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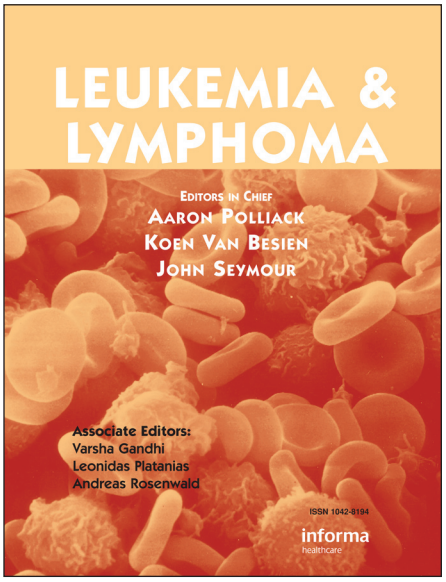

Discontinuation of Alpha-Interferon Treatment in Patients with Chronic Myeloid Leukemia in Long-Lasting Complete Molecular Response

Roberto Latagliata, Angela Romano, Marco Mancini, Massimo Breccia, Ida Carmosino, Federico Vozella, Chiara Montagna, Paola Volpicelli, Federico De Angelis, Luigi Petrucci, Alessandra Serrao, Matteo Molica, Adriano Salaroli, Daniela Diverio, Giuliana Alimena

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ABSTRACT

To evaluate the follow-up after α-interferon (IFN) discontinuation, 23 patients with Chronic Myeloid Leukemia (CML) in stable Complete Molecular Response (CMolR) with IFN were revisited. After a median IFN treatment of 105.8 months (IR 56.1 – 127.3), all patients discontinued IFN for prolonged CMolR (12), intolerance (8) or planned ABMT (3). After 12.5 months, 1 patient developed an extramedullar blast crisis. Four patients needed to start imatinib, all achieving again molecular response. Eighteen patients are still off-therapy (median time from IFN discontinuation 125.5 months, IR 86.9 – 205.3); among these, 5 resulted always BCR-ABL negative, 6 presented a sporadic positivity (BCR-ABL ratio < 0.1) and 7 showed a stable and long-lasting mild positivity (BCR-ABL ratio < 0.5) . *P*atients in prolonged CMolR with IFN have low risk of recurrence after discontinuation; the reappearance of a BCR-ABL positivity < 0.5 did not precede always a relapse, suggesting mechanisms of immunological control induced by IFN.

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Short title: IFN stop in Chronic Myeloid Leukemia

ABSTRACT

To evaluate the follow-up after α-interferon (IFN) discontinuation, 23 patients with Chronic Myeloid Leukemia (CML) in stable Complete Molecular Response (CMolR) with IFN were revisited. After a median IFN treatment of 105.8 months (IR 56.1 – 127.3), all patients discontinued IFN for prolonged CMolR (12), intolerance (8) or planned ABMT (3). After 12.5 months, 1 patient developed an extramedullar blast crisis. Four patients needed to start imatinib, all achieving again molecular response. Eighteen patients are still off-therapy (median time from IFN discontinuation 125.5 months, IR 86.9 – 205.3); among these, 5 resulted always BCR-ABL negative, 6 presented a sporadic positivity (BCR-ABL ratio < 0.1) and 7 showed a stable and long-lasting mild positivity (BCR-ABL ratio < 0.5)**. P**atients in prolonged CMolR with IFN have low risk of recurrence after discontinuation; the reappearance of a BCR-ABL positivity < 0.5 did not precede always a relapse, suggesting mechanisms of immunological control induced by IFN.

Keywords: Chronic Myeloid Leukemia, α-interferon, treatment discontinuation

INTRODUCTION

Before the advent of Tyrosine-kinase inhibitors (TKI), treatment with alpha-interferon (IFN) as first line therapy was widely employed in patients with Chronic Myeloid Leukemia (CML), allowing the achievement of Complete Cytogenetic Response (CCyR) in about 25 – 30% cases and prolonged Complete Molecular Response (CMolR) in some of these cases (< 10%) (1-2).

With the introduction of TKI, IFN was replaced by imatinib in most of the patients (3-5). However, IFN administration was continued in those CML patients who had obtained a prolonged cytogenetic or molecular response. In many of these cases, treatment was successively discontinued for various reasons without further treatment being applied.

While some studies addressing the follow-up of patients who discontinued IFN while in CCyR reported a relapse rate > 50% (6-7-8), nowadays nothing is known about clinical trend for patients who discontinued IFN while in CMolR, and the literature only presents description of sporadic case reports (9) or meeting reports (10).

The aim of our study was the evaluation of a relatively large number of cases in order to describe their clinical and laboratory follow-up, with a particular focus on the possible reappearance of the disease over time.

MATERIALS AND METHODS

Patients eligibility criteria

We considered eligible for our study all patients affected by CML observed at our institution in Rome from January 1986 to December 2002, presenting the following characteristics:

- Treatment with IFN alone or in combination with AraC or other chemotherapeutic procedures
- Achievement of a CMolR confirmed and stable over time
- Discontinuation of therapy with IFN for any reason, followed only by clinical and laboratory observation.

Cytogenetic and molecular evaluation

Cytogenetic analysis was performed on bone marrow cells using standard G-banding techniques on at least 20 metaphases from direct or short-term (24-48 h) cell cultures.

The exam was performed at the onset of the disease and during the whole period of therapy and observation of patients at variable intervals for each individual case (usually at least two evaluations per year), until a stable CMolR was reached.

Fluorescence in-situ hybridization (FISH) was used in those cases in which less than 20 metaphases were available for the standard cytogenetic analysis and was performed using extra-signal for BCR-ABL in two colors, with double fusion probes.

The quantitative molecular analysis was evaluated (on marrow blood until January 2010 and later on peripheral blood) by polymerase chain reaction (RT-Q-PCR) performed according to standard procedures in order to assess the levels of hybrid BCR –

ABL transcript and the results were expressed as the normalized BCR-ABL/ABL ratio based on the international scale (IS).

The qualitative molecular analysis was evaluated by RT-nested PCR in accordance with the standard procedures for the analysis of fusion genes, as described in the BIOMED-16 report.

Definition of cytogenetic and molecular responses

Molecular and cytogenetic responses were classified according to the internationally accepted standard criteria:

- CCyR was defined as the absence of Ph + metaphases. In patients with less than 20 evaluable metaphases, it was defined by FISH analysis as the presence of positive BCR-ABL interphase nuclei in less than 1%;

- Major molecular response (MMolR) was defined as a BCR-ABL / ABL ratio <0.1

- CMolR was defined in different ways according to the evolution of its evaluation along the time. From 1/1990 to 12/2005 it was defined as percentage of BCR-ABL not quantifiable by RT-nested PCR technique. From 1/2006 to 8/2012, CMolR was defined as a peripheral BCR-ABL/ABL transcript ratio below the detection limit of the RQ-PCR analysis; from 8/2012, CMolR was defined as $CMolR^{4.0}$ (BCR-ABL/ABL ratio $\leq 0.01\%^{IS}$), $CMolR^{4.5}$ (BCR-ABL/ABL ratio $\leq 0.0032\%^{IS}$) and $CMolR^{5.0}$ (BCR-ABL/ABL ratio $\leq 0.001\%^{IS}$).

Statistical methods

Data were expressed as mean ± standard deviation (SD) (continuous data with a normal distribution), as median and

interquartile range (IR) (continuous data with non-normal distribution) or as percentage frequency.

Comparisons between data were made using the Student's t test and the χ test, depending on the case, with significance levels of $p < 0.05$.

The Kaplan-Meier product-limit method was used to estimate univariate survival curves, The overall survival (OS) was calculated from the date of suspension of IFN until death from any cause. The event-free survival (EFS) was calculated from the date of suspension of IFN until any of the following events happened: cytogenetic relapse, molecular relapse, evolution in blast crisis or death from any cause.

All calculations were performed using standard statistical package SPSS for Windows (version 18.0, Chicago, IL).

RESULTS

Patient Characteristics

According to eligibility criteria, 23 patients were included in the present analysis. The most important features at diagnosis are listed in Table I.

IFN therapy

IFN was administered as a single agent in 16 patients and in association with autologous bone marrow transplantation (ABMT) in the remaining 7 patients. In the latter group, the ABMT was performed at the time of diagnosis and then followed by IFN administration in 4 patients or after an initial treatment with IFN in the remaining 3 patients.

Median period of IFN treatment was 105.8 months (IR 56.1 – 127.3). Median time to reach CCyR was 21.4 months (IR 14.4 – 37.4), while median time to reach CMolR was 63.7 months (IR 30.3 – 106.0). Median duration of CMolR before IFN discontinuation was 45.7 months (IR 5.9 – 77.3). All pts discontinued treatment due to medical decision for prolonged CMolR (12 pts), intolerance (8 pts) or planned ABMT (3 pts).

Follow up after IFN suspension

Clinical and laboratory follow-up of the 23 patients after discontinuation of IFN treatment is described in Figure 1.

One patient developed a sudden extramedullary lymphoid blast crisis 12.5 months after discontinuation of IFN treatment and died after 4 months due to progression of the disease, despite treatment with dasatinib and chemotherapy.

Four patients needed to start a new treatment with imatinib (2 cases for cytogenetic relapse after 24.8 and 44.0 months after discontinuation of IFN, respectively, 2 cases for molecular relapse after 39.7 and 39.8 months after discontinuation of IFN, respectively), all four obtaining a new molecular response (MMolR in 1 case and CMolR in 3 cases).

The remaining 18 patients are still off-therapy after a median time of 125.5 months after discontinuation of IFN (IR 86.9 - 205.3); among them, 5 pts have persistently resulted negative at the molecular follow-up, 6 pts have presented a sporadic positivity with bcr-abl ratio always < 0.1 and 7 have shown a

mild rise of transcript levels with a long-lasting stable positivity (bcr-abl ratio < 0,5 without further increments).

At the last molecular evaluation, 11/18 patients were in CMolR ($CMolR^{4.0}$ in 6 and $CMolR^{4.5}$ in 5), 4/18 were in MMolR and 3 had a BCR-ABL ratio between 0.5 and 0.1.

The OS of the 23 patients showed a probability of survival at 5 and 10 years of 95.5%, the only event being death of patient who presented blast crisis.

The EFS is described in Figure 2 and showed a probability of 77.4% at 5 years, with a plateau that is maintained over time (last event recorded was after 39.8 months since discontinuation of IFN treatment).

DISCUSSION

Based on the positive results obtained with a targeted molecular treatment, the ultimate cure of patients with discontinuation of treatment is becoming more and more the therapeutic goal for CML. In order to achieve this goal, it is required to obtain a CMolR that is stable over time. Such result is currently achieved in about 20% of patients treated with TKI (3-5), while it was obtained in < 10% of patients treated with IFN, before the introduction of TKI (1-2).

Data in the literature showed that after the discontinuation of imatinib in patients who achieved a stable CMolR, there was a recurrence of the disease in about 50% of cases (11-13): the loss of MMR rather than the fluctuation of BCR-ABL transcript levels below the MMR threshold seems to be a safe indicator of imatinib

restarting in this subset (14). On the other hand, very little is known about patients who discontinued IFN after reaching a stable CMolR; in fact the small number of these patients made it difficult to collect meaningful data, except for individual case reports (9).

Our data are based on a sufficiently large sample size and show that patients with CML in prolonged CMolR during IFN therapy present, after discontinuation of treatment, a very low risk of relapse, with an EFS at 10 years after the therapy suspension of 77.4 %.

The biological reasons for this data, better than that obtained after suspension of Imatinib, should probably be sought in the activation by IFN of a cell-mediated immunity against leukemic stem cells (15-20) which is not observed after treatment with TKIs. In particular, it has been suggested a role for cytotoxic T-cells with high affinity against the proteinase 3 leukemic antigen, whose expansion was demonstrated only in patients responding to IFN treatment (21).

The concept of acquired specific anti-tumor immunosurveillance, capable of controlling small residues of the disease and, therefore, to achieve "functional" cure of the patient, has already been demonstrated in the hematological context in cases of acute myeloid leukemia with t[8;21] translocation; in fact, the persistence of small amounts of the AML-ETO hybrid transcript gene characteristic of this disease was demonstrated in patients

with long-term survival after chemotherapy or allogeneic bone marrow transplantation (22-23).

It should be emphasized that in many patients of our cohort the occurrence of a BCR-ABL positivity > 0.1% < 0.5% did not precede disease relapse, but it was only sporadic or it remained and remains stable over a long period of observation, supporting the hypothesis of the presence of possible immunological mechanisms of control induced by IFN and the ability of self-maintaining over time.

The evidence of the efficacy of IFN in inducing "functional" cure in patients with CML raises the question of its use in combination with TKI (24) that, nowadays, is the first-line therapy. Larger and randomized studies on the use of IFN as a maintenance therapy after obtaining CMolR with TKI, followed by cessation of treatment are thus warranted to provide information on the best role of IFN in these subjects.

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Table Legends

Table I – Patient clinical features at diagnosis

N° of patients	23
M/F	11/12
Median age (years) (IR)	43.3 (35.8 - 51.3)
Median WBC (x 10^9/l) (IR)	39.9 (24.4 – 67.6)
Median PLTS (x 10^9/l) (IR)	360 (300 – 543)
Sokal risk score: Low Intermediate Not evaluable	 18 3 2

FIGURE LEGENDS

Figure 1 – Follow-up after IFN discontinuation

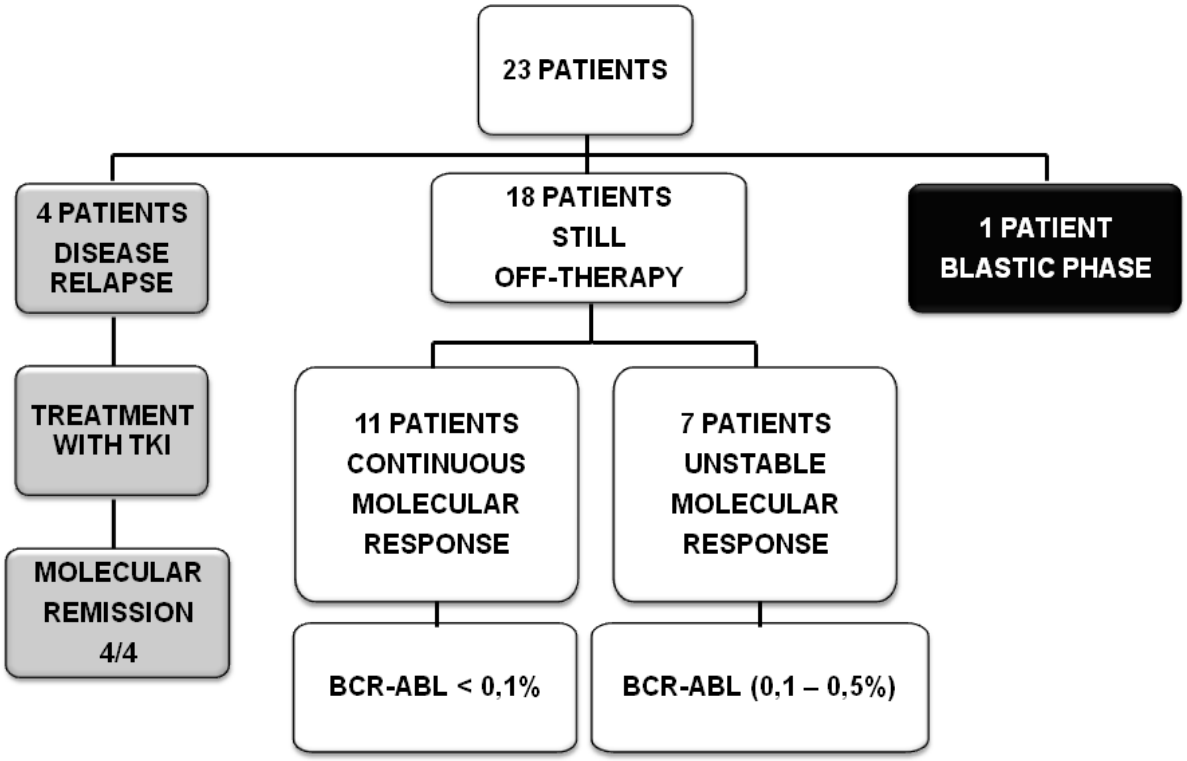

Figure 2 – Event-free survival of the patients after IFN discontinuation