

Imatinib and pegylated human recombinant interferon- α 2b in early chronic-phase chronic myeloid leukemia

Michele Baccarani, Giovanni Martinelli, Gianantonio Rosti, Elena Trabacchi, Nicoletta Testoni, Simona Bassi, Marilina Amabile, Simona Soverini, Fausto Castagnetti, Daniela Cilloni, Barbara Izzo, Antonio de Vivo, Emanuela Messa, Francesca Bonifazi, Angela Poerio, Simona Luatti, Emilia Giugliano, Daniele Alberti, Gianluca Fincato, Domenico Russo, Fabrizio Pane, and Giuseppe Saglio for the GIMEMA Working Party on Chronic Myeloid Leukemia

Since interferon- α and imatinib (IM; STI571, Gleevec, Gleevec) are effective for the treatment of chronic myeloid leukemia (CML), and their mechanisms of action are different, we designed an exploratory study investigating the effects of a standard IM dose (400 mg/d) and a variable pegylated interferon- α (PegIFN) dose (50 μ g/wk, 100 μ g/wk, and 150 μ g/wk). The criteria for dose adjustment were designed so as to ensure the delivery of the IM dose and to protect life quality. There were 76 patients with previ-

ously untreated Philadelphia (Ph)-positive CML enrolled in the study. There were 3 patients who discontinued IM and 45 patients who discontinued PegIFN. The severity of adverse events increased with increasing PegIFN dose. The IM dose could be administered to the patients who were assigned to receive 50 μ g/wk or 100 μ g/wk PegIFN but not to those who were assigned to receive 150 μ g/wk. The median administered dose of PegIFN ranged between 32 μ g/wk and 36 μ g/wk. The cytogenetic re-

sponse was 70% complete (Ph-neg 100%) and 83% major (Ph-neg > 65%). The BCR/ABL transcript was reduced by at least 3 logs in 68% of complete cytogenetic responders. These data of toxicity, compliance, and efficacy may assist in the design and preparation of prospective studies. (Blood. 2004;104:4245-4251)

© 2004 by The American Society of Hematology

Introduction

The introduction of imatinib (IM; STI571, Gleevec, Gleevec), a protein tyrosine kinase inhibitor that suppresses Philadelphia-positive (Ph-pos) cells by a specific interference with the leukemogenic protein encoded by the *bcr/abl* fusion gene, has brought about a revolution in the treatment and the management of Ph-pos chronic myeloid leukemia (CML).¹⁻¹⁰ In the short term, the superiority of IM over interferon- α (IFN- α), the first-choice agent for several years, is overwhelming.¹¹⁻¹⁴ However, IFN- α is effective and may significantly prolong survival. This is particularly true in low-risk patients, where the cytogenetic response (CgR) to IFN- α may be higher than 50% and the survival of complete responders may be longer than 10 years, even if the disease remains detectable at a molecular level.¹⁵⁻¹⁷ On the other hand, the mechanism and the spectrum of IM activity leave doubts that IM alone may be sufficient to control leukemia in the long term. The doubts are based on the observation that the response to IM is related to the phase and the risk of the disease,^{12,13,18} and that resistance may develop through several mechanisms, including point mutations in the *abl* part of the gene, *bcr/abl* gene amplification/overexpression, and the occur-

rence of additional genetic abnormalities.¹⁹⁻²⁵ While the mechanism of action of IM is rather well established,¹⁻⁴ the mechanism of action of IFN- α is less known, and is likely to be multifactorial and different from that of IM. IFN- α can restore the adhesion of leukemic stem cells to marrow stroma, down-regulate the expression of the *bcr/abl* gene, and activate several transcriptional factors that regulate cell proliferation, maturation, and apoptosis.²⁶⁻³¹ Moreover, and maybe more importantly, IFN- α can enhance the recognition and the elimination of the leukemic cells by the immune system.³²⁻³⁵ Although preclinical in vitro studies on the effects of the exposure of Ph-pos cells to IM and IFN- α have provided contrasting data,^{36,37} there is a wide clinical consensus that the combination of IM and IFN- α is worth testing, to prevent the emergence of resistance and to control and eliminate minimal residual disease. In preparation of a prospective randomized study, we designed an exploratory study to provide information on the side effects of the combination, on the deliverable doses, and on patient compliance. The results of this study were the subject of a preliminary report at the last meeting of the American Society of Hematology (ASH),³⁸ and are now reported in full in this paper.

From the Institute of Hematology and Medical Oncology "L. and A. Seràgnoli," University of Bologna; the Division of Hematology and Internal Medicine, Department of Clinical and Biological Science, University of Torino; CEINGE Biotecnologie Avanzate and Department of Biochemistry and Medical Biotechnology, University of Napoli Federico II; Novartis Pharma, Origgio, Italy; Schering-Plough, Milan, Italy; and Chair of Hematology, University of Brescia, Italy.

Submitted March 8, 2004; accepted August 3, 2004. Prepublished online as *Blood* First Edition Paper, August 19, 2004; DOI 10.1182/blood-2004-03-0826.

Supported by COFIN 2003, Fondazione del Monte di Bologna e Ravenna, University of Bologna, Italian Association for Cancer Research (AIRC), National Research Council, Regione Campania, and European LeukemiaNet.

A complete list of the members of the GIMEMA Working Party on Chronic Myeloid Leukemia appears in the "Appendix."

D.A. is an employee of Novartis Pharma, Italy, and G.F. was an employee of Schering-Plough, Italy. These companies supplied the drugs used in this study.

Reprints: Michele Baccarani, Institute of Hematology and Medical Oncology "L. and A. Seràgnoli," University of Bologna, Via Massarenti, 9-40138 Bologna, Italy; e-mail: baccarani@med.unibo.it.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2004 by The American Society of Hematology

Patients and methods

This was an exploratory, phase 2 study of the Italian Cooperative Study Group (ICSG) on CML (currently GIMEMA Working Party on CML) to investigate the treatment of CML with IM and IFN- α . The purpose of the study was to explore which dose of IFN- α could be combined with a standard dose of IM, and to investigate the toxicity profile of the combination as well as the compliance to the treatment. The response to treatment was also assessed.

The protocol was designed, promoted, and sponsored by the ICSG with the support of Novartis Pharma and Schering Plough, which ensured the supply of the study drugs, free of charge, for the duration of the study. The study and the protocol were approved by the independent ethics committee of S. Orsola-Malpighi Hospital in Bologna, hence by the ethic committees of all participating centers, and was designed and performed according to the Declaration of Helsinki and to good clinical practice.

Patients

Patients were eligible if they had a confirmed diagnosis of Ph-pos CML in early chronic phase (CP), were previously untreated with either study drug, were younger than 70 years old, if their performance status (according to the World Health Organization [WHO]) was 0-1, and if they were able and willing to provide written informed consent, including a specific mention to pregnancy and to procreation. Exclusion criteria were hepatitis B virus (HBV), hepatitis C virus (HCV), or HIV positivity, alcohol or drug addiction, and severe unrelated disease.

Study drugs and treatment protocol

Study drugs were IM (also known as STI571, currently registered as Glivec or Gleevec; Novartis Pharma, East Hanover, NJ), and a pegylated preparation of human recombinant interferon- α 2b (PegIFN; PegIntron; Schering Plough, Kenilworth, NJ). PegIFN had already been tested in the treatment of CML³⁹⁻⁴¹ and was preferred to conventional IFN- α because of the weekly injection schedule.

The treatment protocol was designed so as to ensure that all the patients could receive the standard or recommended dose of IM (400 mg/d). The scheduled dose of PegIFN was 50 μ g/wk in a first cohort of 27 patients, 100 μ g/wk in a second cohort of 18 patients, and 150 μ g/wk in a third cohort of 31 patients. The number of cases in each cohort was not identical because the enrollment was competitive, with a grace period of 15 days after the 20th case of each cohort, when additional patients could be enrolled. The 2

drugs were begun simultaneously (day 1). Treatment trial time was 1 year. Subsequent treatment was free, with the recommendation to continue with both drugs in case of response and if tolerated.

Dose adaptation and discontinuation

The basic principle of dose adaptation was to save the dose of IM over that of PegIFN. Stringent criteria were adopted for safety requirements, with attention to the severity of the adverse events (AEs) and also to the recurrence of the events in the same patient (Table 1).

For operational purposes, AEs were divided into hematologic (Hema) and nonhematologic (non-Hema). All the Hema AEs were attributed a priori to both drugs. In case of a Hema AE grade 1 or 2, no action was taken. Notice that in case of a Hema AE grade 3 third time, or grade 4 first time, PegIFN was allowed to be reassumed and continued only at the lowest dose level (50 μ g/wk), and that in case of a Hema AE grade 3 fourth time, or grade 4 second time, PegIFN was discontinued forever. In case of a non-Hema AE, the investigators were required to identify which drug was responsible for that event. In case of a grade 3 non-Hema AE, PegIFN was allowed to be reassumed and continued only at the lowest dose level (50 μ g/wk), while in case of repeated grade 3 or grade 4 non-Hema AEs, PegIFN was discontinued forever. IM was discontinued forever only after 2 grade 3 or one grade 4 non-Hema AEs attributable to IM itself (Table 1).

Required studies

A visit, with blood counts and differential, was scheduled before treatment. Visits were weekly during the first month, every 2 weeks during the second and third months, and monthly thereafter. The karyotype of bone marrow cells was examined by standard banding techniques before treatment, every 3 months during the first year of treatment, and every 6 months thereafter. Fluorescent in situ hybridization (FISH) analysis for 9q deletion was performed prior to treatment in 66 of 76 cases.

Molecular studies were performed on marrow cells prior to treatment, every 3 months during the first year of treatment, and every 6 months thereafter.

Molecular methods

Patient samples were shipped by overnight courier to the Group Central Laboratory in Bologna where the cell pellets were isolated and stored. The samples were divided into 3 groups and each group was assigned for molecular assay to one of the 3 reference laboratories of the ICSG, in

Table 1. Criteria for dose discontinuation and dose adaptation

Adverse event	Attributed to	Action taken	Subsequent dose level	
			Imatinib	PegIFN
Hema				
Grade 3, first time	Both drugs	D/C both drugs until recovery	400 mg at recovery	Cohort dose level 1 week after recovery
Grade 3, second time	Both drugs	D/C both drugs until recovery	300 mg at recovery, 400 mg 1 week after recovery	Cohort dose level 1 week after recovery
Grade 3, third time, or grade 4, first time	Both drugs	D/C both drugs until recovery	300 mg at recovery, 400 mg 1 week after recovery	50 μ g 2 weeks after recovery
Grade 3, fourth time, or grade 4, second time	Both drugs	D/C both drugs until recovery	300 mg one week after recovery	D/C forever
Non-Hema				
Grade 3, first time	IM	D/C IM until recovery	300 mg at recovery, 400 mg 2 weeks after recovery	No change
Grade 3, first time	PegIFN	D/C PegIFN until recovery	No change	50 μ g at recovery
Grade 3, first time	Both drugs	D/C both drugs until recovery	300 mg at recovery, 400 mg 2 weeks after recovery	50 μ g at recovery
Grade 3, second time, or grade 4, first time	IM	D/C both drugs until recovery	D/C forever	Cohort dose level at recovery
Grade 3, second time, or grade 4, first time	PegIFN	D/C both drugs until recovery	400 mg at recovery	D/C forever
Grade 3, second time, or grade 4, first time	Both drugs	D/C both drugs until recovery	300 mg at recovery	D/C forever

Notice that all Hema adverse events (AEs) are attributed to both drugs *a priori*, requiring the discontinuation (D/C) of both drugs until recovery to grade 1 or less. In contrast, in the case of non-Hema AEs, the investigators were required to identify which agent was likely to be responsible and to take actions accordingly. No action was taken for Hema AEs grade 2, whereas for non-Hema AEs grade 2 the responsible agent was discontinued until recovery to grade 1 or less (see text for other details).

Bologna, Turin, and Naples. All the samples of the same patient were analyzed in the same laboratory. Quantitative assays were performed using a real-time quantitative reverse transcriptase–polymerase chain reaction (QRT-PCR) assay, which has been described and reported in detail elsewhere.^{42–44} Briefly, leukocyte pellets were isolated from bone marrow aspirates by lysis of red blood cells upon their arrival at the Bologna laboratory. Cell pellets were washed twice in saline solution and resuspended in aliquots of 1×10^6 in 600 μL of 4 M guanidium isothiocyanate solution. The aliquots were stored at -20°C until shipment to the other reference laboratories (Naples and Turin). The QRT-PCR method independently measured in each sample the copy number of the mRNAs for the *P210^{BCR/ABL}* protein and for $\beta 2$ -microglobulin ($\beta 2m$), which served as a control gene to verify and adjust for sample-to-sample RNA quality variations. Each sample was analyzed in triplicate, and threshold cycle (Ct; number of PCR cycles necessary to achieve a target-specific fluorescence detection threshold) values were averaged. Reaction conditions and primer and probe sequences for QRT-PCR of *BCR/ABL* and $\beta 2m$ transcripts were designed, tested, and standardized within the framework of a European Union (EU) concerted action.^{42,43} For each amplification run, a *BCR/ABL* and $\beta 2m$ standard curve were independently generated by assaying, in parallel with the samples, 10-fold serial dilutions (from 10^6 to 10^2 copies, each in triplicate) of plasmid DNA calibrators containing the target sequences diluted in a solution of *E coli* RNA (20 ng/ μL). Plasmid dilutions were purchased from IPSOGEN (Marseille, France). Given the mean Ct values for each sample, the copy number of *BCR/ABL* and $\beta 2m$ transcripts was derived by interpolation to the appropriate standard curve, and the result was expressed as the ratio of *BCR/ABL* mRNA copies to $\beta 2m$ mRNA. Since the level of $\beta 2m$ mRNA is approximately 2 logs higher than the level of the other 2 control genes selected within the EU concerted action, *ABL* and beta-glucuronidase, the ratio of *BCR/ABL* to $\beta 2m$ was multiplied by 100. The lowest limit of sensitivity of the method was set at 0.000 01 (corresponding to a Ct of 38.5 for the *BCR/ABL* transcript). Patient RNA samples that repeatedly gave $\beta 2m$ Cts higher than 25.7 (corresponding approximately to 10 000 molecules) were operationally considered degraded and were excluded from further evaluation. This ensured that in all samples assayed, the dynamic range of detectability of the technique was at least 4 logs.

Definitions

Chronic phase (CP) was distinguished from accelerated phase and blastic phase (AP, BP) on the basis of the percentage of blast cells in peripheral blood (less than 10% vs more than 10%) and of the involvement of nonhematopoietic tissues or organs (not involved vs involved), as in prior studies.⁴⁵ The relative risk of the patients was calculated and defined as low, intermediate, or high, according to both the Sokal and Hasford (European) formulations.^{46,47} The hematologic response was defined as complete (CHR) if the white blood cell (WBC) count was less than $10 \times 10^9/\text{L}$, if the platelet count was less than $450 \times 10^9/\text{L}$, and if no granulocyte precursors were counted in the differential. Moreover, it was required that the spleen

was not palpable. The cytogenetic response was based on the evaluation of a minimum of 20 marrow cell metaphases and was graded according to the proportion of Ph-negative (Ph-neg) metaphases, as complete (100% Ph-neg), partial (66%–99% Ph-neg), minor (34%–65% Ph-neg), and minimal or none ($< 34\%$ Ph-neg). The term major CgR (MCgR) grouped together both partial and complete CgRs (65%–100% Ph-neg). Molecular response was graded according to the log reduction of *BCR-ABL* transcript amount. Survival and progression-free survival were determined according to the method of Kaplan and Meier.⁴⁸ Namely, survival was calculated from the date of enrollment to the date of death. Progression-free survival was calculated from the date of enrollment to the date of progression to AP or BP, or to death. AEs were identified and graded according to the WHO scale and were divided into Hema AEs and non-Hema AEs. The frequency of AEs was calculated and expressed either as the percent of the patients who suffered at least once from a given AE or as the number of AEs per patient, because several patients complained of the same or of different AEs at the same time or more than once. The compliance to the doses was calculated as the ratio between the administered dose and the scheduled dose, taking into account all dose reductions and all the cases of discontinuation, both temporary and permanent.

Results

Patients

During a 5-month period, 18 centers enrolled 78 patients. There were 2 patients who were enrolled in the second cohort who were not eligible: one patient was *BCR/ABL*-positive but Ph-neg, and one patient was already in AP. There were 27 patients enrolled in the first cohort (PegIFN 50 $\mu\text{g}/\text{wk}$), 18 patients enrolled in the second cohort (PegIFN 100 $\mu\text{g}/\text{wk}$), and 31 patients enrolled in the third cohort (PegIFN 150 $\mu\text{g}/\text{wk}$). Details of the patients are reported in Table 2. All were in early CP, less than 3 months from diagnosis. There were 59 patients (78%) who had been pretreated with hydroxyurea. About 50% were low risk (45% by Sokal score, 51% by Hasford score), while high-risk patients were 24% by Sokal's score and 12% by Hasford's score. Additional cytogenetic abnormalities were found in 19 cases (25%), and included 9q deletions (12 cases), variant translocations (5 cases), and others (4 cases) (data not shown in Table 2).

Adverse events (AEs)

Neutropenia, grades 3 and 4, was recorded in 63% of cases, with a frequency of 1.69 events per patient (Table 3). It increased in frequency from the first cohort (1.37) to the second and the third cohort (1.66 and 1.99, respectively). Thrombocytopenia (grade 3

Table 2. Patient characteristics

	All patients	First cohort	Second cohort	Third cohort
No. patients	76	27	18	31
Sex, no. males/no. females	44/32	17/10	12/6	15/16
Age, mean (range), y	47 (18–68)	47 (19–64)	47 (18–67)	47 (28–68)
Time from diagnosis to enrollment, mean (range), d	50 (9–90)	51 (10–90)	43 (9–90)	50 (14–90)
Prior treatment with hydroxyurea, no. patients (%)	59 (78)	20 (74)	14 (78)	25 (81)
No prior treatment, no. patients (%)	17 (22)	7 (26)	4 (22)	6 (19)
Relative risk (according to Sokal)				
Low, no. patients (%)	34 (45)	14 (52)	9 (50)	11 (35)
Intermediate, no. patients (%)	24 (31)	9 (33)	4 (22)	11 (35)
High, no. patients (%)	18 (24)	4 (15)	5 (28)	9 (29)
Relative risk (according to Hasford)				
Low, no. patients (%)	39 (51)	14 (52)	9 (50)	16 (52)
Intermediate, no. patients (%)	28 (37)	11 (41)	8 (44)	9 (29)
High, no. patients (%)	9 (12)	2 (7)	1 (6)	6 (19)

Table 3. Hematologic and nonhematologic adverse events

	All patients		First cohort		Second cohort		Third cohort	
	No. per patient	%	No. per patient	%	No. per patient	%	No. per patient	%
Hema AEs								
Neutropenia, grade 3/4	63	1.69	54	1.37	66	1.66	68	1.99
Thrombocytopenia, grade 3	28	0.39	18	0.22	33	0.44	31	0.52
Anemia, grade 3	1	0.04	4	0.11	—	—	—	—
Non-Hema AEs								
Grade 2	38	1.95	52	2.18	22	1.78	35	1.84
Grade 3	38	0.99	22	0.59	33	1.00	55	1.32
Grade 4	3	0.03	—	—	11	0.11	—	—
Total	79	2.97	74	2.77	66	2.89	90	3.16

The frequency of adverse events (AEs) has been calculated both as the percent of patients who suffered at least one of the adverse events (%) and as the number of AEs per patient. For all patients, n = 76; first cohort, 27; second cohort, 18; and third cohort, 31.

only) occurred in 28% of cases, with a frequency of 0.22 events per patient in the first cohort, 0.44 events per patient in the second cohort and 0.52 events per patient in the third cohort. Grade 3 anemia occurred in one patient only.

Non-Hema AEs, grades 2 to 4, were recorded in 79% of cases, with a frequency of 2.97 events per patient (Table 3). The frequency of these AEs was not different in the 3 cohorts, but the severity increased along with the increasing dose of PegIFN (grade 3 non-Hema AEs were reported in 22%, 33%, and 55% of patients of the first, second, and third cohorts, with a frequency of 0.59, 1.00, and 1.32, respectively; Table 3). Among non-Hema AEs, constitutional events (pain, fatigue, asthenia, fever, flu-like syndrome) were the most frequent and occurred in 43% (grade 2) and 36% (grade 3) of cases, with a clear increase of grade 3 events from the first cohort (11% of cases) to the third cohort (60% of cases; Table 4). Skin AEs, including rash and pruritus, occurred in 39% of cases (22% grade 2 and 17% grade 3) without any detectable relationship with PegIFN dose. Neurologic or psychiatric problems, mainly depression, were experienced by 22% of patients. Nausea, oral mucositis, abdominal pain, or diarrhea developed in 21% of patients. Edema, generalized or local, was recorded in 16% of cases, with only one case of grade 3 (Table 4).

Treatment discontinuation and dose reduction

IM was permanently discontinued in 3 of 76 patients (4%) because of generalized edema and skin rash (one patient) and of skin rash (2

patients). PegIFN was permanently discontinued in 45 of 76 cases (59%), of which 15 of 27 cases (55%) were in the first cohort, 11 of 18 (61%) were in the second cohort, and 19 of 31 (61%) were in the third cohort. Hema AEs (neutropenia and thrombocytopenia) caused PegIFN discontinuation in 30% of cases, but only after the first quarter. Non-Hema AEs caused PegIFN discontinuation in 29% of cases, and that occurred more frequently during the first quarter (14%), than during the second quarter (8%) and the second half-year (7%).

PegIFN dose was reduced to 50 µg/wk in 5 of 18 patients (28%) of the second cohort (and all these 5 patients did not tolerate and discontinued the reduced dose), and in 17 of 31 patients (55%) of the third cohort (and 9 of these 17 patients did not tolerate, and therefore discontinued the reduced dose).

The ratio between the administered and the scheduled dose of each drug is reported in Table 5, showing that the dose of IM was maintained at 87% to 90% of the scheduled dose in the first 2 cohorts, but was decreased (77%) in the third cohort. In contrast, the median administered dose of PegIFN was always substantially lower than scheduled, and was similar in all 3 cohorts, ranging between 32 µg/wk and 36 µg/wk.

Response and course

A CHR was achieved in all but 2 patients, who progressed to AP after 3 months. A CCgR was obtained in 53 of 76 patients (70%) and a PCgR was obtained in the other 10 patients, for a MCgR rate of 63 of 76 (83%). The CgR rate was not different in the 3 PegIFN cohorts and was not different in the patients who discontinued PegIFN versus those patients who did not discontinue PegIFN. All 13 patients with a 9q deletion and/or a variant translocation achieved an MCgR, with only one exception. On the contrary, only 55% of high-risk patients (as calculated according to either the Sokal or Hasford score)^{46,47} achieved an MCgR.

The molecular response of the patients who achieved a CCgR is reported in Figure 1, showing a progressive decrease of the amount of the BCR/ABL transcript from a median value of 0.31 at baseline to 0.000 09 after 12 months.

In 47% of all 76 patients and 68% of CCgRs, there was a reduction of the BCR/ABL transcript level of 3 logs or more, and at 12 months the amount of the transcript was below the level of sensitivity of the method in 11 of 76 cases (14%).

Six months after the end of the study (18 months after the first dose of IM and PegIFN), overall survival was 98.7%, progression-free survival was 97.4%, and 70% of patients were in continuous CCgR.

Table 4. Frequency of non-Hema AEs by type

	All patients	First cohort	Second cohort	Third cohort
Adverse events, no. (grade)	2 (3/4)	2 (3/4)	2 (3/4)	2 (3/4)
Constitutional (muscle cramps, myalgia, arthralgia, musculoskeletal pain, fatigue, asthenia, fever, headache, flu-like syndrome), no. (%)	43 (36)	45 (11)	56 (33)	33 (60)
Skin (rash and related events, pruritus), no. (%)	22 (17)	22 (15)	5 (17)	33 (19)
Neuro-psychiatric (polyneuropathy, depression), no. (%)	13 (9)	19 (7)	5 —	13 (16)
Gastrointestinal (nausea, vomiting, mucositis, abdominal pain, diarrhea), no. (%)	17 (4)	22 —	28 —	7 (9)
Edema (generalized, superficial, orbital, facial, weight gain), no. (%)	15 (1)	26 (4)	— —	12 —
Liver (AST/ALT increase), no. (%)	4 (4)	4 —	6 (5)	4 (6)
Other, no. (%)	9 (5)	— —	6 (5)	16 (10)

The frequency has been calculated as the percent of cases who suffered at least one adverse event (AE), grade 2 or grade 3/4. Notice that the frequencies that are shown in this table are greater than the frequencies shown in Table 3, because several patients suffered from the same AE, grade 2 and grade 3/4. For all patients, n = 76; first cohort, 27; second cohort, 18; and third cohort, 31.

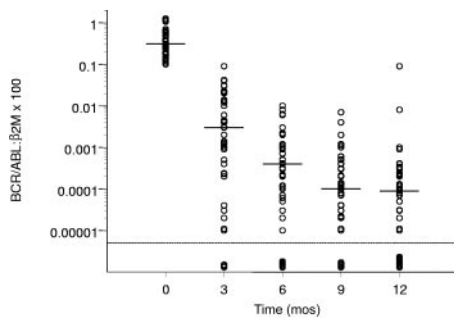


Figure 1. Molecular response in the patients who achieved a CCgR. Horizontal bars represent median values. The molecular response is assessed as the ratio between BCR/ABL and $\beta 2m$ RNA $\times 100$, as described in "Patients and methods." The lowest limit of sensitivity of the method was set at 0.00001.

Discussion

This study was planned and designed with the aim of evaluating the feasibility of the treatment of Ph-pos CML with a combination of IM standard dose and PegIFN, and to provide information on 3 points. The first point concerned the hemopoietic toxicity. Although neither drug has a great hematologic toxicity, the combination could result in cases of marrow aplasia or could lead to an undesired reduction of IM dose due to recurrent or chronic neutropenia or thrombocytopenia. The second point concerned the nonhematologic side effects of the combination. IFN is a lymphokine that has a number of side effects. Some of these side effects, although less severe, are seen also with IM. The combination could result in an increase of the frequency and/or the severity of the side effects, lead to an undesired reduction of drug doses (particularly of IM dose), or even require treatment discontinuation. A third important point was the identification of the PegIFN dose, and in particular, of a dose being both safe and free from undesirable and recurrent AEs.

The main result of this study is that 45 of 76 patients (59%) required a permanent discontinuation of PegIFN during the first year of treatment. This figure is about 3 times higher than the figures of IFN- α discontinuation in many prior studies of IFN- α alone or in combination with low dose arabinosyl cytosine (LDAC).^{45,49-53} The causes of discontinuation of PegIFN were multifactorial in many cases, but were accounted for almost equally by Hema (30%) and non-Hema AEs (29%).

Neutropenia, grade 3 or 4, was very frequent in all 3 cohorts. However, there were neither cases of severe or prolonged hemopoietic failure nor cases of severe infection. Grade 4 non-Hema AEs were rare (3% of cases, 0.03 events per patient) and no treatment-related death occurred. Therefore, we can conclude that the combination treatment was safe, at least with a careful application of the rules for dose adaptation and treatment discontinuation, as described in Table 1. However, the frequency of grade 3 Hema AEs and of grades 2 and 3 non-Hema AEs was remarkable, required frequent visits, and contributed substantially to limit patient compliance.

One would have expected the frequency and the severity of all AEs to rise with increasing the scheduled dose of PegIFN. As a matter of fact, the severity of AEs increased, being maximal in the third cohort (PegIFN 150 $\mu\text{g}/\text{wk}$), whereas the frequency of all AEs did not increase. Also, the rate of PegIFN discontinuation was not different in the 3 cohorts (55%, 61%, and 61%, respectively). This may be explained by the treatment plan, which accounted for PegIFN dose reduction before discontinuation (Table 1). Thus, the

Table 5. Median administered dose versus scheduled dose

Cohort	Imatinib			PegIFN		
	Scheduled, mg/d	Administered Median, mg/d	%	Scheduled, $\mu\text{g}/\text{wk}$	Administered Median, $\mu\text{g}/\text{wk}$	%
First	400	360	90	50	36	72
Second	400	350	87	100	35	35
Third	400	310	77	150	32	21

The administered dose of IM was significantly lower (Student *t* test) in the third cohort than in the second cohort ($P = .03$) and the first cohort ($P = .01$).

dose of PegIFN was decreased to 50 $\mu\text{g}/\text{wk}$ in 28% of the second cohort patients and in 55% of the third cohort patients, reducing substantially the dose difference that was originally scheduled, so that the median PegIFN dose that was actually delivered was similar in the 3 cohorts (Table 5). However, IM dose was not the same in the 3 cohorts, with a significant reduction of the dose in the third cohort (median 310 mg/d, corresponding to 77% of the scheduled dose), suggesting that the attempt to deliver a higher PegIFN dose (150 $\mu\text{g}/\text{wk}$) resulted in a reduction of the deliverable IM dose, which may compromise efficacy.

The major types of non-Hema AEs (Table 4) were those that can be expected from IFN- α alone and from IM alone. We may try to offer a comparison of the frequency of the side effects of this combination with the frequency of the side effects of IM alone, using the data of a study of 191 late CP patients with CML who were treated with IM alone at the same institutions⁴⁴ (Table 6). In the present study (IM+PegIFN), the frequency of all non-Hema AEs was 3 to 5 times greater than in the prior study of IM alone, with the exception of liver toxicity. Interestingly, the events that may be attributed mainly to IM were more frequent with the combination. Thus, skin rash and related events occurred with a frequency of 0.74 events per patient versus 0.13 with IM alone. Edema, generalized and superficial, occurred with a frequency of 0.21 events per patient versus 0.08 with IM alone. Therefore, it is wise to consider the possibility that the simultaneous administration of the 2 agents may enhance the toxicity of either agent. Another possibility that is worth discussing is that the combination was poorly tolerated because it was not perceived by patients and doctors as the best available treatment modality. This probably occurred in the recent International Randomized Study of Interferon and STI571 (IRIS) study, where the compliance to control

Table 6. Frequency of non-Hema AEs, by type, in this study and in a prior study of IM alone, 400 mg/day

Adverse events, grades 2, 3, and 4	No. adverse events per patient	
	This study, IM + PegIFN	Prior study, ⁴⁴ IM alone
Constitutional (muscle cramps, myalgia, arthralgia, musculoskeletal pain, fatigue, asthenia, fever, headache, flu-like syndrome)	1.04	0.28
Skin (rash and related events, pruritus)	0.74	0.13
Neuro-psychiatric (polyneuropathy, depression)	0.39	0.08
Gastrointestinal (nausea, vomiting, mucositis, abdominal pain, diarrhea)	0.26	0.12
Edema (generalized, superficial, orbital, facial, weight gain)	0.21	0.08
Liver	0.10	0.10

The frequency was calculated as the number of adverse events (AEs) per patient, over a 1-year period. For this study, $n = 76$; for the study using IM alone, $n = 191$.

treatment, IFN plus LDAC, was lowered by the perception that this treatment was less effective, and was outdated.¹¹ We do not think that these considerations apply to the present study, because all the patients received the best perceived drug (IM) at a time when it was not yet available for CML treatment, and the treatment proved to be effective very rapidly, since within 3 months all patients but 2 were in CHR and 61% of them had already achieved an MCgR.

Even though we have also reported the data on response, there are no means to compare the results of this study to the results that have been reported with IM alone as a first-line treatment of CP CML. However, it may be of interest to point out that in the IM arm of the international IRIS study, after a median treatment time of 19 months, the CgR rate was 76% complete and 87% major, and a molecular response of 3 logs or more was achieved in 57% of all patients.¹¹⁻¹² In a pilot study of 30 patients who received first-line treatment with IM (400 mg/d) and LDAC, the CCgR at one year was 70% and the MCgR was 83%.⁵⁴

In conclusion, we have found that a combination of IM standard dose (400 mg/d) with PegIFN at a dose ranging from 50 µg/wk to 150 µg/wk was devoid of severe AEs and did not result in an unpredictable toxicity, but was associated with considerable neutropenia, thrombocytopenia, and non-Hema toxicity. This required a reduction of the dose of PegIFN to 50 µg/wk in many of the patients who were assigned to receive 100 µg/wk or 150 µg/wk and a permanent discontinuation of PegIFN in 59% of all patients. We also observed that administering PegIFN at 150 µg/wk might cause a substantial reduction of IM dose. It is possible that modifying the dose and the schedule of either agent, as well as using conventional IFN-α or other pegylated IFN-α preparations, the toxicity profile, the compliance, and the results may change, in one sense or in another. Also, it should not be overlooked that we

report here only on the short-term, one-year response to the treatment. Although the response was similar to that reported with IM alone,^{11,12} it is not possible to anticipate if there would be any effects on progression-free survival or survival. In any case, these data may assist in the design and preparation of prospective studies.

Acknowledgments

We are indebted to Barbara Giannini, Emanuela Ottaviani, Carolina Terragna, and Chiara Nicci (Bologna) for technical help. We acknowledge Ursula Giussani (Cytogenetic Laboratory, Bergamo), Gian Carlo Discepoli (Cytogenetic Laboratory, Pediatric Hospital "Salesi"), and Ancona and Mario Sessarego (DIMI, Genova), for their contributions to molecular cytogenetic studies. The technical assistance of Mrs Katia Vecchi and Mrs Maira Marsili (Bologna) is kindly acknowledged.

Appendix

The following members of the Italian Cooperative Study Group (ICSG) on CML (currently known as the GIMEMA Working Party on CML) have actively participated in this study: Antonio Peta and Franco Iuliano (Catanzaro), Renato Fanin and Mario Tiribelli (Udine), Tiziano Barbui and Renato Bassan (Bergamo), Eugenio Gallo and Patrizia Pregno (Torino), Francesco Lauria and Monica Bocchia (Siena), Pietro Leoni and Serena Rupoli (Ancona), Antonio Bosi and Valeria Santini (Firenze), Marco Gobbi and Maurizio Miglino (Genova), Giorgina Specchia (Bari), Federico Papineschi (Pisa), Giuliana Alimena (Roma), Dario Ferrero (Torino), Anna Marina Liberati (Perugia), Ester Pungolino (Milano), Elisabetta Abruzzese (Roma), and Adele Capucci (Brescia).

References

- 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;2:561-566.
- Buchdunger E, Zimmermann J, Mett H, et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. *Cancer Res*. 1996;56:100-104.
- Gambacorti-Passerini C, le Coutre P, Mologni L, et al. Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL⁺ leukemic cells and induces apoptosis. *Blood Cells Mol Dis*. 1997;23:380-394.
- Deininger MW, Goldman JM, Lydon N, Melo JV. The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells. *Blood*. 1997;90:3691-3698.
- Druker BJ, Talpaz M, Resta D, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*. 2001;344:1031-1037.
- Druker BJ, Sawyers CL, Kantarjian HM, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med*. 2001;344:1038-1042.
- Sawyers CL, Hochhaus A, Feldman E, et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood*. 2002;99:3530-3539.
- Talpaz M, Silver RT, Druker BJ, et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. *Blood*. 2002;99:1928-1937.
- Kantarjian HM, Sawyers CL, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med*. 2002;346:645-652.
- Kantarjian HM, Cortes JE, O'Brien SG, et al. Imatinib mesylate 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.
- 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.
- Hughes TP, Kaeda J, Branford S, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med*. 2003;349:1421-1423.
- Goldman JM. Chronic myeloid leukemia: still a few questions. *Exp Hematol*. 2004;32:2-10.
- Savage DG, Antman KH. Imatinib mesylate: a new oral targeted therapy. *N Engl J Med*. 2002;346:683-693.
- The Italian Cooperative Study Group on Chronic Myeloid Leukemia. Long term follow-up of the Italian Trial of Interferon alfa-2a versus conventional chemotherapy in chronic myeloid leukemia. *Blood*. 1998;92:1-9.
- Bonifazi F, de Vivo A, Rosti G, et al. Chronic myeloid leukemia and interferon-alpha: a study of complete cytogenetic responders. *Blood*. 2001;98:3074-3081.
- Kloke O, Opalka B, Niederle N. Interferon alfa as primary treatment of chronic myeloid leukemia: long-term follow-up of 71 patients observed in a single center. *Leukemia*. 2000;14:389-392.
- Rosti G, Trabacchi E, Bassi S, et al. Risk and early cytogenetic response to imatinib and interferon in chronic myeloid leukemia. *Haematologica*. 2003;88:256-259.
- Gambacorti-Passerini C, Gunby RH, Piazza R, et al. Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukemias. *Lancet Oncol*. 2003;4:75-85.
- Hochhaus A, Kreil S, Corbin A, et al. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia*. 2002;16:2190-2196.
- Sawyers CL. Research on resistance to cancer drug Gleevec. *Science*. 2001;294:1834.
- Mahon FX, Deininger MW, Schultheis B, et al. Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. *Blood*. 2000;96:1070-1079.
- Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science*. 2001;293:876-880.
- Branford S, Rudzki Z, Walsh S, et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with poor prognosis. *Blood*. 2003;102:276-283.
- Tipping AJ, Deininger MW, Goldman JM, Melo JV. Comparative gene expression profile of chronic myeloid leukemia cells innately resistant to imatinib mesylate. *Exp Hematol*. 2003;31:1073-1080.
- Andrews III DF, Singer JW, Collins SJ. Effects of recombinant alpha-interferon on the expression of the bcr-abl fusion gene in human chronic myelogenous human leukemia cell lines. *Cancer Res*. 1987;47:6629-6632.

27. Dowding C, Guo AP, Osterholz J, Siczkowski M, Goldman J, Gordon M. Interferon-alpha overrides the deficient adhesion of chronic myeloid leukemia primitive progenitor cells to bone marrow stromal cells. *Blood*. 1991;78:499-505.
28. Bhatia R, McCarthy JB, Verfaillie CM. Interferon-alpha restores normal $\beta 1$ integrin-mediated inhibition of hematopoietic progenitor proliferation by marrow microenvironment in chronic myelogenous leukemia. *Blood*. 1996;87:3883-3891.
29. Hochhaus A, Yan XH, Willer A, et al. Expression of interferon regulatory factor (IRF) genes and response to interferon-alpha in chronic myeloid leukemia. *Leukemia*. 1997;11:933-939.
30. Pane F, Mostarda I, Selli C, et al. BCR/ABL mRNA and the P210BCR/ABL protein are down-modulated by interferon- α in chronic myeloid leukemia patients. *Blood*. 1999;2200-2207.
31. Schmidt M, Hochhaus A, Nitsche A, Hehlmann R, Neubauer A. Expression of nuclear transcription factor interferon consensus sequence binding protein in chronic myeloid leukemia correlates with pretreatment risk features and cytogenetic response to interferon-alpha. *Blood*. 2001;97:3648-3650.
32. Deng M, Daley GQ. Expression of interferon consensus sequence binding protein induces potent immunity against BCR/ABL-induced leukemia. *Blood*. 2001;97:3491-3497.
33. Cortes I, Fayad L, Kantarjian H, O'Brien S, Lee MS, Talpaz M. Association of HLA phenotype and response to interferon-alpha in patients with chronic myelogenous leukemia. *Leukemia*. 1998;12:455-462.
34. Oka T, Sastry KJ, Nehete P, et al. Evidence for specific immune response against P210 BCR-ABL in long-term remission CML patients treated with interferon. *Leukemia*. 1998;12:155-163.
35. Burchert A, Wölfel S, Schmidt M, et al. Interferon-alpha, but not the ABL-kinase inhibitor imatinib (STI 571), induces expression of myeloblastin and specific T-cell response in chronic myeloid leukemia. *Blood*. 2003;101:259-264.
36. Kano Y, Akutsu M, Tsunoda S, et al. In vitro cytotoxic effects of a tyrosine kinase inhibitor STI571 in combination with commonly used antileukemic agents. *Blood*. 2001;97:1999-2007.
37. Thiesing JT, Ohno-Jones S, Kolibaba KS, Druker J. Efficacy of STI571, an abl tyrosine kinase inhibitor, in conjunction with other antileukemic agents against bcr-abl-positive cells. *Blood*. 2000;96:3195-3199.
38. Baccarani M, Rosti G, Trabacchi E, et al. Imatinib and pegylated-a2b interferon in early chronic phase Ph⁺ chronic myeloid leukemia: 12-months results of a prospective phase I/II study by the Italian Cooperative Study Group on CML (ICSG on CML) [abstract]. *Blood*. 2003;102:638a.
39. Talpaz M, O'Brien S, Rose E, et al. Phase 1 study of polyethylene glycol formulation of interferon alpha-2b (Schering 54031) in Philadelphia chromosome-positive chronic myelogenous leukemia. *Blood*. 2001;98:1708-1713.
40. Michallet M, Delain M, Maloisel F, et al. Phase III trial of PEG Intron vs Interferon alpha-2b for initial treatment of chronic myelogenous leukemia (CML) [abstract]. *Blood*. 2001;98:348a.
41. Michallet M, Maloisel F, Delain M, et al. Pegylated recombinant interferon alpha-2b vs recombinant interferon alpha-2b for the initial treatment of chronic phase chronic myelogenous leukemia: a phase III study. *Leukemia*. 2004;18:309-315.
42. Van Dongen JJ, Gabert JA, Delabesse E, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease: report of the BIOMED-1 concerted action: investigation of minimal residual disease in acute leukemia. *Leukemia*. 1999;13:1901-1928.
43. Gabert J, Van Der Velden VH, Bi H, et al. Standardization and quality control studies of "real-time" quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) of fusion gene transcripts for residual disease detection in leukemia: A Europe Against Cancer Program. *Leukemia*. 2003;17:2318-2357.
44. Rosti G, Martinelli G, Bassi S, et al. Molecular response to imatinib in late chronic phase chronic myeloid leukemia. *Blood*. 2004;103:2284-2290.
45. The Italian Cooperative Study Group on Chronic Myeloid Leukemia. Interferon alfa-2a as compared with conventional chemotherapy for the treatment of chronic myeloid leukemia. *N Engl J Med*. 1994;330:820-825.
46. Sokal JE, Cox EB, Baccarani M, et al. Prognostic discrimination in good risk chronic granulocytic leukemia. *Blood*. 1984;63:789-799.
47. Hasford J, Pfirrmann M, Hehlmann R, et al. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. *J Natl Cancer Inst*. 1998;90:850-858.
48. Kaplan EL, Meier P. Nonparametric estimation for incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
49. Hehlmann R, Heimpel H, Hasford J, et al. Randomized comparison of interferon-alpha with busulfan and hydroxyurea in chronic myelogenous leukemia. *Blood*. 1994;84:4064-4077.
50. Allan NC, Richards SM, Shepherd PC. UK Medical Research Council randomised, multicentre trial of interferon-alpha n1 for chronic myeloid leukaemia: improved survival irrespective of cytogenetic response. *Lancet*. 1995;345:1392-1397.
51. The Benelux CML Study Group. Randomized study on hydroxyurea alone versus hydroxyurea combined with low-dose interferon-alpha 2b for chronic myeloid leukemia. *Blood*. 1998;91:2713-2721.
52. Baccarani M, Rosti G, de Vivo A, et al. A randomized study of interferon-alpha vs interferon-alpha and low dose arabinosyl cytosine in chronic myeloid leukemia. *Blood*. 2002;99:1527-1535.
53. 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;337:223-229.
54. Gardembas M, Rousselot P, Tulliez M, et al. Results of a prospective phase 2 study combining imatinib mesylate and cytarabine for the treatment of Philadelphia-positive patients with chronic myelogenous leukemia in chronic phase. *Blood*. 2003;102:4298-4305.



blood[®]

2004 104: 4245-4251

doi:10.1182/blood-2004-03-0826 originally published online
August 19, 2004

Imatinib and pegylated human recombinant interferon- α 2b in early chronic-phase chronic myeloid leukemia

Michele Baccarani, Giovanni Martinelli, Gianantonio Rosti, Elena Trabacchi, Nicoletta Testoni, Simona Bassi, Marilina Amabile, Simona Soverini, Fausto Castagnetti, Daniela Cilloni, Barbara Izzo, Antonio de Vivo, Emanuela Messa, Francesca Bonifazi, Angela Poerio, Simona Luatti, Emilia Giugliano, Daniele Alberti, Gianluca Fincato, Domenico Russo, Fabrizio Pane and Giuseppe Saglio

Updated information and services can be found at:

<http://www.bloodjournal.org/content/104/13/4245.full.html>

Articles on similar topics can be found in the following Blood collections

[Clinical Trials and Observations](#) (4796 articles)

[Neoplasia](#) (4182 articles)

[Oncogenes and Tumor Suppressors](#) (795 articles)

Information about reproducing this article in parts or in its entirety may be found online at:

http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:

<http://www.bloodjournal.org/site/misc/rights.xhtml#reprints>

Information about subscriptions and ASH membership may be found online at:

<http://www.bloodjournal.org/site/subscriptions/index.xhtml>