

Delayed cytogenetic response with prolonged interferon- α treatment in chronic myeloid leukemia patients: quantification of BCR-ABL transcript by competitive reverse transcription-polymerase chain reaction

Interferon- α (IFN) induces a major reduction of the Philadelphia chromosome (Ph) positive clone in about 40% of patients with chronic myelogenous leukemia (CML) in chronic phase (CP) and in a substantial minority a complete cytogenetic response (CCyR), predicting a favorable outcome.^{1,2} Most of the major CyR are obtained within one year from starting therapy; we present data concerning patients in whom late major and complete CyR were achieved after prolonged treatment with IFN.

Twenty-two CML patients in CP diagnosed between September 1986 to December 1993 (9M/13F; median age 51 years, range 30-69) were treated with IFN. They had received minimal or no treatment before IFN. The percentage of Ph negative metaphases (at least 25 metaphases examined if possible, but fewer in the case of insufficient metaphases as often happens in IFN-treated CML patients) was used to classify the response as major (67-99%) or complete (100%).² Any major CyR obtained more than 14 months after starting IFN therapy was considered a delayed response. The detection of BCR/ABL transcript by reverse transcription polymerase chain reaction

(RT-PCR) and competitive RT-PCR was performed as described elsewhere.³⁻⁵ The 22 patients treated with IFN had an estimated median survival of 114 months (range 14-168) and at 5 years the overall survival probability was 61% (95% C.I., 41% to 82%). Fifteen of the 22 IFN-treated patients did not have a cytogenetic response. Twelve patients (11 cytogenetically non-responders) died of blastic crisis after a median of 41 months (range 14-131). One patient (#7) had a major CyR after 26 months, maintained it for 37 months and then became resistant and died. Ten of the IFN-treated patients are still alive in CP (median observation time 115 months; range 92-168); 4 of them are cytogenetically non-responders and alive after 93, 100, 120 and 130 months (Sokal's score:⁶ 1.16, 0.79, 0.69, 0.93), the other 6 patients reached a CCyR. Seven patients (32%) (Table 1) reached the major and/or complete CyR, achieved as a delayed response (> 14 months) in six cases in which the IFN treatment has been prolonged at the maximum tolerated doses even in the absence of major CyR in the first 14 months of IFN therapy. It should be noted that the maximum tolerated doses in five cases corresponded to a low daily dosage⁷ when the major CyR was obtained. The response achieved by patient #2 is one of the most delayed described to date.⁸ Only one of the 7 patients (#5) reached an early CCyR after 10 months of IFN therapy. The 6 Ph negative patients proved BCR-ABL positive by PCR assay.^{9,10} During the molecular follow-up two patients (#1 and #5) became PCR negative (Figure 1) 122 months after beginning IFN therapy (74 months after Ph negativity) and after 36 months of IFN therapy (25 months after Ph negativity), respectively. Patient #1 is still PCR negative, patient #5 was intermittently PCR pos-

Table 1. Findings at diagnosis, dosage and duration of IFN therapy and time to reach major and/or complete cytogenetic response in CML patients.

Pt	Sex M/F	BCR/ABL L type	Age (years)	Sokal's score	IFN dosage (months)	Follow-up (months)	Cytogenetic response (months since starting IFN therapy)	
							Major	Complete
1	M	b2a2	46	0.62	9MU/die (9) 12MU/die (11) 9MU/die (146+)	168	16	48
2	F	b3a2	56	0.72	9MU/die (9) 3MU/die(138+)	151	61	97
3	F	b2a2	49	0.83	3MU/die (5) 9MU/die (11) 4.5MU/die (102+)	120	22	57
4	M	b3a2	30	0.50	3MU/die (109+)	110	36	60
5	F	b3a2	47	0.69	6MU/die (1) 1.5 MU/die (84+)	94		10
6	F	b2a2	50	0.65	9MU/die (15) 1.5 MU/die (69+)	92	49	59
7	F	b3a2	60	1.03	9MU/die (4) 4.5MU/die (13) 3MU/die (74)	118*	26	

Pt, patient; M, male; F, female; MU/die, MegaUnits (10⁶) per day. *This patient died. The discrepancy between the follow-up and the sum of periods of IFN therapy is due to delay of IFN therapy or to discontinuation of therapy because of intervening side effects.

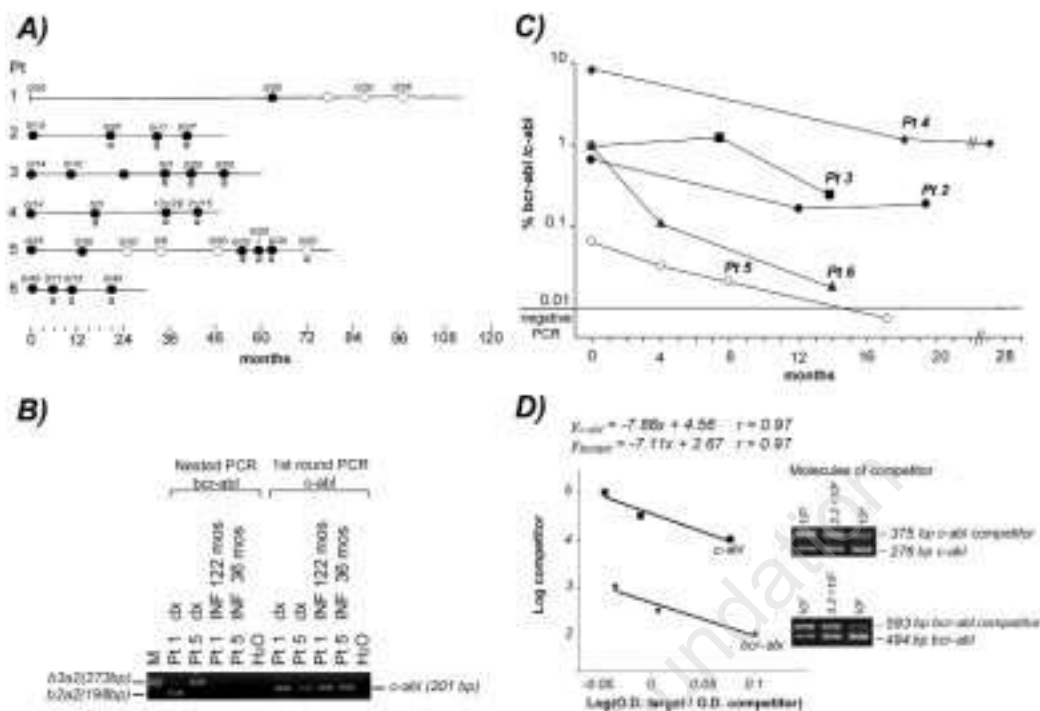


Figure 1. (A) Graphic representation of molecular and cytogenetic follow-up of IFN-treated patients after reaching Ph negativity. The numbers refer to the Ph positive metaphases over the total metaphases analyzed. Q identifies the samples analyzed by competitive PCR. The solid circles denote PCR positivity, the open circles PCR negativity. (B) Nested RT-PCR of BCR-ABL transcript present in bone marrow cells of patient #1 and patient #5 at diagnosis and respectively after 122 and 36 months of IFN treatment. (C) Monitoring with competitive PCR of minimal residual disease (samples denoted by Q) of five IFN-treated patients who reached Ph negativity. The BCR-ABL transcript level is expressed as ratio % of number of molecules of BCR-ABL/number of molecules of c-abl. (D) Representative quantification by competitive PCR of BCR-ABL and c-abl transcript level present in bone marrow cells. The regression curves give the equivalence point between the number of molecules of target transcript and competitor. This number, corrected by a factor 1.2 (for b3a2) or 1.36 (for abl) is expressed as number of molecules per μg of RNA.

itive/negative maintaining a low ratio % and then reverted again to PCR negativity. Five of the 6 Ph negative patients were also studied with competitive PCR evidencing a decreasing trend in the BCR-ABL/c-abl ratios % (Figure 1). A ratio % close to 1 or higher occurred, in the majority of cases, in patients who actually had a low number of metaphases and in whom it was not, therefore, possible to evaluate cytogenetics. Patient #4 had a high ratio % although cytogenetically he had 5 Ph negative metaphases out of 5. The ratio %, however, decreased when the patient had 13 Ph positive metaphases out of 29 and the same ratio % was maintained with 2 Ph positive metaphases out of 15.

In conclusion, our series seems to suggest that it could be useful to continue IFN therapy even when a cytogenetic response is not obtained quickly and/or the IFN dose has to be reduced because of side effects. Quantitative PCR shows that, with IFN treatment at the maximum tolerated dose, the level of residual disease, even after many months of CCyR, tends to decrease over time although, of course, more patients are needed to confirm these findings. Patients might become PCR negative or reach a stable plateau at a very low level of residual disease at which

relapse is unlikely, as evidenced by the transplant experience. Competitive PCR in our patients seems to give a better quantitative representation of residual disease when there are too few metaphases for significant evaluation, as often happens in IFN-treated patients.

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Key words: chronic myelogenous leukemia, interferon- α , cytogenetics, BCR-ABL transcript, competitive RT-PCR.

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