52. Moritz T, Niederle N, Baumann J *et al.* Phase I study of recombinant human tumor necrosis factor alpha in advanced malignant disease. *Cancer Immunol Immunother* 1989, 29, 144-150.
53. Carlo-Stella C, Cazzola M, Ganser A *et al.* Synergistic antiproliferative effect of recombinant interferon-gamma with recombinant interferon-alpha on chronic myelogenous leukemia hematopoietic progenitor cells (CFU-GEMM, CFU-Mk, BFU-E and CFU-GM). *Blood* 1988, 72, 1293-1299.
54. Raefsky EL, Platanias LC, Zoumbos NC *et al.* Studies of interferon as regulator of hematopoietic cell proliferation. *J Immunol* 1985, 135, 2507-2512.
55. Talpaz M, Kurzrock R, Kantarjian HM *et al.* Therapy of Philadelphia positive chronic myelogenous leukemia with recombinant alpha A interferon and recombinant gamma interferon. *Blood* 1988, 72, 229a.
56. Niederle N, Wandl U, Kloke O *et al.* Efficiency of interferon alpha (IFN alfa-2b) and IFN gamma in chronic myelogenous leukemia (CML). *Proc Am Soc Clin Oncol* 1989, 8, 185.

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Evolving Modalities of Treatment with Interferon Alfa-2b for Ph^1-Positive Chronic Myelogenous Leukaemia

Enrica Morra, Giuliana Alimena, Mario Lazzarino, Anna Marina Liberati, Enrico Montefusco, Paolo Bernasconi, Marco Mancini, Emilio Donti, Serena Merante, Ferdinando Dianzani, Fausto Grignani, Carlo Bernasconi and Franco Mandelli

We have administered interferon alfa-2b, alone or in combination with chemotherapy, to 126 Ph^1-positive chronic myelogenous leukaemia patients. Of 71 early chronic phase (CP) patients (< 12 months from diagnosis), 41 (58%) obtained a complete haematological response (CHR). Daily interferon was more effective than intermittent administration. In previously untreated patients, the response was significantly influenced by risk status at diagnosis. Thirty-four out of 71 (48%) patients improved cytogenetically, the median of Ph^1+ mitoses declining from 100% to 66% with complete Ph^1-suppression in one case. Of 46 late CP patients (> 12 months from diagnosis), 32 (70%) achieved CHR with interferon alone or combined with chemotherapy. All 10 patients with disease well controlled by chemotherapy obtained stable CHR with interferon alone. Of 36 partial responders to conventional chemotherapy, 22 (61%) obtained CHR on interferon plus low-dose hydroxyurea. Ph^1 mosaicism was reached by 16 (35%) late CP patients (median Ph^1+ cells 75%). Of nine accelerated phase patients on interferon plus chemotherapy, one attained CHR, and two responded partially. At a median follow up of 36 months, of 41 CHR patients in early CP, 15 are controlled on interferon, 12 have had autologous bone marrow transplantation (BMT), and two allogeneic BMT. Blastic transformation (BT) has occurred in eight of 41 CHR patients (19%) versus 17 of 30 (57%) non-responders and partial responders to interferon. At a median follow up of 22 months, of 32 late CP patients obtaining CHR, 26 remain on interferon, one had allogeneic BMT, one had autologous BMT, and one developed BT (versus five out of 14 with less than CHR). These studies confirm the haematological and cytogenetic efficacy of interferon in CML and indicate that the disease status at the start of treatment is critical in determining the success of therapy.
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INTRODUCTION

DURING the last 20 years neither conventional chemotherapy, nor more aggressive chemotherapy have substantially modified the natural evolution of chronic myelogenous leukaemia (CML) [1, 2]. The suppressive effect on the Ph^1-positive clone, sometimes observed after intensive chemotherapy, is usually transient [3, 4]. To date, only allogeneic bone marrow transplantation (BMT) has led to the stable, complete suppression of the Ph^1 cells with restoration of normal haemopoiesis. This approach, however, is limited to a minority of CML patients [5]. Since 1983, several studies with partially purified human alpha interferon and with recombinant interferon have demonstrated significant activity in CML patients, leading in some cases to the partial or complete

Correspondence to: E. Morra.
E. Morra is at the Division of Hematology, Istituto Scientifico Policlinico S. Matteo, 27100 Pavia, Italy. G. Alimena, E. Montefusco, M. Mancini and F Mandelli are at the Institute of Hematology, University "La Sapienza", Rome, M. Lazzarino, P. Bernasconi, S. Merante and C. Bernasconi are at the Division of Hematology, Istituto Scientifico Policlinico S. Matteo, Pavia, A.M. Liberati, E. Donti and F. Grignani are at the Institute of Internal Medicine, University of Perugia, and F Dianzani is at the Institute of Virology, University "La Sapienza", Rome, Italy.

suppression of Ph¹-positive metaphases. This suppression has been achieved progressively without provoking the cytopenic effects of chemotherapy [6-17].

Following the first encouraging results obtained with leucocyte interferon [6, 7], in 1985 we started our first co-operative study to test the haematological and cytogenetic efficacy of recombinant interferon alfa-2b, administered three times a week (t.i.w.) at doses of 2 and 5 million units (MU)/m² in Ph¹ + CML patients. Seventy-four patients entered this first study [12].

Later, to improve the haematological and cytogenetic results, we used interferon daily in newly diagnosed patients or in patients in whom intermittent interferon had obtained only a partial response or had failed to maintain a complete response [14].

More recently, we tested daily interferon alone or in combination with hydroxyurea (HU) in 46 CML patients in the late chronic phase of their disease [18].

PATIENTS AND METHODS

We have treated with recombinant interferon alfa-2b (Intron A, Schering-Plough) a total of 126 patients with Ph¹-positive CML. Of these, 71 were in the early chronic phase (less than 12 months from diagnosis), 46 were in the late chronic phase (more than 12 months from diagnosis), and nine were in the accelerated phase.

CML patients in early chronic phase

This group of 71 patients included 35 previously untreated patients and 36 who had been given conventional chemotherapy for a median duration of 7.5 months (range 3-12 months). All patients were randomized to receive intermittent interferon at doses of 2 or 5 MU/m² t.i.w. Patients assigned to the lower dose who showed increased white blood cell (WBC) counts after 4 weeks or no reduction after 8 weeks were crossed over to the higher dose. Patients receiving the intermittent regimen who achieved a partial haematological response persisting unmodified over 6 months, or an unstable complete haematological response (CHR), were moved to daily interferon administration at doses ranging from 2 to 5 MU/m². The last eight newly-diagnosed patients admitted to the study were started with daily interferon.

CML patients in late chronic phase

This group consists of 46 patients previously treated with conventional chronic phase chemotherapy for over 12 months (median 32 months, range 13-97 months). Of these, 10 patients whose disease was well controlled by conventional chemotherapy (nearly normal blood counts, minimal or absent splenomegaly, 100% Ph¹-positive marrow metaphases) were switched to daily interferon alone at doses ranging from 3 to 9 MU/day (median 4 MU/day). The other 36 patients who showed only partial haematological response to chemotherapy received interferon (median dose 3.5 MU/day, range 2-10 MU/day) in addition to ongoing HU (0.5 to 2 g/day, median dose 1 g/day).

CML patients in accelerated phase

Nine patients in accelerated phase had interferon 5 MU/m² t.i.w. concurrently with chemotherapy (HU ± 6-mercaptopurine (6-MP)) and, in the case of partial haematological response or unstable CHR, were crossed over to the daily schedule.

Criteria for response.

CHR was defined as the normalization of peripheral blood counts and disappearance of all clinical symptoms and signs of disease, including splenomegaly, with persistence of Ph¹-positive mitoses in the bone marrow. Partial haematological response (PHR) was defined as the reduction of WBC count by more than 50% of pretreatment levels (to less than 20 x 10⁹/L), and reduction by more than 50% of splenomegaly. Complete remission is defined as haematological and clinical remission with complete eradication of the Ph¹-positive marrow metaphases.

RESULTS

CML patients in early chronic phase

Of 71 patients, 41 (58%) obtained CHR in a median time of 3 months. In this subset of patients in early chronic phase the response rate did not differ significantly according to previous treatment, CHR being achieved in 22/35 (63%) of previously untreated and in 19/36 (53%) of pretreated patients (Table 1).

Table 1. Overall results of treatment with interferon alfa-2b as a single agent in early chronic phase CML patients

	Total patients *n*	CHR *n* (%)	Ph¹ mosaicism *n* (%)	Ph¹ mitoses median (%) (range)
Untreated	35	22 (63)	19 (54)	58 (15-95)
Pretreated < 12 months	36	19 (53)	15 (41)	76 (0-95)
Total	71	41 (58)	34 (48)	66 (0-95)

As shown in Table 2, the complete response rate increased after

Table 2. Response to interferon alfa-2b in early chronic phase CML according to interferon dose and schedule

Schedule	Dose (MU/m²)	Total patients *n*	CHR *n*	CHR (%) *n*	Patients crossed over *n*
Intermittent	2	33	8	(24)	
	5	30	14		
				(41)*	
			7		21
Daily	2→5	8	7		
				(71)*	
			5		9
Total		71	41	(58)	

*Expressed as the percentage of patients showing CHR from the original patients in the group plus those crossed over into the group.

escalation of intermittent interferon from lower to higher doses and after crossing the patients from intermittent to daily administration. When previously untreated patients are divided among the three risk categories of the prognostic classification of Sokal *et al.* [19], the rate of response to interferon appears to be influenced significantly by the initial risk status (Table 3).

Table 3. Effect of initial risk status on response to interferon alfa-2b in previously untreated CML patients

Risk category*	Total patients *n*	CHR *n*	(%)
Low risk	16	14	(87)
Intermediate risk	12	7	(58)
High risk	7	1	(14)
			$P < 0.01$

* According to Sokal *et al.* [19]:
Relative risk: Exp 0.0116 (age - 43.4) + 0.0345 (spleen - 7.51)

$$+ 0.188 \left[\left(\frac{\text{plts}}{700}\right)^2 - 0.563\right] + 0.0887 \text{ (\% blasts - 2.10)}$$

Low risk = relative risk < 0.8;
Intermediate risk: relative risk = 0.8 to 1.2;
High risk = relative risk > 1.2

A cytogenetic improvement was observed in 34/71 patients (48%), the median percentage of Ph¹-positive metaphases declining from 100% to 66% (range 0-95) (Table 1). The complete suppression of Ph¹ chromosome observed in one case after 14 months of treatment was confirmed by Southern blot analysis.

CML patients in late chronic phase

Of the entire group of 46 patients, 32 (70%) obtained CHR in a median time of 4 months. Of 10 patients with the disease well-controlled by chemotherapy, all achieved a stable CHR with interferon alone, with normal blood counts and absence of splenomegaly in a median time of 2 months. In seven of them Ph¹-positive mitoses declined from 100% to a median of 72% (range 14-90%) after a median time of 13 months. Of the remaining 36 patients with only partial haematological control, 22 (61%) obtained stable CHR in a median time of 8 months after receiving combination of interferon with low-dose HU. Of 16 cytogenetically evaluable CHR patients, nine showed some degree of Ph¹ mosaicism (median Ph¹-positive cells 75%, range 18-90%) after a median time of 14 months (Table 4).

Table 4. Overall results of treatment with interferon alfa-2b ± HU in late chronic phase CML patients

	Total patients *n*	CHR *n* (%)	Ph¹ mosaicism *n*	Ph¹ mitoses median % (range)
Patients with stable CP (interferon alone)	10	10 (100)	7/10	72 (14-90)
Patients with unstable CP (interferon + HU)	36	22 (61)	9/16*	75 (18-90)

*No. patients tested from total.
CP = Chronic phase

CML patients in accelerated phase

Of the nine patients in accelerated phase receiving interferon plus chemotherapy, one attained CHR and two PHR. In these three patients the concurrent chemotherapy dosage was reduced on starting interferon, and could be completely discontinued after 8 months in the single case showing CHR. This patient had a transient reduction of Ph¹-positive marrow metaphases from 100% to 71% at the eighth month of treatment.

Follow up

After a median follow up of 36 months, 15 of the 41 early chronic phase patients who had achieved CHR with interferon remain in continuous disease control on interferon; 12 underwent autologous BMT; two received allogeneic BMT; four were taken off study for toxicity or late resistance to interferon; and eight developed blastic transformation (BT). BT occurred in 17 of 30 patients (57%) who did not respond or responded only partially to interferon, compared to eight of 41 patients (19%) who achieved CHR ($P < 0.01$) (Table 5).

*Table 5. Outcome of 71 CML patients in **early chronic phase** treated with interferon alfa-2b as a single agent at a median follow up of 3 years*

Response to interferon	Total patients *n*	On interferon	Autologous BMT	Allogeneic BMT	Off study	BT
CHR	41	15	12	2	4	8
						$P < 0.01$
PHR + NR	30	2	1	4	6	17

NR = No response

After a median follow up of 22 months, 26 of 32 late chronic phase patients achieving CHR are still on interferon (24 in CHR and two in PHR); one underwent autologous BMT and one allogeneic BMT; three were taken off study because of toxicity and one developed BT. Of the 14 patients obtaining less than CHR, five developed BT ($P < 0.01$) (Table 6).

Overall, 13 patients treated with interferon for a median of 21 months and showing varying degrees of Ph¹ mosaicism (median 65%, range 16-93%) were submitted to autologous BMT. Two

*Table 6. Outcome of 46 CML patients in **late chronic phase** treated with interferon alfa-2b ± HU at a median follow up of 22 months*

Response to interferon	Total patients *n*	On interferon	Autologous. BMT	Allogeneic. BMT	Off study	BT
CHR	32	26	1	1	3	1
						$P < 0.01$
PHR + NR	14	5	—	1	3	5

show complete suppression of the Ph^1 clone after a median of 12 months from BMT [20,21].

Toxicity

Apart from flu-like symptoms occurring transiently in the majority of patients at the start of interferon therapy, other relatively frequent side effects requiring dose reduction or temporary discontinuation were: thrombocytopenia, hepatotoxicity, leucopenia, neurotoxicity, minimal to moderate hair loss, skin desquamation, and weight loss. All these side effects were mild-to-moderate and their incidence and severity were related to the interferon dose [14]. Leucopenia and thrombocytopenia occurred more frequently during combination therapy with interferon plus HU, leading to a dose reduction of both interferon and HU in most patients [18].

DISCUSSION

This report shows that interferon alfa-2b induces a haematological response and cytogenetic improvement in a substantial proportion of chronic phase CML patients.

In CML patients in early chronic phase the response rate was influenced significantly by the interferon dose and schedule. In fact, daily administration of interferon proved to be more effective than the intermittent schedule [12, 14]. Pretreatment with conventional chemotherapy for less than 12 months did not influence response. The risk status at diagnosis, assessed according to the Sokal *et al.* criteria [19], appeared critical in determining the success of interferon therapy (87% complete response rate in low-risk versus 14% in high-risk patients).

In CML patients in late chronic phase disease who were well controlled by chemotherapy, interferon alone was able to maintain completely normal blood counts and to induce some degree of Ph^1 suppression. In heavily pre-treated patients with only partial response to chemotherapy, the combination of interferon with HU was effective in inducing a stable haematological response in 60% of cases and Ph^1 mosaicism in a substantial proportion of responders. This indicates a synergism between interferon and this cytotoxic agent.

In our experience, interferon as a single agent seems an effective treatment for CML patients with low-risk disease. However, more prolonged observation and comparison with conventionally treated patients of the same risk category will assess whether these haematological and cytogenetic results will be translated into improved survival and postponement of blastic transformation. As observed also by others [10], in most patients with intermediate- and high-risk CML, interferon alone seems of limited benefit. In these cases [10], as well as in those in late chronic phase [18], the combination of interferon with chemotherapy may improve haematological and cytogenetic results.

Another promising approach is the combination of interferon with autologous BMT [20,21]. In this modality, interferon can be used both before and after autologous reinfusion of Ph^1-negative or partially negative marrow cells. Through the reciprocal potentiation of the two procedures, a greater proportion of patients may achieve and maintain suppression of the leukaemic clone. Continued follow up of patients submitted to autologous transplant will give information on the long-term effect of this combined approach.

These observations suggest that to maximize the benefit from its anti-neoplastic potential in CML, interferon should be integrated with other anti-neoplastic therapies. This issue constitutes an important area to be explored in future studies.

1. Champlin RE, Golde DW. Chronic myelogeneous leukemia: recent advances. *Blood* 1985, **65**, 1039-1047.
2. Lazzarino M, Morra E, Brusamolino E *et al.* Hydroxyurea and 6-mercaptopurine in the treatment of chronic granulocytic leukemia. *Am J Clin Oncol (CCT)* 1986, **9**, 45-49.
3. Cunningham I, Gee T, Dowling M *et al.* Results of treatment of Ph^1 chronic myelogenous leukemia with an intensive treatment regimen (L-5 protocol). *Blood* 1979, **53**, 375-395.
4. Kantarjian HM, Vellekoop L, McCredie KB *et al.* Intensive combination chemotherapy (ROAP 10) and splenectomy in the management of chronic granulocytic leukemia. *J Clin Oncol* 1985, **3**, 192-200.
5. Goldman JM, Perley JF, Jones L *et al.* Bone marrow transplantation for patients with chronic myeloid leukemia. *N Engl J Med* 1986, **314**, 202-220.
6. Talpaz M, McCredie B, Mavligit GM *et al.* Leukocyte interferon induced myeloid cytoreduction in chronic myelogenous leukemia. *Blood* 1983, **62**, 689-692.
7. Talpaz M, Mavligit GM, Keating MJ *et al.* Human leukocyte interferon to control thrombocytosis in chronic myelogenous leukemia. *Ann Intern Med* 1983, **99**, 789-792.
8. Talpaz M, McCredie K, Kantarjian H *et al.* Chronic myelogenous leukemia: hematologic remissions with alpha interferon. *Br J Haematol* 1986, **64**, 87-95.
9. Talpaz M, Kantarjian H, McCredie K *et al.* Clinical investigation of human alpha interferon in chronic myelogenous leukemia. *Blood* 1987, **69**, 1280-1288.
10. Talpaz M, Kantarjian H, Kurzrock R *et al.* Update on therapeutic options for chronic myelogenous leukemia. *Semin Hematol* 1990, 27 **(Suppl 4)**, 31.
11. Niederle N, Kloke O, May D *et al.* Treatment of chronic myelogenous leukemia with recombinant interferon alfa-2b. *Invest New Drugs* 1987, 5 **(Suppl)**, 19-25.
12. Morra E, Alimena G, Liberati AM *et al.* Recombinant alpha-2 interferon in the treatment of chronic myelogenous leukemia (CML). *Proc Am Soc Clin Oncol* 1987, **6**, 145 (Abstract 571).
13. Gastl G, Aulitzky W, Tilg G *et al.* Recombinant Interferon α-2 for treatment of chronic myelogenous leukemia in benign and accelerated phase. *J Interferon Res* 1987, 7, 737.
14. Alimena G, Morra E, Lazzarino M *et al.* Interferon alpha-2b as therapy for Ph^1-positive chronic myelogeneous leukemia: a study of 82 patients treated with intermittent or daily administration. *Blood* 1988, **72**, 642-647.
15. Freund M, Von Wussow P, Diedrich H *et al.* Recombinant human interferon (IFN) alpha-2b in chronic myelogenous leukaemia. Dose dependency of response and frequency of neutralizing antibodies. *Br J Haematol* 1989, **72**, 350.
16. Baccarani M, Russo D, Zuffa E *et al.* An update of the multicentre study of Roferon-A versus conventional chemotherapy in chronic myeloid leukemia. Fourth International Conference on Malignant Lymphoma, June 6-9, 1990, Lugano. Satellite symposium on *"New Aspects on Interferon Therapy in Haematological Malignancies and Lymphomas"* 1990, 159.
17. Ozer H, Dear K, Testa J *et al.* Prolonged administration of subcutaneous α-interferon induces major clinical and complete cytogenetic remissions in untreated Philadelphia positive (Ph^1+) chronic myelogenous leukemia. *Proc Am Soc Clin Oncol* 1990, 203a.
18. Morra E, Alimena G, Lazzarino M *et al.* Interferon alpha-2b alone or in combination with hydroxyurea as treatment for Ph-positive chronic myelogenous leukemia in late chronic phase. Proceedings Meeting ASH, Boston, *Blood* 1990, **76** (Suppl 1), 302a.
19. Sokal JE, Baccarani M, Tura S *et al.* Prognostic discrimination in good-risk chronic granulocytic leukemia. *Blood* 1984, **63**, 789-799.
20. Meloni G, De Fabritiis P, Alimena G *et al.* Autologous bone marrow or peripheral blood stem cell transplantation for patients with chronic myelogenous leukemia in chronic phase. *Bone Marrow Transplant* 1989, 4 **(Suppl 4)**, 92-94.
21. Alimena G, Morra E, Lazzarino M *et al.* Interferon alpha-2b as therapy for patients with Ph^1 positive chronic myelogenous leukemia. *Eur J Haematol* 1990, 45 (Suppl 52),25-28.