10-30 x 10^3/μl, 1g; and 5-10 x 10^3/μl, 0g), or HU alone. Thus, patients received no HU until their WBC count rose above 5-10 x 10^3/μl. Eleven of 12 patients on IFN plus HU achieved a haematological response, including nine with complete haematological remission (CHR) (A1: $n = 0$, A2: $n = 4$, A3: $n = 3$, A4: $n = 2$) and two with partial haematological remission (PHR) (B1: $n = 0$, B2: $n = 2$), while none of the nine patients on HU alone achieved a remission with the above-mentioned doses of HU according to our criteria (A[CHR]: WBC count maintained below 9 x 10^3/μl without blast and no symptoms or signs associated with CML; subclassified according to the percentage of Ph^1 chromosome: A1, Ph^1 chromosome present in all analyzable metaphases; A2, Ph^1 chromosome present in 35-59%; A3, 5-34%; A4, Ph^1 chromosome absent from all analyzable metaphases. B[PHR]: B1, reduction of peripheral WBC count by at least 50% to < 20 x 10^3/μl; B2, normalization of peripheral WBC count but persistence of immature form or clinically palpable splenomegaly. Failure: patients who failed to achieve a PHR or CHR as defined above). Using two sets of primer pair sequences encoding bcr-abl mRNA (A primer: 5'-GGAGCTGCAGATGCTGACCAAC-3' encoding bcr exon II; B primer: 5'-TCAGACCCTGAGGCTCAAAGTC-3' encoding abl exon II; A' primer: 5'-CCTGATCTCCTCTGACTATGAG-3' encoding bcr exon I; B' primer: 5'-TCCAGCGAGAAGGTTTTCCTTG-3' encoding abl exon I), we performed the cDNA-PCR analysis, which revealed persistent bcr-abl mRNA expression in the peripheral mononuclear cells from two patients who achieved CHR induced by IFN. We suggest that IFN therapy should be continued at least until molecular remission is achieved.

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Interferon Therapy for Ph^1-positive Chronic Myelogenous Leukaemia Patients Relapsing After T-cell Depleted Allogeneic Bone Marrow Transplantation

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INTRODUCTION

A STUDY was undertaken to investigate the effectiveness of alpha interferon in the management of Ph^1-positive chronic myelogenous leukaemia (CML) patients relapsing after T-cell depleted allogeneic bone marrow transplantation (BMT).

PATIENTS AND METHODS

Twenty patients with a median age of 35 years (range 11 to 42 years) were entered into the study, including 17 in chronic phase, two in accelerated phase, and one in blastic phase. All patients had undergone allogeneic BMT a median of 10 months (range 3 to 44 months) from diagnosis of CML. Five patients had haematological relapse and 15 had cytogenetic relapse following BMT. Patient characteristics at the time of relapse are shown in Table 1.

On relapse, patients were treated with recombinant interferon alfa-2b 5 million units (MU) subcutaneously three times a week. In non-responding patients, the schedule was altered to daily dosing and the dose was adjusted according to haematological response and side effects.

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Table 1. Patient characteristics at time of relapse

	Type of relapse			
	Haematological		Cytogenetic	
Patients *n*	5		15	
Months from BMT to relapse:				
median (range)	12	(8-24)	7	(4-18)
Cytogenetic examinations from BMT to relapse:				
total *n*	20		90	
n/pt (mean)	4		6	
time intervals (days)	120		45	
Months from relapse to IFN therapy:				
median (range)	1.5	(0.5-5)	3.25	(0-10)
Cytogenetic examinations from relapse to IFN therapy:				
total *n*	5		54	
n/pt (mean)	1		3.6	
Percentage of Ph^1-positive metaphases:				
At time of relapse: median (range)	100	(90-100)	30	(8-90)
At time of IFN start: mean (range)	100	(90-100)	50	(9-100)

RESULTS

Response to interferon

Within 2 to 3 months after starting alpha interferon treatment, all five patients with haematological relapse had achieved haematological remission (HR), which lasted for a median of 16 months (range 3 to 16 months) (Table 2). Alpha interferon was given for a median of 18 months (range 6 to 19 months). At 6 to 48 months from relapse, three patients were alive (one in HR and two with chronic-phase disease) and two had died following development of acute myeloid leukaemia blast crisis.

Among the 15 cytogenetic relapsers, seven (47%) were still in HR a median of 37 months (range 3 to 45 months) after starting interferon, including two that also regained cytogenetic remission (CR) (Table 2). The other eight patients (53%) progressed to haematological relapse after 21 months (range 6 to 46 months). At 3 to 45 months after cytogenetic relapse, all seven of the patients maintaining HR were still alive, while four of the eight haematological relapsers had died following development of blast crisis.

Table 2. Type of relapse

	Haematological relapse	Cytogenetic relapse/ maintaining HR	Cytogenetic relapse/ progressing to HR	Total cytogenetic relapse
Patients *n* (%)	5	7* (47)	8†(53)	15
Patients achieving HR *n*	5			
Months from start IFN to HR: median (range)	3 (2-3)			
Months in HR: median (range)	16 (3-16)	37 (3-45)	21 (6-46)	33 (3-46)
Months on IFN therapy: median (range)	18 (6-19)	32 (2-39)	21 (6-40)	26 (2-40)
Months from haematological relapse: median (range)	43 (6-48)			
Months from cytogenetic relapse: median (range)		37 (3-45)	28.5 (12-48)	34(3-48)
Alive/dead	3 ≠ /2**	7/10	4/4	11/4

HR = haematological remission.
* Cytogenetic remission.
† Four chronic phase, four blast crisis (two ALL, one mixed, one ANLL).
≠One haematological remission, two chronic phase.
** Two AML - blast crisis.

Table 3. Cytogenetic analysis in two patients achieving cytogenetic remission on alpha interferon therapy

Patients. *n.* (sex D/R)	BM cells	Percentage of metaphases							
16	Ph^1 pos	50	10	45	ND	0		Cytogenetic	0
(M/M)	Ph^1 neg	50	90	55	ND	100		remission	100
21	Ph^1 pos	50	50	35	50	10	0	Cytogenetic	0
(M/F)	Ph^1 neg/D	50	50	65	50	90	100	remission	100
	Ph^1 neg/R	0	0	0	0	0	0		0
Months on IFN		0	2	4	6	8	10	36	45

ND = not done; D = donor; R = recipient.

Cytogenetic analysis

Molecular studies were performed on bone marrow samples of the two patients achieving CR on alpha interferon. Table 3 shows the percentage of Ph^1-positive metaphases at different stages in these two patients. Southern blot hybridization of the m-bcr showed, in both cases, total disappearance of the rearranged bands detected at diagnosis (Fig. 1a). In patient number 21, however, amplification of the bcr-abl mRNA junction using the polymerase chain reaction (PCR) technique revealed the presence of residual disease at the time that interferon was discontinued (Fig. 1b). Evidence of chimeric haemopoiesis was obtained by cytogenetic analysis in patient 21 (who had a sex-mismatched donor) and by comparing donor and patient DNA hypervariable polymorphic patterns in patient number 16 (sex-matched donor) (Fig. 1c).

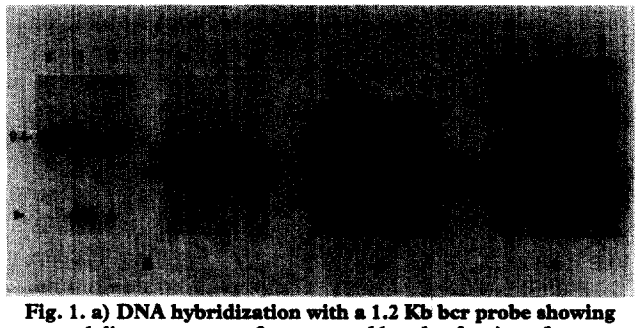

Fig. 1. a) DNA hybridization with a 1.2 Kb bcr probe showing total disappearance of rearranged bands after interferon treatment in patients 16 and 21 (lanes 1 and 2, respectively). b) Detection of bcr-abl junction after PCR mRNA amplification and hybridization with a specific oligonucleotide probe in patient 21. c) Chimeric haemopoiesis demonstrated in patient 16 with a hypervariable mini-satellite DNA probe (33.15) showing identical polymorphic bands in donor (lane 2) and recipient cells (lanes 1 and 3).
c = Control samples. K562 leukaemic DNA was utilized as positive control in PCR studies. Rearranged bands are indicated by the arrows.

Survival and remission duration

Kaplan-Meier curves reveal a 0.40 probability of survival in all patients 60+ months from BMT (Fig.2). Survival at 60+

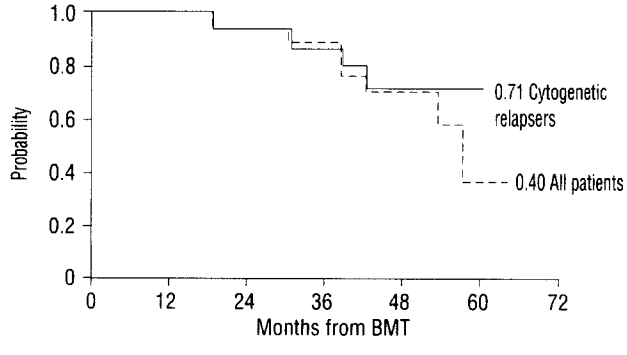

Fig. 2. Response to post-BMT alpha interferon therapy: Kaplan-Meier survival curves in all relapsing patients (haematological and cytogenetic relapse) and in patients with cytogenetic relapse following T-cell depleted allogeneic BMT.

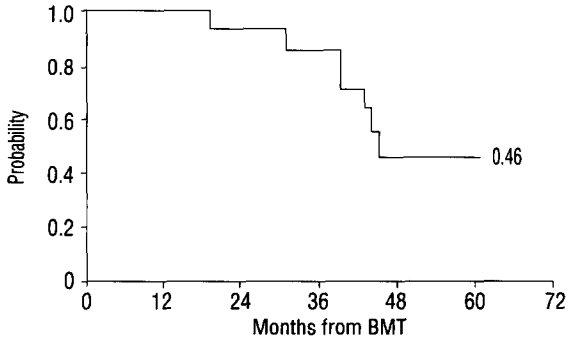

Fig. 3. Response to post-BMT alpha interferon therapy: continuous haematological remission in CML patients with cytogenetic relapse following T-cell depleted allogeneic BMT (Kaplan-Meier curve).

months was better in cytogenetic relapsers (Fig. 2), of whom nearly half also remained in continuous HR (Fig. 3).

CONCLUSIONS

These results suggest that alpha interferon is an effective alternative to conventional chemotherapy in CML patients with haematological relapse after BMT, and that alpha interferon should be considered the treatment of choice for CML patients with a persistent cytogenetic relapse occurring after BMT. A second BMT should be considered only in patients with haematological relapse not responding to interferon therapy. Taking into account the high percentage of Ph^1-positive cells, their persistence and increase during the follow up and, finally, the progression towards haematological disease in some cases, the cytogenetic relapse cannot be considered "transient" in our study.

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Treatment of Ph^1-Positive Chronic Myelogenous Leukaemia with Recombinant Interferon Alfa-2b: A Case Report of Complete Cytogenetic Response

Shigeru Shirakawa, Tohru Kobayashi, Kazuhiro Nishii and Kenkichi Kita

ABSTRACT

A STUDY was undertaken in which eight patients aged 22 to 80 years with Ph^1-positive chronic myelogenous leukaemia (CML) were treated with interferon alfa-2b at an initial dose of 4 million units (MU)/m^2 per day.

One patient in accelerated phase was pretreated with vindesine and prednisone, and showed an increase in white blood cell count during interferon treatment. In addition, one of the seven patients in chronic phase, who was previously untreated, dropped out because of skin eruption. According to the response criteria of Alimena *et al.* [1], three of the other six patients in chronic phase achieved haematological response (two complete, one partial) and two had cytogenetic improvements (one complete, one minor).

Adverse effects included fever (six patients), malaise (two), anorexia (one), delirium (one), liver disorder (two) and skin eruption (one).

We present here the pre-treatment features and clinical course of a patient who achieved complete cytogenetic response (CCR). A 32-year-old female patient, admitted to hospital because of marked hepatosplenomegaly (spleen 11 cm under the navel) and anaemia, was diagnosed as having Ph^1-positive CML and treated with interferon alfa-2b 6 MU/body daily for 4 months and twice weekly thereafter. Her pretreatment blood cell counts were as follows: red blood cells 2.17 x 10^6/μL; haemoglobin 6.6 g/dL; white blood cells (WBC) 206,700/μL; and platelets 610 x 10^3/μL.

The patient achieved complete haematological response [1] at the 32nd week of interferon administration, together with resolution of hepatosplenomegaly.

Cytogenetic and molecular biological analyses revealed partial suppression of the Ph^1 chromosome at the 38th week (75% Ph^1-positive) and complete cytogenetic response (CCR, 0% Ph^1-positive) at the 140th week of interferon treatment.

In conclusion, long-term administration of interferon alfa-2b alone induced complete suppression of the Ph^1 chromosome in one patient with Ph^1-positive CML, and the duration of this CCR is more than 4 months. The combination of alpha interferon with other treatments in order to enhance cytogenetic improvement should be investigated.

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