ABL independent mechanisms of tumor growth. If, for example, the marrow of an *early* CP patient contained co-existing populations of Ph$^+$ and Ph$^-$ (normal) stem cells, then treatment with imatinib should induce Ph-negativity. On the other hand, many patients in this series had been treated for some years before receiving imatinib; thus their marrows may have contained predominantly Ph$^+$ stem cells and only low numbers of residual Ph$^-$ normal cells. If, in some cases, there was also present a population of more *transformed* Ph$^+$ cells that were relatively resistant to imatinib, the results of giving imatinib to such patients would depend on the size and viability of the residual Ph$^-$ population and aggressiveness of the transformed population. In the absence of residual Ph$^-$ stem cells, suppression by imatinib of the untrans-

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formed CP clone would be likely to induce neutropenia and would also permit expansion of the more transformed clone. The more transformed clone could carry cytogenetic abnormalities acquired before starting the imatinib or during imatinib treatment. Whatever the case the more transformed cells bearing the abnormalities would have a proliferative advantage. The fact that CE may occur early after beginning imatinib (median 146 days) and the fact that the abnormalities seen are identical to those during IFNα treatment support the notion that they were acquired before the start of treatment with imatinib.

The presence of a deletion in the derivative chromosome 9[19] was investigated retrospectively in patients who developed *isolated* CE. Only one of the ten had the deletion in spite of poor outcome for this sub-group. This could be explained if our analysis focused on *selected* patients who achieved remission on imatinib and then developed isolated CE, while other patients whose leukemia cells had a 9q+ deletion might have been less likely to have achieved complete hematologic remission. In three patients we observed karyotypic changes in Ph⁻ cells, namely trisomy 8, del(5) (q13q22) and monosomy 7. CE in Ph⁻ cells has been reported previously in patients treated with IFNα[24-28] but its occurrence seems to be very rare. In a series of 5,100 cytogenetic studies performed on 1,146 patients treated with IFNα at the Hammersmith Hospital we found only one patient with CE in Ph⁻ cells; this consisted of trisomy 8 together with del(7)(q21q32).

Conversely, at least three other groups have recently reported finding CE in Ph⁻ cells in patients treated with imatinib.[29-30] Gambacorti-Passerini *et al.*[29] reported three patients among 36 CP and accelerated phase patients achieving major or complete cytogenetic responses who developed cytogenetic changes in Ph⁻, BCR-ABL-negative cells; the changes in these three patients were del(7)(q22), trisomy 8 and trisomy 8 plus del (7)(q22q32). Meeus *et al.*[30] described two patients with CE in Ph⁻ cells out of 200 patients with CP, accelerated phase and blastic phase; one had monosomy 7 and the other had trisomy 8. More recently Deininger *et al.* have created a registry accounting for 26 patients reported by seven centers.[31] This phenomenon is extremely difficult to explain. It could reflect an underlying *genomic instability* that affects both Ph⁺ and Ph⁻ (presumably normal) cells. Alternatively it could be the result of imatinib inhibiting the presumed pro-apoptotic function of the normal Abl protein.[32] In this scenario cells acquiring *random* karyotypic changes, which would normally be forced towards apoptosis, survive and proliferate when the Abl protein is inactivated. This would suggest that these aberrant Ph⁻ cells do not belong to the malignant clone and thus the finding would have no prognostic implication. However only a multicenter analysis will confirm the increased incidence and clarify the clinical relevance of this finding.

In summary, we speculate that the finding of additional cytogenetic changes in Ph⁺ metaphases in patients apparently responding well to imatinib could reflect a generally increased incidence of ill-defined cytogenetic or molecular events in a given myeloid population and may thus be a reliable predictor of progression to advanced disease. We recently stated that the optimal usage of imatinib will become better defined as more experience is gained.[33] Early identification of CE may help the decision to adopt alternative therapeutic approaches.

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Pre-publication Report & Outcomes of Peer Review

Contributions

All authors provided substantial contributions to the conception and design of the study, acquisition of evidence, analysis and interpretation of data. All authors also participated in drafting the article and revising it critically, and gave final approval of the version to be published. SM and DM contribute equally to the study.

SM, DM, EO and JFA were responsible for the conduction of the clinical trial, data collection and the project's design; RS, NF, PK and MB were responsible for analysis of results; AK, VDM, FD and JMG were responsible for manuscript preparation.

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Disclosures

Conflict of interest: two of the authors (JMG; JFA) have received travel expenses and honoraria for participation in meetings sponsored by Novartis. The Hammersmith Hospitals Trust was reimbursed by Novartis for the costs of entering the patients into Novartis-led clinical studies.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received September 2, 2002; accepted January 2, 2003.

In the following paragraphs, the Editor-in-Chief summarizes the peer-review process and its outcomes.

What is already known on this topic

The emergence of non-random karyotypic abnormalities in addition to t(9;22) is well described in CML patients treated with hydroxyurea or IFNα in which it is regarded as indicating disease progression.

What this study adds

This study suggests that acquisition of clonal evolution increases the risk of disease progression also in CML patients in incomplete hematologic remission on imatinib mesylate.

Caveats

Conclusions of this study are based on a relatively small series of patients.

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