American Journal of Hematology 56:126–128 (1997)

Polycythemia Vera Treated with Recombinant Interferon-alpha 2a: Evidence of a Selective Effect on the Malignant Clone

Paolo Massaro, Paolo Foa,* Mauro Pomati, Maria Luisa LaTargia, Alessandra Iurlo, Claudia Clerici, Sarah Caldiera, Monica Fornier, and Anna Teresa Maiolo

Istituto di Scienze Mediche, Università degli Studi, Milan, Italy

We periodically analyzed bone-marrow cytogenetic features in 8 patients belonging to a series of 38 subjects with polycythemia vera (PV), all treated with recombinant interferon-alpha 2a (rIFN-alpha) at a weekly dose of 9,000,000 IU. Six out of these 8 patients never showed any chromosome alterations, while 2 displayed at diagnosis the presence of trisomy 8 in all bone-marrow metaphases. Interestingly enough, in these 2 patients rIFN-alpha treatment was able to induce not only complete hematological response but also the disappearance of trisomy 8, as shown by conventional cytogenetic investigation and fluorescence in situ hybridization performed on bone-marrow cells after 1 year of treatment. This finding indicates that, as previously shown in chronic myeloid leukemia, in PV rIFN-alpha can also eradicate the malignant clone by means of a selective effect on bone-marrow transformed cells. Am. J. Hematol. 56:126–128, 1997. © 1997 Wiley-Liss, Inc.

Key words: polycythemia vera; interferon-alpha; cytogenetics; fluorescence in situ hybridization

INTRODUCTION

Polycythemia vera (PV) is a hematopoietic stem-cell disorder characterized by unregulated hyperproliferation of bone-marrow precursors. The main therapeutic goal in PV is reduction of excessive peripheral blood-cell counts; unfortunately, treatment options so far available are far from being optimal, since phlebotomy, radioactive phosphorus, and antiproliferative drugs all have relevant side effects [1,2].

Interferon-alpha is a cytokine widely used for treatment of myeloproliferative syndromes such as essential thrombocythemia, idiopathic myelofibrosis, and chronic myelogenous leukemia (CML). In CML it has been shown that interferon-alpha, besides inducing hematological response, can also determine in about ⅓ of treated patients a significant cytogenetic conversion which results in a more favorable prognosis [3]. Recent reports seem to indicate that interferon-alpha might also be a promising agent for treatment of PV, as it is able to induce normalization of hematological parameters [4–6]; however, as far as interferon-alpha's effect on chromosomal alterations in PV is concerned, published data are restricted to two case reports [7,8]. We describe here our findings on 8 patients with PV treated with recombinant interferon-alpha 2a and submitted to periodical cytogenetic analysis of bone-marrow cells.

© 1997 Wiley-Liss, Inc.

MATERIALS AND METHODS

Patients and Treatment

After informed consent was obtained, 38 patients with PV diagnosed according to the Polycythemia Vera Study Group criteria [1] were treated at our institution with recombinant interferon-alpha 2a (Roferon, Hoffmann LaRoche, Basel, Switzerland) at a starting dose of 3,000,000 IU s.c. three times a week. In all patients, hematocrit was first brought into the normal range (<52% for males; <48% for females) by venesection; pharmacological treatment was then begun with recombinant interferon-alpha 2a (rIFN-alpha). Patients were

*Correspondence to: Paolo Foa, M.D., Istituto di Scienze Mediche, Università degli Studi, Via F. Sforza 35, 20122 Milan, Italy. E-mail: profpfoa@imiucca.csi.unimi.it

Received 20 May 1997; Accepted 27 May 1997.

monitored monthly by clinical examination, blood cell counts, and complete hematochemical evaluation. Response to rIFN-alpha treatment was assessed every 3 months: complete response (CR) was defined as persistence of normal hematocrit values without venesection; partial response (PR) was defined as a >50% reduction of phlebotomy requirement. Out of these 38 patients, 8 could be periodically analyzed in terms of bone-marrow conventional cytogenetic investigation and fluorescence in situ hybridization (FISH).

Cytogenetics

Karyotype analysis was performed on unstimulated, Colcemid (GIBCO, Grand Island, NY)-treated bone-marrow cells from overnight cultures. Metaphases were stained by conventional trypsin-Giemsa method. Fifty metaphases were scored for each preparation.

Fluorescence In Situ Hybridization

FISH analysis was performed on bone-marrow cells kept in culture without mitogens for 48 hr, by means of a biotinylated probe specific for detecting alpha satellite sequences on the centromeric region of chromosome 8 (D8Z2; Oncor, Gaithersburg, MD). Denaturation, hybridization, and posthybridization steps were carried out as detailed elsewhere [9]. The probe was detected using fluorescein isothiocyanate (FITC). Nuclei were counterstained with propidium iodide, using p-phenylenediamine as antifade. Cells were analyzed by a confocal laser scanning microscope (Bio-Rad MRC 600, Bio-Rad Microscience Division, Herts, England) equipped with a krypton-argon laser and mounted on a Nikon Optiphot 2 microscope. Two wavelengths were used: 488 nm for FITC and 568 nm for propidium iodide. A total of 400 nuclei was scored for each sample; the hybridization signal appeared as a distinct green spot on red nuclei.

RESULTS

As stated above, out of a series of 38 subjects with PV all treated with rIFN-alpha at a weekly dose of 9,000,000 IU, 8 patients were periodically submitted to bone-marrow cytogenetic investigation. Six of these 8 patients never showed any chromosome alteration, while two displayed at diagnosis the presence of trisomy 8 in all bone-marrow metaphases. The main characteristics of these 2 cases are summarized in Table I. At diagnosis, both patients displayed a typical hematological and clinical picture of PV with increased levels of erythrocytes, leukocytes, and platelets, elevated red cell mass, splenomegaly, and diffuse pruritus. In both cases, bone-marrow aspiration and biopsy showed an abnormal proliferation of the erythroid compartment without fibrosis, while cytogenetic analysis demonstrated the presence of trisomy 8 in all metaphases. Treatment with rIFN-alpha was then instituted, which led to a progressive normalization of hematological parameters and spleen volume, associated with disappearance of pruritus; both patients achieved CR respectively after 6 (patient 1) and 9 (patient 2) months of therapy. Bone-marrow cytogenetic features were reevaluated 12 months after the beginning of rIFN-alpha therapy, when both patients were still under treatment and in CR. As reported in Table I, according to conventional cytogenetic analysis of bone-marrow cells, complete disappearance of trisomy 8 was recorded in both patients; this finding was further confirmed by means of FISH.

TABLE I. Characteristics of the Two Patients With PV Displaying Cytogenetic Alterations at Diagnosis (Trisomy 8)*

Parameter	Case 1	Case 2
Sex	M	F
Age at diagnosis	42	65
Hemoglobin (g/dl)	16.7	19.5
Hematocrit (%)	51.2	60.1
Erythrocyte number (10^9/l)	7.110	8.610
Leukocyte number (10^9/l)	16.7	12.9
Platelet number (10^9/l)	1.270	940
Red cell mass increase	Yes	Yes
Spleen enlargement	Yes	Yes
Percent of mitosis with trisomy 8 before rIFN-alpha therapy	100	100
Mean rIFN-alpha weekly dose (IU)	9×10^6	9×10^6
Hematological response after 1 year of treatment	CR	CR
Percent of mitosis with trisomy 8 after 1 year of treatment (cytogenetics)	0	0
Percent of nuclei with trisomy 8 after 1 year of treatment (FISH)	0	0

*M, male; F, female; IU, international units; CR, complete response; FISH, fluorescence in situ hybridization.

DISCUSSION

Our results indicate that in patients with PV, rIFN-alpha can induce not only CR but also complete disappearance of a clonal cytogenetic alteration. Interestingly enough, in our study normalization of chromosome pattern during rIFN-alpha therapy was proved not only by means of conventional cytogenetic analysis on mitotic figures but also by means of fluorescence in situ hybridization, a quantitative technique which allows detection of specific chromosomal alterations not only in cells undergoing metaphase but also in interphase nuclei [10]. Our data suggest that as previously proved in CML [3], in PV, rIFN-alpha is also able to eradicate the malignant clone by means of a selective effect on bone-marrow transformed cells. It remains to be assessed whether in PV, as already shown in CML [3], bone-marrow cytogenetic conversion is associated with a more favorable long-term prognosis.

REFERENCES

1. Berk PD, Goldberg JD, Donovan PB, Fruchtman SM, Berlin NI, Wassermann LR: Therapeutic recommendations in polycythemia vera based on Polycythemia Vera Study Group protocols. Semin Hematol 23:132–143, 1986.
2. Weinfeld A, Swolin B, Westirf J: Acute leukemia after hydroxyurea therapy in polycythemia vera and allied disorders: Prospective study of efficacy and leukaemogenicity with therapeutic implications. Eur J Haematol 52:134–139, 1994.
3. Italian Cooperative Study Group on Chronic Myeloid Leukemia: Interferon alpha-2a as compared with conventional chemotherapy for the treatment of chronic myeloid leukemia. N Engl J Med 330:820–825, 1994.
4. Sacchi S, Leoni P, Liberati M, Riccardi A, Tabilio A, Tartoni P, Messora C, Vecchi A, Bensi L, Rupoli S, Ucci G, Falzetti F, Grignani F, Martelli MF: A prospective comparison between treatment with phlebotomy alone and with interferon-alpha in patients with polycythemia vera. Ann Haematol 68:247–250, 1994.
5. Foa P, Massaro P, Ribera S, Iurlo A, Mezzanotte C, Giandalia D, Maiolo AT: Role of interferon alpha-2a in the treatment of polycythemia vera. Am J Hematol 48:55–57, 1995.
6. Taylor PC, Dolan J, Ng JP, Paul B, Collin R, Reilly JT: Efficacy of recombinant interferon-alpha (rIFN-alpha) in polycythemia vera: A study of 17 patients and an analysis of published data. Br J Haematol 92:55–59, 1996.
7. Hino M, Futami E, Okuno S, Miki T, Nishizawa Y, Morii H: Possible selective effect of interferon alpha-2b on a malignant clone in a case of polycythemia vera. Ann Haematol 66:161–162, 1993.
8. Messora C, Bensi L, Vecchi A, Longo R, Giacobbi F, Temperani P, Bevini M, Emilia G, Sacchi S: Cytogenetic conversion in a case of polycythemia vera treated with interferon-alpha. Br J Haematol 86: 402–404, 1994.
9. Kibbelaar RE, van Kamp H, Dreef EJ, Wessels JW, Beverstock GC, Raap AK, Fibbe WE, den Ottolander GJ, Kluin PM: Detection of trisomy eight in hematological disorders by in situ hybridization. Cytogenet Cell Genet 56:132–136, 1991.
10. Arnoldus EPJ, Wiegant J, Noordermeer IA, Wessels JV, Beverstock GC, Grosveld GC, van der Ploeg M, Raap AK: Detection of the Philadelphia chromosome in interphase nuclei. Cytogenet Cell Genet 54:108–111, 1990.