Treatment of Chronic Myelogenous Leukemia with Interferons: Hematologic, Cellular, and Genetic Investigations*

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A. Introduction

Chronic myelogenous leukemia (CML) is a stem cell disorder characterized by the accumulation of immature precursors of the granulocytic and monocytic lineage. Most commonly, the disease is treated with chemotherapeutic agents, preferably busulfan and hydroxyurea. High-dose chemotherapy followed by allogeneic bone marrow transplantation may eradicate the malignant clone and offers the possibility of cure [1, 2].

In recent years, interferons (IFNs) have been successfully used as a novel therapeutic approach [3]. We have therefore started a study using IFN-alpha-2b alone or in combination with IFN-gamma. In addition to the clinical follow-up, the effects of IFNs were scored by molecular analysis and studies made on the growth of myeloid progenitor cells.

B. Materials and Methods

I. Patients' Characteristics

Two groups of patients with chronic-phase CML were treated. In the first group (48 patients), most patients were pretreated, while in the second group (24 patients) all patients were untreated. Of the first group all patients and in the second group 19 patients were evaluable for response.

II. Treatment Schedule

Recombinant human IFN-alpha-2b (Intron A°) with a specific activity of more than 1×10^8 units/mg protein was provided by Schering Inc., Kenilworth, New Jersey, United States. The drug was administered subcutaneously. During the introduction period, the daily dose was 4×10^6 units/m^2 body surface area. The dose was gradually reduced 2–4 weeks after initiation of IFN treatment. After normalization of white blood cell counts, the patients received individual doses ranging from 1×10^6 units every other day to 10×10^6 units daily. Some patients received a combination of IFN-alpha and -gamma at an initial dose of 4×10^6 units/m^2 IFN-alpha-2b and 50 µg IFN-gamma. IFN-gamma was provided by Biogen SA, Geneva, Switzerland, and had a specific activity of $2-4 \times 10^8$ units/mg protein.

III. Response Criteria

Response to IFN treatment was evaluated according to standard criteria [3]:

- Complete remission (CR): normalization of all clinical and hematologic parameters plus complete suppression of the Philadelphia (Ph') chromosome in all analyzable metaphases
- Hematologic remission (HR): a normalization of total and differential leukocyte counts, platelets, serum lactate dehydrogenase levels, and spleen size

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* This work was supported by the Meyer-Struckmann Foundation

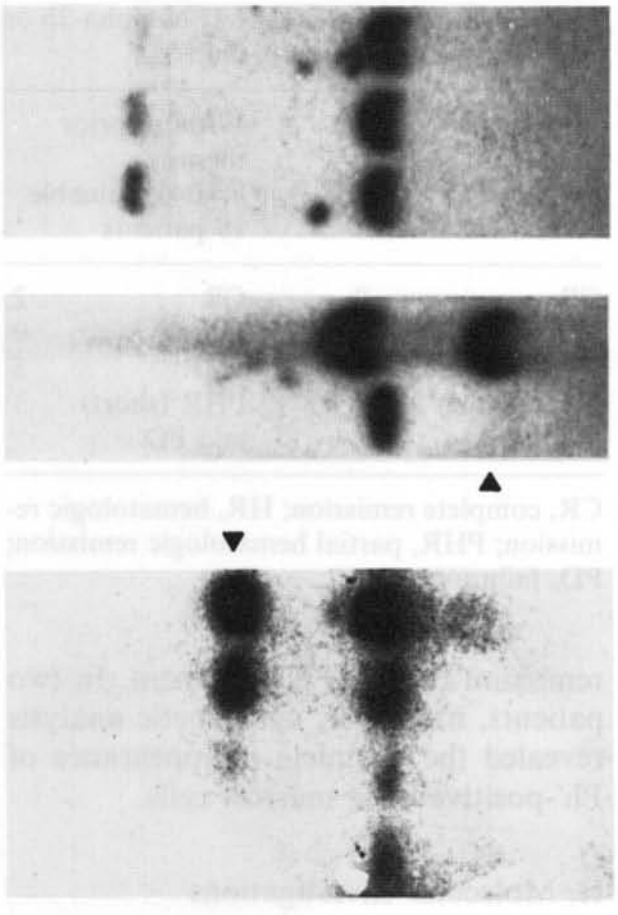

Fig. 1. Southern blot analysis of *bcr* rearrangements of two patients before and during IFN therapy. *First number*, number of patient; *m*, bone marrow; *b*, peripheral blood; *second number*, number of sample. Sample 51m4 was taken 14 months, sample 62b3 9 months, after initiation of therapy. *Arrows* indicate Ph' specific bands. *Bgl*II-digested DNA was hybridized to the 5' *bcr*-specific probe. For control, C, *Pvu*II-digested normal donor DNA was mixed with DNA from a leukemia patient at different ratios.

found in the erythroid compartment BFU-E. One patient showed initially an increase of BFU-E and CFU-GEMM. In this patient, however, all colony types did decrease later on during IFN treatment. Two patients with accelerated phase disease at the beginning of IFN-alpha-2b treatment did not respond to IFN administration. This failure correlated with an increase of granulocytic progenitor cell proliferation in vitro.

D. Discussion

The effects of IFN were evaluated in two groups of patients with chronic-phase CML. In the first group of patients, the minimal necessary dosage to keep patients in hematologic remission was administered as maintenance therapy after induction of remission. Most patients responded to IFN therapy. However, no disappearance or significant reduction of Ph'-positive metaphases was obtained with this therapeutic regimen (for details see [10, 11]). In a second study, therefore, higher maintenance doses were administered. So far, most patients have responded to IFN therapy. In two patients, moreover, a total loss of Ph'-positive bone marrow cells could be demonstrated by cytogenetic and molecular *bcr* analysis with a limit of detection of 1% – 5% [3, 12]. Thus, the more aggressive therapy might perhaps be able to alter the course of the disease. So far, no superiority of combination therapy with IFN-alpha plus IFN-gamma over monotherapy with IFN-alpha has been proven. One has to keep in mind, however, that the number of patients tested is very low and the time of observation rather short.

The stem cell analyses showed a good correlation between the clinical response and the reduction of myeloid progenitor cell proliferation in vitro. Therefore, these in vitro determinations might serve as predictive tests for determining the in vivo response. The availability of the molecular analysis of the Ph' chromosome allows a highly sensitive screening

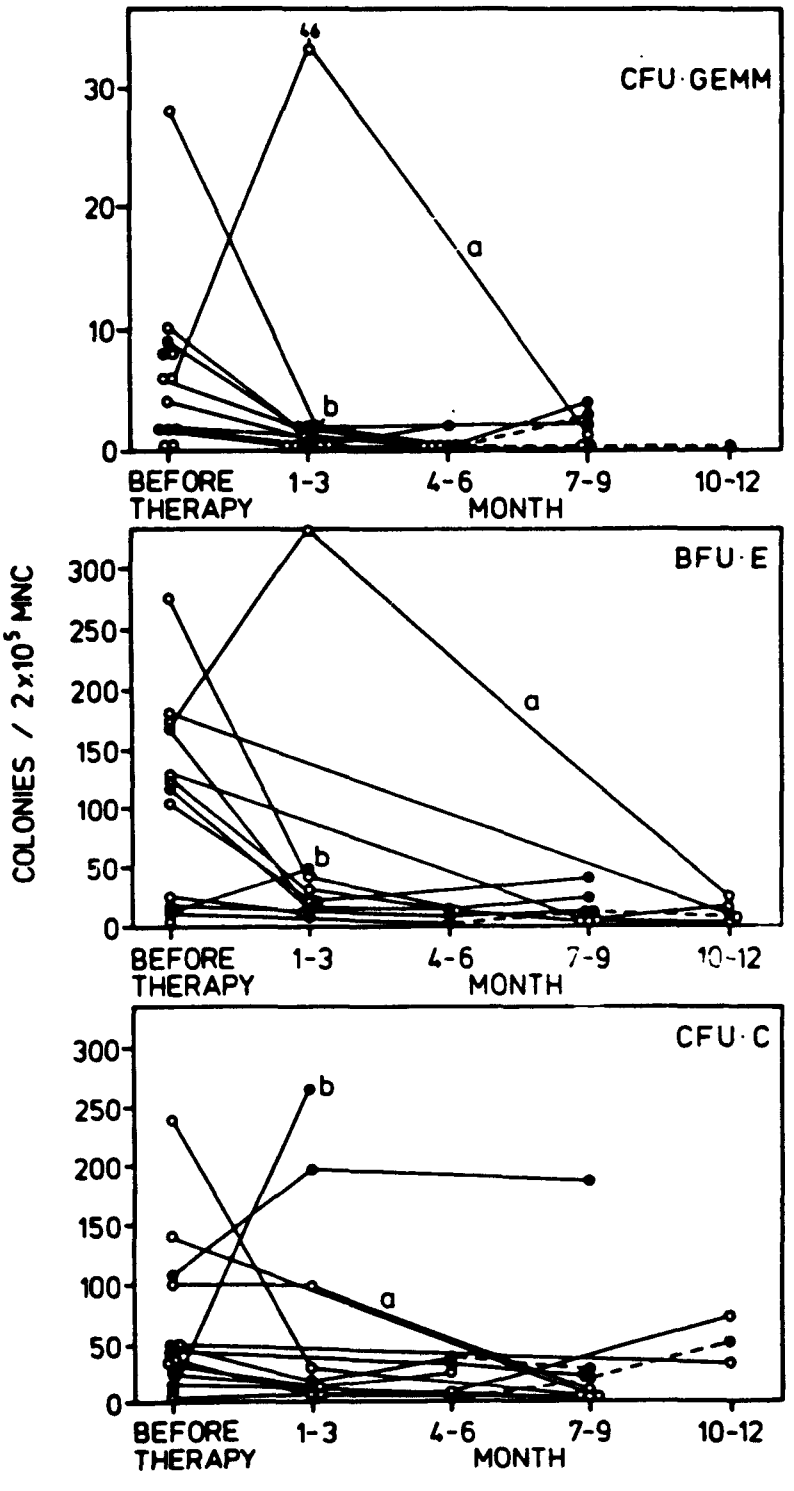

Fig. 2. Colony-forming potential in bone marrow of CML patients undergoing IFN therapy. (Redrawn from [8])

method which is independent of the presence of dividing cells and the preparation of metaphases. Moreover, the recently developed technique of PCR [13] will increase the sensitivity by about three to four orders of magnitude.

Acknowledgments. We thank Ms. C. Oberle, Ms. B. Muss, and Ms. B. Flöter for technical assistance, and Ms. C. Middendorf for preparing the manuscript.

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