

ORIGINAL ARTICLE

Frequency of Major Molecular Responses to Imatinib or Interferon Alfa plus Cytarabine in Newly Diagnosed Chronic Myeloid Leukemia

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ABSTRACT

BACKGROUND

In a randomized trial, 1106 patients with chronic myeloid leukemia (CML) in chronic phase were assigned to imatinib or interferon alfa plus cytarabine as initial therapy. We measured levels of BCR-ABL transcripts in the blood of all patients in this trial who had a complete cytogenetic remission.

METHODS

Levels of BCR-ABL transcripts were measured by a quantitative real-time polymerase-chain-reaction assay. Results were expressed relative to the median level of BCR-ABL transcripts in the blood of 30 patients with untreated CML in chronic phase.

RESULTS

In patients who had a complete cytogenetic remission, levels of BCR-ABL transcripts after 12 months of treatment had fallen by at least 3 log in 57 percent of those in the imatinib group and 24 percent of those in the group given interferon plus cytarabine ($P=0.003$). On the basis of the rates of complete cytogenetic remission of 68 percent in the imatinib group and 7 percent in the group given interferon plus cytarabine at 12 months, an estimated 39 percent of all patients treated with imatinib but only 2 percent of all those given interferon plus cytarabine had a reduction in BCR-ABL transcript levels of at least 3 log ($P<0.001$). For patients who had a complete cytogenetic remission and a reduction in transcript levels of at least 3 log at 12 months, the probability of remaining progression-free was 100 percent at 24 months, as compared with 95 percent for such patients with a reduction of less than 3 log and 85 percent for patients who were not in complete cytogenetic remission at 12 months ($P<0.001$).

CONCLUSIONS

The proportion of patients with CML who had a reduction in BCR-ABL transcript levels of at least 3 log by 12 months of therapy was far greater with imatinib treatment than with treatment with interferon plus cytarabine. Patients in the imatinib group with this degree of molecular response had a negligible risk of disease progression during the subsequent 12 months.

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N Engl J Med 2003;349:1423-32.

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CHRONIC MYELOID LEUKEMIA (CML) IS a clonal disease of the hematopoietic stem cell in which a reciprocal translocation, t(9;22)(q34;q11), forms the Philadelphia chromosome (Ph) and creates a novel fusion gene, BCR-ABL.¹ This gene expresses an activated tyrosine kinase that is central to the pathogenesis of CML.^{2,3} The median survival among patients with CML is three to six years, with most deaths resulting from progression of the disease into a blastic phase. Survival among patients treated with interferon alfa is superior to that among patients treated with hydroxyurea.⁴ The addition of cytarabine to interferon alfa may further improve median survival, although randomized studies have shown conflicting results.^{5,6} The degree to which survival is prolonged among patients who are receiving interferon-based therapy can be linked to the reduction in leukemic-cell burden. Patients who have a complete cytogenetic remission, defined as the absence of Ph-positive cells in metaphase among at least 20 cells in metaphase in a bone marrow aspirate, have a better prognosis — more than 70 percent are still alive after 10 years — than do patients who do not have a complete cytogenetic remission.^{7,8} The development of accurate techniques to measure the BCR-ABL transcripts in peripheral blood or bone marrow by a quantitative reverse-transcriptase polymerase chain reaction (PCR) has allowed patients in complete cytogenetic remission to be stratified further.⁹⁻¹⁶ The level of BCR-ABL transcripts can predict the duration of remission in patients who have a complete cytogenetic remission during therapy with interferon alfa.^{17,18}

Imatinib mesylate (Gleevec, Novartis) is a tyrosine kinase inhibitor that blocks the kinase activity of BCR-ABL, thus inhibiting the proliferation of Ph-positive progenitors.^{19,20} Imatinib has shown activity against all phases of CML, though responses are most substantial and durable in patients who are in the chronic phase.²¹⁻²⁶

The International Randomised Study of Interferon versus STI571 (IRIS), which randomly assigned 1106 patients with newly diagnosed CML to receive 400 mg of imatinib per day or interferon alfa (target dose, 5 million U per square meter per day) plus 10-day cycles of cytarabine at a dose of 20 mg per square meter per day every month, completed 18 months of follow-up of all patients in July 2002. The rates of complete cytogenetic remission were 73.8 percent in the imatinib group and 8.5 per-

cent in the group given interferon plus cytarabine.²⁶ In the present study, we examined the reduction of disease burden in all patients enrolled in the IRIS study who had a complete cytogenetic remission by measuring the levels of BCR-ABL transcripts. We used real-time quantitative PCR to measure the level of BCR-ABL transcripts in the blood of patients when they first had a complete cytogenetic remission and at subsequent times in both groups, so that we could compare the patterns of response and determine the prognostic value of a molecular response.

METHODS

The IRIS study was approved by the ethics committee at each center, and all patients gave written informed consent. The study was funded by Novartis and was designed by the investigators and representatives of Novartis. The data were collected with the data-management and statistical-support systems of Novartis and analyzed and interpreted by a statistician from Novartis in close collaboration with the investigators from the PCR laboratories. All authors had access to the primary data.

Patients 18 to 70 years of age were enrolled within six months after receiving a diagnosis of CML in the chronic phase. Patients could have received no previous treatment for the disease except hydroxyurea and anagrelide. A total of 553 patients were randomly assigned to receive imatinib and 553 to receive interferon plus cytarabine. Patients could cross over to the other group if strict definitions of treatment failure or intolerance were met. Details of the study design, conduct, and treatment plan have been reported previously.²⁶

MOLECULAR ANALYSIS

Molecular analysis was carried out in three laboratories according to their proximity to the referring center: Adelaide, Australia; London; and Seattle. No samples obtained after crossover to the other treatment group were included in this analysis.

BLOOD SAMPLING AND PREPARATION

At each time point 20 ml of peripheral blood was collected. The base-line sample was obtained just before the administration of study drug. Subsequent samples were obtained from patients who had a complete cytogenetic remission. Samples collected within two weeks after a documented complete cytogenetic remission were defined as having been

obtained at the time of remission for the purposes of this analysis. Thereafter, samples were collected every 3 months until the completion of the PCR study after 24 months of therapy. Peripheral-blood samples were processed either at the coordinating laboratory in Australia or in a central data-and-processing laboratory (Covance) in Switzerland or in the United States. Total RNA was extracted from cellular pellets, and the complementary DNA was synthesized as described previously.^{13,15,27}

REAL-TIME QUANTITATIVE PCR

Detailed descriptions of quantitative PCR methods used in the Adelaide, Seattle, and London laboratories have been published previously.^{13,15} At each laboratory, BCR was used as the control gene and BCR-ABL values were expressed as a percentage of the BCR transcript levels. Normalizing the results to the BCR value compensated for variations in the quality of the RNA and for differences in the efficiency of the reverse-transcriptase reaction. Nested PCR techniques were used to confirm the results in samples defined as having undetectable BCR-ABL levels.

STANDARDIZATION OF PCR VALUES

To compare quantitative PCR results obtained by the three laboratories, the primary BCR-ABL values calculated as a percentage of BCR were converted to reflect the reduction in the value with use of a standardized logarithmic (base 10) scale. This was done in each laboratory by first calculating the median value of 30 samples collected from patients with newly diagnosed chronic-phase CML who had not yet begun taking the study drug (the same 30 samples were tested in each laboratory). The median value was used as the standardized base line at each laboratory. The reduction in BCR-ABL levels from the standardized base-line value was calculated for each sample. For example, if the standardized base-line value at one center was a BCR-ABL:BCR ratio of 36 percent, a ratio of 0.036 percent represented a reduction of 3 log from the standardized base-line value. It was not necessary to know the BCR-ABL level of a patient at base line to calculate the subsequent reduction, because the calculation was based on the standardized base-line value. There were no statistically significant differences among the three laboratories after reductions had been calculated relative to the standardized median pretreatment BCR-ABL:BCR ratio for each laboratory.

QUALITY OF SAMPLES

The transcripts of the BCR control gene reflect the degree of degradation of the sample. To judge the quality of the RNA in the sample, each laboratory determined its own acceptable level of BCR control gene transcripts and discarded samples with values below this level. The following criteria were used to determine whether a sample had undetectable levels of BCR-ABL transcripts: there were sufficient BCR transcripts in the sample to ensure a lower limit of sensitivity of more than 4.5 log below base line, BCR-ABL transcripts were undetectable by nested reverse-transcriptase PCR, and these results were then confirmed in a second laboratory.

STATISTICAL ANALYSIS

The rates of complete cytogenetic remission and a reduction from base line in BCR-ABL transcripts of at least 3 log were compared between groups with the use of Fisher's exact test. The difference between groups in the extent of the reductions in BCR-ABL transcripts at or after complete cytogenetic remission was evaluated with use of the Wilcoxon rank-sum test. The differences between laboratories were evaluated with use of the Kruskal-Wallis test. Long-term clinical data from 128 patients who were treated for 12 months without complete cytogenetic remission were included in the analysis of the time to progression according to the level of their response at 12 months. Among patients in the imatinib group, the time to progression was compared between patients who did not have a complete cytogenetic remission within 12 months and those who did, with or without reduction in BCR-ABL transcripts of at least 3 log, with use of the Kaplan-Meier method, and the difference in the levels of response was evaluated with use of the log-rank test. Progression was defined as death, the development of accelerated-phase or blast-crisis CML, an increasing white-cell count, or the loss of complete hematologic or major cytogenetic response.²⁶ The prognostic scores were calculated according to the method of Sokal et al.²⁸ and Hasford et al.²⁹

RESULTS

CHARACTERISTICS OF THE PATIENTS

After a median of 19 months of follow-up, 408 patients in the imatinib group and 47 in the group given interferon plus cytarabine had had a complete cytogenetic remission. Of these 455 patients, 370

had quantitative PCR data available for analysis (333 in the imatinib group and 37 in the group given interferon plus cytarabine). The characteristics of all patients who had a complete cytogenetic remission and the subgroup of patients who had quantitative PCR data available were similar, suggesting that the population analyzed is representative of the group with a complete cytogenetic remission (Table 1). A total of 1140 samples were included in the analysis: 1058 from patients in the imatinib group and 82 from patients in the group given interferon plus cytarabine. The median number of samples per patient was three (range, one to seven; 10 percent of patients in the imatinib group who had quantitative PCR data had more than four samples available for analysis, as compared with none of the patients in the group given interferon plus cytarabine).

LEVELS OF BCR-ABL TRANSCRIPTS AT THE TIME OF A COMPLETE CYTOGENETIC REMISSION

At the time of a complete cytogenetic remission, the median reduction from base line in the levels of BCR-ABL transcripts was 2.5 log (25th to 75th percentile, 2.0 to 3.2) in the imatinib group and 2.2 log (25th to 75th percentile, 1.5 to 2.6) in the group given interferon plus cytarabine ($P=0.036$) (Fig. 1). Thirty-two percent of the 120 patients in the imatinib group had a reduction of at least 3 log at the time of their complete cytogenetic remission, as compared with none of the 12 patients in the group given interferon plus cytarabine ($P=0.019$).

LEVELS OF BCR-ABL TRANSCRIPTS AFTER A COMPLETE CYTOGENETIC REMISSION

Three months after patients had entered a complete cytogenetic remission, the median reduction from base line in BCR-ABL transcripts was 2.9 log in the imatinib group (range, 0.3 to >4.5; 25th to 75th percentile, 2.4 to 3.6) and 2.1 in the group given interferon plus cytarabine (range, 0.2 to 3.8; 25th to 75th percentile, 1.8 to 2.4) ($P<0.001$) (Fig. 1). BCR-ABL transcript levels decreased further during follow-up, with a median reduction of 3.7 log in the imatinib group and 2.5 log in the group given interferon plus cytarabine 15 months after the achievement of a complete cytogenetic remission ($P=0.01$). Among patients in the imatinib group, the median transcript level was significantly lower 18 months after a complete cytogenetic remission than 12 months afterward ($P=0.002$). This difference was

also reflected by the difference in the frequency of a reduction of at least 3 log at 12 and 18 months (69 percent vs. 81 percent, $P=0.003$). As of this writing, it is not yet possible to determine whether BCR-ABL transcript levels will continue to fall after 18 months in complete cytogenetic remission.

LEVELS OF BCR-ABL TRANSCRIPTS ACCORDING TO THE DURATION OF THERAPY

Reductions in BCR-ABL transcripts of at least 3 log were achieved faster among patients in the imatinib group than among those in the group given interferon plus cytarabine. Among all patients who were in complete cytogenetic remission at six months, 42 percent of those in the imatinib group had a reduction of at least 3 log, as compared with only 13 percent of those in the group given interferon plus cytarabine ($P=0.03$). This result is consistent with the rapidity of a complete cytogenetic response among patients treated with imatinib. On the basis of the rates of complete cytogenetic remission at six months (50 percent in the imatinib group and 3 percent in the group given interferon plus cytarabine) and the assumption that patients without a complete cytogenetic remission have not had a reduction in transcript levels of at least 3 log, we estimated that 21 percent of all patients who were treated with imatinib had a reduction from baseline levels of at least 3 log (42 percent of the 50 percent who had a complete cytogenetic remission at six months) as compared with less than 1 percent of all patients who received interferon plus cytarabine (13 percent of the 3 percent who were in remission at six months) ($P<0.001$).

One year after the start of treatment, 137 of 240 patients in the imatinib group who had a complete cytogenetic remission (57 percent) had a reduction in the levels of BCR-ABL transcripts of at least 3 log, as compared with 6 of 25 of such patients in the group given interferon plus cytarabine (24 percent) ($P=0.003$). On the basis of the 12-month rates of complete cytogenetic remission (68 percent and 7 percent, respectively) and the assumption that patients without a complete cytogenetic remission have not had a reduction of at least 3 log, we estimated that 39 percent of all patients in the imatinib group (57 percent of the 68 percent who had a complete cytogenetic remission at 12 months) had a reduction from base line in BCR-ABL transcript levels of at least 3 log, whereas only 2 percent of all patients receiving interferon plus cytarabine (24 per-

Table 1. Base-Line Characteristics of All Patients Who Had a Complete Cytogenetic Remission and of All Patients Who Had a Complete Cytogenetic Remission and Polymerase-Chain-Reaction (PCR) Data Available.

Characteristic	Complete Cytogenetic Remission		Complete Cytogenetic Remission and PCR	
	Imatinib (N=408)	Interferon + Cytarabine (N=47)	Imatinib (N=333)	Interferon + Cytarabine (N=37)
Age				
Median — yr	51	49	51	50
Range — yr	18–70	21–70	18–70	21–70
≥60 yr — no. (%)	87 (21)	7 (15)	67 (20)	5 (14)
Sex — no. (%)				
Male	247 (61)	25 (53)	198 (59)	21 (57)
Female	161 (39)	22 (47)	135 (41)	16 (43)
ECOG performance status — no. (%)*				
Data missing	4 (1)	0	3 (1)	0
0	316 (77)	42 (89)	259 (78)	33 (89)
1	82 (20)	5 (11)	66 (20)	4 (11)
2	6 (1)	0	5 (2)	0
Interval since diagnosis — mo				
Median	2.1	1.9	2.1	2.2
Range	0.1–10.4	0.1–6.3	0.1–10.4	0.1–6.3
Sokal risk group — no. (%)				
Total evaluated	286 (70)	35 (74)	231 (69)	27 (73)
Low	165 (58)	23 (66)	133 (58)	16 (59)
Intermediate	81 (28)	10 (29)	64 (28)	9 (33)
High	40 (14)	2 (6)	34 (15)	2 (7)
Hasford risk group — no. (%)				
Total evaluated	281 (69)	35 (74)	226 (68)	27 (73)
Low	129 (46)	25 (71)	101 (45)	17 (63)
Intermediate	127 (45)	9 (26)	104 (46)	9 (33)
High	25 (9)	1 (3)	21 (9)	1 (4)
Additional chromosomal abnormalities — no. (%)				
Data missing	20 (5)	3 (6)	17 (5)	3 (8)
No	345 (85)	42 (89)	283 (85)	33 (89)
Yes	43 (11)	2 (4)	33 (10)	1 (3)
Splenomegaly — no. (%)	81 (20)	6 (13)	63 (19)	4 (11)
Spleen size ≥10 cm below costal margin — no. (%)	19 (5)	0	15 (5)	0
White-cell count — ×10 ⁻³ /mm ³				
Median	17.8	21.0	17.8	22.3
Range	1.6–421.3	3.1–144.4	1.9–421.3	3.1–144.4
Platelet count — ×10 ⁻³ /mm ³				
Median	335	326	337	313
Range	61–2950	127–1493	61–2950	127–1493
Hemoglobin — g/dl				
Median	13.0	13.8	13.1	13.9
Range	6.9–17.0	9.3–19.4	6.9–17.0	9.3–19.4
Peripheral-blood blasts — no. (%)				
<3%	60 (15)	5 (11)	49 (15)	4 (11)
≥3%	12 (3)	1 (2)	11 (3)	1 (3)
Bone marrow blasts — no. (%)				
<5%	241 (59)	23 (49)	195 (59)	17 (46)
≥5%	44 (11)	6 (13)	39 (12)	5 (14)

* ECOG denotes Eastern Cooperative Oncology Group.

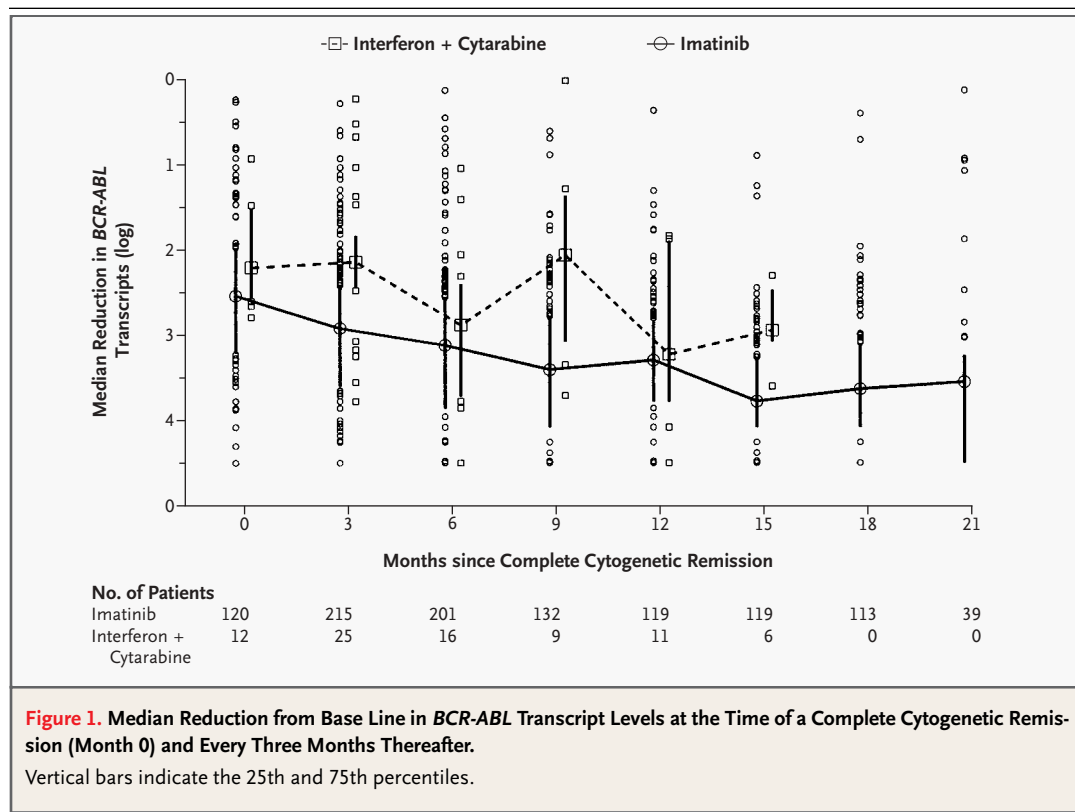


Figure 1. Median Reduction from Base Line in *BCR-ABL* Transcript Levels at the Time of a Complete Cytogenetic Remission (Month 0) and Every Three Months Thereafter.

Vertical bars indicate the 25th and 75th percentiles.

cent of 7 percent who were in complete cytogenetic remission at 12 months) did so ($P < 0.001$) (Fig. 2). Since several patients in the group given interferon plus cytarabine crossed over to imatinib, shortening their follow-up, we estimated that the rate of complete cytogenetic remission was 12 percent at 12 months using the Kaplan–Meier method.²⁶ Even when we used the estimated rate of 12 percent at 12 months (rather than the observed rate of 7 percent), the estimated percentage of patients with a reduction of at least 3 log was still only 3 percent in the group given interferon plus cytarabine.

PATIENTS WITH UNDETECTABLE LEVELS OF *BCR-ABL* TRANSCRIPTS

On the basis of stringent criteria, no *BCR-ABL* transcripts were detected on at least one occasion in 12 of 333 patients with a complete cytogenetic remission (4 percent). An additional 20 patients with a complete cytogenetic remission (6 percent) had undetectable levels of *BCR-ABL* transcripts, but the quality of the RNA sample was not adequate to ensure that the lower level of sensitivity was more than 4.5 log.

SOKAL RISK GROUP AND MOLECULAR RESPONSE AMONG PATIENTS TREATED WITH IMATINIB

The percentages of patients in the imatinib group with high-risk, intermediate-risk, and low-risk Sokal scores²⁸ who had a complete cytogenetic remission within 12 months were 49 percent, 67 percent, and 76 percent, respectively ($P < 0.001$). At 12 months, 38 percent of those in the high-risk group had had a reduction from base line of at least 3 log in *BCR-ABL* transcripts, as compared with 45 percent of those in the intermediate-risk group and 66 percent of those in the low-risk group ($P = 0.007$).

CLINICAL COURSE AFTER A REDUCTION IN *BCR-ABL* TRANSCRIPTS OF AT LEAST 3 LOG

After a median follow-up of 25 months and a maximal follow-up of 31 months, there was evidence of progression in 56 of the 553 patients in the imatinib group. We conducted a landmark analysis of patients without progression who were still receiving treatment at 12 months. We compared the 128 patients who had not had a complete cytogenetic remission at 12 months with the 240 who had had a complete cytogenetic remission and who also had

a quantitative PCR sample available at 12 months. The remaining patients were not included in the analysis: 50 either had disease progression or had discontinued imatinib for other reasons before 12 months of treatment, and 135 had no quantitative PCR sample available. Progression occurred in 26 of the 365 patients included in the landmark analysis: 1 died during treatment, 8 had progression to the accelerated or blast phase of CML, and in 17 the complete hematologic or major cytogenetic response was lost. For patients who had a complete cytogenetic remission and reduction in BCR-ABL transcript levels of at least 3 log at 12 months, the probability of remaining progression-free was 100 percent at 24 months, as compared with 95 percent for patients who had a complete cytogenetic remission with a reduction of less than 3 log and 85 percent for patients who did not have a complete cytogenetic remission ($P < 0.001$) (Fig. 3).

DISCUSSION

The IRIS study demonstrated that the rates of hematologic and cytogenetic responses were higher among patients with newly diagnosed chronic-phase CML who were treated with imatinib than among those who were treated with interferon plus cytarabine. Our molecular studies provide evidence that imatinib treatment causes rapid and substantial reductions in the leukemic load. After 12 months of treatment, an estimated 39 percent of patients in the imatinib group had a reduction in the levels of BCR-ABL transcripts of at least 3 log, as compared with 2 percent of patients in the group given interferon plus cytarabine. These results should be interpreted with caution, however, since the actual number of patients in the group given interferon plus cytarabine who had a complete cytogenetic remission was relatively small.

The frequency of achieving a reduction in BCR-ABL transcripts of 3 log or greater was highest among patients in the imatinib group with low Sokal risk scores. The Sokal prognostic score is widely accepted as a good predictor of the cytogenetic response of patients who are receiving interferon-based therapy,^{4,6} and our data suggest that it may also prove to be a good predictor of molecular response to imatinib.

Since imatinib induces a cytogenetic remission in most patients with CML in chronic phase, the next logical goal would be the reduction of the number of BCR-ABL transcripts to an undetectable level

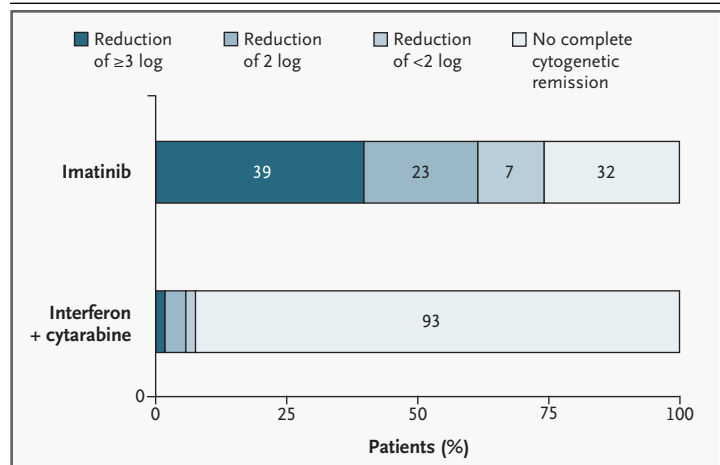


Figure 2. Estimated Percentages of All Study Patients in Each Group with a Reduction from Base Line in BCR-ABL Transcript Levels of at Least 3 Log, 2 Log, or Less Than 2 Log after 12 Months of Treatment.

An estimated 39 percent of all patients in the imatinib group had a reduction of at least 3 log at 12 months (20 percent had a reduction of 3 to less than 4 log and 19 percent had a reduction of at least 4 log), as compared with an estimated 2 percent of patients in the group given interferon plus cytarabine ($P < 0.001$).

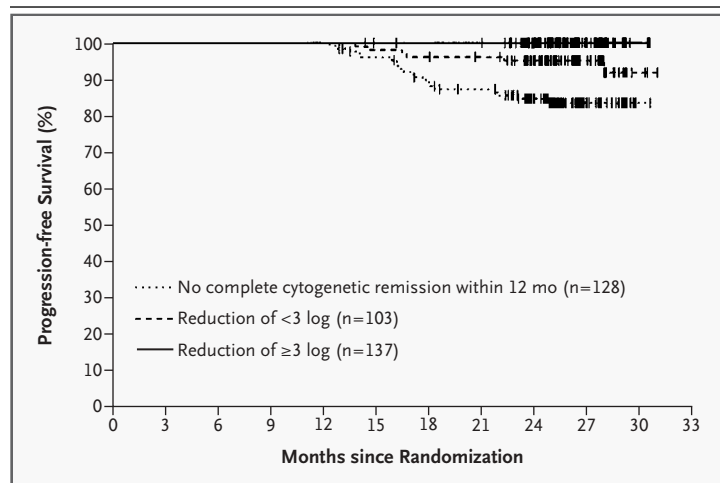


Figure 3. Actuarial Probability of Progression-free Survival among 128 Patients Who Were Treated with Imatinib for 12 Months without a Complete Cytogenetic Remission and 240 Patients Who Had a Complete Cytogenetic Remission and Had Polymerase-Chain-Reaction Data Available, According to the Extent of the Reduction from Base Line in BCR-ABL Transcript Levels.

$P < 0.001$ for the overall comparison, $P = 0.013$ for the comparison of patients without a complete cytogenetic remission with those with a reduction of at least 3 log, and $P = 0.007$ for the comparison of patients with a reduction of less than 3 log with those with a reduction of at least 3 log.

— that is, a complete molecular remission. However, the term “complete molecular remission” is imprecise and should be used with caution. Several million leukemic cells could be present but not detected by means of current quantitative PCR assays,^{18,30} and the sensitivity of the assay varies between samples and between laboratories. For these reasons we avoided using the term “complete molecular remission” and instead reported the number of patients who had undetectable levels of BCR-ABL transcripts and then specified the significance of this finding in terms of the extent of the reduction. Overall, we can conclude that at least 3 percent of patients in the imatinib group had BCR-ABL transcript levels that were more than 4.5 log below the baseline value as of the most recent follow-up analysis. This low proportion is in contrast to the situation after allogeneic hematopoietic stem-cell transplantation, in which most patients have undetectable levels of BCR-ABL transcripts.^{9,15,31-36} The observation that the vast majority of patients who take imatinib have substantial molecular responses in the first 6 months but still have measurable disease after 12 to 18 months of therapy is consistent with experimental observations that imatinib acts mainly by inhibiting proliferation rather than by inducing apoptosis. Longer follow-up is needed to determine whether imatinib can eventually eradicate the leukemic clone.

Other studies that have used quantitative PCR to monitor patients with chronic-phase CML who are taking imatinib have focused mainly on patients who could not tolerate interferon or who had refractory disease. Those studies found a strong correlation between cytogenetic analysis and quantitative PCR,

which is consistent with findings that most patients who have a complete cytogenetic remission have uniformly low blood levels of BCR-ABL transcripts and a steady downward trend in the levels of BCR-ABL transcripts over time.^{37,38} The incidence of undetectable levels of BCR-ABL transcripts has, however, varied, possibly because of the variable sensitivity of the assays used and differences in the duration of imatinib therapy.³⁸⁻⁴⁰

In this study, patients in the imatinib group who had a reduction in the level of BCR-ABL transcripts of at least 3 log had a negligible risk of disease progression over the subsequent 12 months. We propose that a reduction in BCR-ABL transcript levels of at least 3 log be used to define a major molecular response. This term defines a level of response that can be verified in any quantitative PCR laboratory after appropriate adjustment of locally derived values to the standardized log scale. Longer follow-up should determine whether the high frequency of major molecular responses seen with imatinib therapy will be associated with prolonged progression-free and overall survival. Meanwhile, the frequency of major molecular responses achieved in this study should serve as a benchmark against which future studies aiming to optimize therapy for CML can be measured.

Presented in part at the 44th Annual Meeting of the American Society of Hematology, Philadelphia, December 6–10, 2002.

All investigators, including the academic authors, report having received grant support from Novartis Pharma for the conduct of the study.

We are indebted to the coinvestigators as well as to the members of the medical, nursing, and research staff at study centers, the clinical trial monitors, and the data managers and programmers at Novartis for their contributions; and to Renaud Capdeville, Ian Beppu, Sue Saunders, Charlene So, and Carsten Goessl for their invaluable collaboration.

APPENDIX

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REFERENCES

1. Sawyers CL. Chronic myeloid leukemia. *N Engl J Med* 1999;340:1330-40.
2. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science* 1990;247:1079-82.
3. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* 1990;247:824-30.
4. Chronic Myeloid Leukaemia Trialists' Collaborative Group. Interferon alpha versus chemotherapy for chronic myeloid leukaemia: a meta-analysis of seven randomised trials. *J Natl Cancer Inst* 1997;89:1616-20.
5. Guilhot F, Chastang C, Michallet M, et al. Interferon alpha-2b combined with cytarabine versus interferon alone in chronic myelogenous leukemia. *N Engl J Med* 1997;337:223-9.
6. Baccarani M, Rosti G, de Vivo A, et al. A randomized study of interferon-alpha versus interferon-alpha and low-dose arabinosyl cytosine in chronic myeloid leukaemia. *Blood* 2002;99:1527-35.
7. Bonifazi F, de Vivo A, Rosti G, et al. Chronic myeloid leukemia and interferon-alpha: a study of complete cytogenetic responders. *Blood* 2001;98:3074-81.
8. Mahon F, Delbrel X, Cony-Makhoul P, et al. Follow-up of complete cytogenetic remission in patients with chronic myeloid leukemia after cessation of interferon- α . *J Clin Oncol* 2002;20:214-20.
9. Cross NCP, Feng L, Chase A, Bungey J, Hughes TP, Goldman JM. Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. *Blood* 1993;82:1929-36.
10. Lion T, Gaiger A, Henn T, et al. Use of quantitative polymerase chain reaction to monitor residual disease in chronic myelogenous leukemia during treatment with interferon. *Leukemia* 1995;9:1353-60.
11. Hochhaus A, Lin F, Reiter A, et al. Quantification of residual disease in chronic myeloid leukemia patients on interferon- α therapy by competitive polymerase chain reaction. *Blood* 1996;87:1549-55.
12. Emig M, Saussele S, Wittor H, et al. Accurate and rapid analysis of residual disease in patients with CML using specific fluorescent hybridization probes for real time quantitative RT-PCR. *Leukemia* 1999;13:1825-32.
13. Branford S, Hughes TP, Rudzki Z. Monitoring chronic myeloid leukaemia therapy by real-time quantitative PCR in blood is a reliable alternative to bone marrow cytogenetics. *Br J Haematol* 1999;107:587-99.
14. Preudhomme C, Revillion F, Merlat A, et al. Detection of BCR-ABL transcripts in chronic myeloid leukemia (CML) using a 'real time' quantitative RT-PCR assay. *Leukemia* 1999;13:957-64.
15. Radich JP, Gooley T, Bryant E, et al. The significance of bcr-abl molecular detection in chronic myeloid leukemia patients "late," 18 months or more after transplantation. *Blood* 2001;98:1701-7.
16. van Rhee F, Marks DI, Lin F, et al. Quantification of residual disease in Philadelphia-positive acute lymphoblastic leukemia: comparison of blood and bone marrow. *Leukemia* 1995;9:329-35.
17. Kurzrock R, Estrov Z, Kantarjian H, Talpaz M. Conversion of interferon-induced, long-term cytogenetic remissions in chronic myelogenous leukemia to polymerase chain reaction negativity. *J Clin Oncol* 1998;16:1526-31.
18. Hochhaus A, Reiter A, Saussele S, et al. Molecular heterogeneity in complete cytogenetic responders after interferon-alpha therapy for chronic myelogenous leukemia: low levels of minimal residual disease are associated with continuing remission. *Blood* 2000;95:62-6.
19. Buchdunger E, Zimmerman J, Mett H, et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylamino-pyrimidine derivative. *Cancer Res* 1996;56:100-4.
20. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 1996;2:561-6.
21. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001;344:1031-7.
22. Goldman JM, Druker BJ. Chronic myeloid leukaemia: current treatment options. *Blood* 2001;98:2039-42.
23. Sawyers CL, Hochhaus A, Feldman E, et al. Imatinib induces durable hematologic and cytogenetic responses in patients with chronic myelogenous leukemia and myeloid blast crisis: results of a phase II study. *Blood* 2002;99:3530-9.
24. Talpaz M, Silver RT, Druker BJ, et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukaemia: results of a phase 2 study. *Blood* 2002;99:1928-37.
25. Kantarjian H, Sawyers C, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 2002;346:645-52. [Erratum, *N Engl J Med* 2002;346:1923.]
26. O'Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 2003;348:994-1004.
27. Mughal TI, Yong A, Szydlo RM, et al. Molecular studies in patients with chronic myelogenous leukaemia in remission 5 years after allogeneic stem cell transplant define the risk of subsequent relapse. *Br J Haematol* 2001;115:569-74.
28. Sokal JE, Baccarani M, Russo D, Tura S. Staging and prognosis in chronic myelogenous leukemia. *Semin Hematol* 1988;25:49-61.
29. Hasford J, Pfirrmann M, Hehlmann R, et al. A new prognostic score for survival of patients with chronic myelogenous leukemia treated with interferon alfa. *J Natl Cancer Inst* 1998;90:850-8.
30. Morley A. Quantifying leukemia. *N Engl J Med* 1998;339:627-9.
31. Hughes TP, Morgan GJ, Martiat P, Goldman JM. Detection of residual leukemia after bone marrow transplant for chronic myeloid leukemia: role of the polymerase chain reaction in predicting relapse. *Blood* 1991;77:874-8.
32. Roth MS, Antin JH, Ash R, et al. Prognostic significance of Philadelphia chromosome-positive cells detected by the polymerase chain reaction after allogeneic bone marrow transplant for chronic myelogenous leukemia. *Blood* 1992;79:276-82.
33. Lin F, van Rhee F, Goldman JM, Cross NCP. Kinetics of increasing BCR-ABL tran-

- script numbers in chronic myeloid leukemia patients who relapse after bone marrow transplantation. *Blood* 1996;87:4473-8.
34. Mackinnon S, Barnett L, Heller G. Polymerase chain reaction is highly predictive of relapse in patients following T cell-depleted allogeneic bone marrow transplantation of chronic myeloid leukemia. *Bone Marrow Transplant* 1996;17:643-7.
35. Miyamura K, Tahara T, Tanimoto M, et al. Long persistent *bcr-abl* positive transcript detected by polymerase chain reaction after marrow transplant for chronic myelogenous leukemia without clinical relapse: a study of 64 patients. *Blood* 1993;81:1089-93.
36. Olavarria E, Kanfer E, Szydlo R, et al. Early detection of BCR-ABL transcripts by quantitative reverse transcriptase-polymerase chain reaction predicts outcome after allogeneic stem cell transplantation for chronic myeloid leukemia. *Blood* 2001;97:1560-5.
37. Kantarjian HM, Cortes JE, O'Brien S, et al. Imatinib mesylate therapy in newly diagnosed patients with Philadelphia chromosome-positive chronic myelogenous leukemia: high incidence of early complete and major cytogenetic responses. *Blood* 2003;101:97-100.
38. Hughes TP, Branford S. Molecular monitoring of chronic myeloid leukemia. *Semin Hematol* 2003;40:Suppl 3:62-8.
39. Wang L, Pearson K, Pillitteri L, Ferguson JE, Clark RE. Serial monitoring of BCR-ABL by peripheral blood real-time polymerase chain reaction predicts the marrow cytogenetic response to imatinib mesylate in chronic myeloid leukaemia. *Br J Haematol* 2002;118:771-7.
40. Merx K, Muller MC, Kreil S, et al. Early reduction of BCR-ABL mRNA transcript levels predicts cytogenetic response in chronic phase CML patients treated with imatinib after failure of interferon alpha. *Leukemia* 2002;16:1579-83.

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