Eur J Cancer Clin Oncol, Vol. 18, No. 10, pp. 929–935, 1982.
Printed in Great Britain.

0277-5379/82/100929-07$03.00/0

Administration of Fibroblast Interferon to Patients with Advanced Breast Cancer: Possible Effects on Skin Metastasis and on Hormone Receptors*

P. POUILLART,† T. PALANGIE,† M. JOUVE,† E. GARCIA-GIRALT,† W. H. FRIDMAN,† H. MAGDELENAT,† E. FALCOFF‡ and A. BILLIAU§‖

†*Section Médicale et Hospitalière and* ‡*Section de Biologie de l'Institut Curie, Paris, France and* §*Rega Institute for Medical Research, Leuven, Belgium*

Abstract—*Eleven patients with metastasized breast cancer received* 8 *intramuscular injections of* 6×10^6 *units of human fibroblast interferon over a period of* 40 days. *The injections did not cause local irritation or inflammation. Fever occurred in only* 1 *of the* 11 *patients. Although several types of metastases were monitored, only skin nodules consistently* (10 *out of* 11 *patients) exhibited changes that were suggestive of a therapeutic effect of the treatment regimen: either a simple decrease in size of some nodules or central necrosis accompanied by an inflammatory reaction. NK-activity of peripheral blood leukocytes was significantly increased after administration of the first dose; the effect of subsequent injections was less clear. Receptors for estrogens and progestogens were increased in the tumor biopsies of* 2 *out of* 2 *and* 5 *out of* 6 *patients tested respectively.*

INTRODUCTION

PARENTERAL administration of interferons has been shown to inhibit the development of experimental tumors in mice [1–4]. This effect is believed to be due to enhancement of various host responses to the tumor cells, one of which is the enhancement of natural killer cell (NK-) activity [5–7]. Clinical trials in man using leukocyte interferon have produced encouraging results in that shrinking of tumor masses or remissions of lymphoproliferative disorders have been noted in some of the treated patients [8, 9]. Trials with fibroblast interferon have been started later and therefore less data are available at present (for a review see ref. [10]).

In a study on terminal patients with advanced cancer, we have reported that single intramuscular injections of fibroblast interferon at dosages of 8×10^6 units were well tolerated and caused enhancement of NK-activity of peripheral white blood cells to about the same extent as similar doses of leukocyte interferon [11].

The present study describes the clinical and biological observations of 11 patients with metastasized breast cancer during and after administration of a series of 8 injections of 6×10^6 units of fibroblast interferon over a period of 40 days.

MATERIALS AND METHODS

Eleven patients with disseminated breast cancer, refractory to cytotoxic chemotherapy and to hormonal therapy, were entered into the trial. All patients had measurable skin metastases; in addition, other metastatic localizations were also present. The overall disease status is presented in Table 1. When they were entered into the trial, specific treatment had already been stopped for more than 1 month.

Accepted 27 May 1982.

*This work was supported by grants from the INSERM (Institut National de la Santé et de la Recherche Médicale) (47-77-79, 78-5-082), the DGRST (Délégation Générale à la Recherche Scientifique et Technique), Comité-Intersection of the Curie Institute, and the Cancer Research Foundation of the Belgian ASLK/GCER (General Savings and Retirement Fund). The authors are grateful to the Institut Pasteur (Paris) for the gift of leukocyte interferon.

‖Reprint requests and correspondence: A. Billiau, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium.

Table 1. *Status of breast cancer patients entering into phase* II *therapeutic trial of fibroblast interferon*

Patient No.	Code	Age (years): At tumor diagnosis	At interferon treatment	Metastatic lesions in: Skin	Lung	Pleural cavity (effusion)	Bone	Liver	Contralateral breast gland	Lymph nodes	Karnofsky index	NK-activity*
1	LAF	49	50	+	+	−	−	−	+	+	80	N.D.
2	ESC	47	55	+	−	−	−	−	−	+	90	3
3	GRO	46	52	+	−	−	+	+	−	−	60	1
4	BOY	61	63	+	+	+	−	−	+	−	60	9
5	JEZ	33	40	+	−	+	−	+	−	−	70	3
6	SOL	38	42	+	−	−	+	−	−	−	80	3
7	NOE	58	62	+	+	+	−	−	−	−	60	1
8	DEM	50	54	+	−	+	−	−	+	−	80	8
9	BOU	32	34	+	−	+	−	−	−	+	60	8
10	DES	53	60	+	−	−	+	−	+	+	60	12
11	FRA	61	68	+	−	+	−	−	−	+	80	14

*Lytic units.
N.D. = not determined.

Only patient No. 8 was still receiving a daily dose of 150,000 units of retinol.

Human fibroblast interferon was prepared at the Rega Institute from MG–63 cells according to procedures described earlier; it had a specific activity of $\sim 10^6$ units/mg protein [12]. Injections of 6×10^6 international units were given intramuscularly with 5-day intervals. After 8 successive injections treatment was arrested for 1 month. Four of the patients (Nos 2, 8, 9 and 11) were then given a course of leukocyte interferon treatment (8 intramuscular injections of 6×10^6 units with 5-day intervals).

Serum levels of β_2-microglobulin and NK-activity were determined according to procedures described in a previous study [11]. NK-activity was expressed in lytic units, which represent the number of effector cells per 10^6 lymphocytes needed to induce 50% specific [^{51}Cr] release by K–562 target cells. Estrogen (ER) and/or progesterone (PR) receptors were determined as described elsewhere [13] on small biopsy samples of cutaneous nodules removed under local anesthesia. Briefly, the metastatic tissue was homogenized in 5 mM phosphate buffer (pH 7.6) containing 0.4 M KCl (to extract nuclear and cytosolic receptors simultaneously) and centrifuged for 1 hr at 105,000 *g*. Duplicate supernatant aliquots (100 μl) were incubated with tritium-labeled ligands ([^{3}H]-R2858, 90 Ci/mmol, New England Nuclear, for ER and [^{3}H]-R5020, 90 Ci/mmol, New England Nuclear, for PR) at one single saturating concentration (1 nM for ER and 2 nM for PR). The unbound fraction of ligand was adsorbed for 10 min on dextran-coated charcoal (DCC) and centrifuged for 10 min at 800 *g*. The radioactivity of aliquots (150 μl) of the DCC supernatants was measured by liquid scintillation counting. Receptor levels, representing both cytosolic and nuclear receptors, were expressed in fmol of radioligand specifically bound to their receptor per mg of tissue DNA. The DNA content of the biopsy sample was assayed by fluorimetry [14] on the 105,000-*g* pellet.

RESULTS

Tolerance

The intramuscular interferon injections were painless and there was no induration or erythema at the injection site. With the exception of patient No. 4, who developed ~38.5°C body temperature after each injection, no fever occurred. No allergic reactions were noted. Late in the course of therapy (i.e., after 5–7 injections) some of the patients complained of asthenia. However, in the 6-week course of treatment no weight loss was observed. Since the patients received blood transfusions when the hemoglobin level was below 12 g/100 ml, the asthenia was probably not related to anemia as a result of multiple blood sampling. Other hematological values (total and differential leukocyte counts and thrombocyte counts) as well as blood biochemistry (γ-glutamyl transpeptidase, alkaline phosphatase, transaminase, lactic dehydrogenase, creatinine, urea and glucose) were not notably changed during the interferon administration.

Alterations in tumor status associated with treatment

The alterations in disease status occurring during interferon treatment are summarized in Table 2.

In all patients except one (No. 8) the systemic administration of fibroblast interferon was associated with unexpected changes in the skin metastases. In patients Nos 1, 6 and 7 the skin nodules progressively decreased in size and some of them disappeared completely. In the other cases (Nos 2–5, 9, 10 and 11) necrosis occurred in the centre of each nodule. No inflammatory reaction was observed around the lesions. In patients Nos 3, 4 and 10 no further changes were seen. In the other cases (Nos 2, 5, 9 and 11) the necrotic process progressed and led to a large ulceration.

In 1 patient (case No. 7) out of 3 with skin lymphangitis (cases Nos 5, 7 and 8) the treatment was associated with regression of the edema, warmth, erythema and tenderness. In another case (No. 5) a diffuse superficial necrosis developed in the lymphangitic zone after as little as 2 injections of interferon.

Of 3 patients with lung metastases only 1 (case No. 1) showed some evidence of a possible effect of interferon. This patient had numerous small nodules and 3 larger nodules (diameters of 3×4, 3×3 and 4×5 cm) in the lung. At the end of treatment with interferon the small lesions were decreased in size and number; the larger nodules had progressed.

Six of the patients had a pleural effusion. In 4 of these (cases Nos 4, 5, 8 and 9) no obvious changes occurred during treatment. In 1 of the patients (case No. 2) the effusion volume slightly decreased. Patient No. 5 had a large effusion, necessitating regular evacuations. After the 5th injection of interferon no further evacuations were necessary for a period of 6 weeks.

Five of the patients had enlarged and indurated lymph nodes. During treatment 4 out of these (cases Nos 1, 2, 5 and 11) developed infiltrations and necrotic ulcerations of the skin area overlying the affected nodes. Concomitantly, the lymph nodes became smaller.

In patients Nos 1–10 all beneficial changes that had occurred during treatments were abrogated within 15–30 days following treatment arrest. In one patient (case No. 11) the improvement associated with interferon therapy lasted for more than 3 months.

Two of the patients (cases Nos 2 and 3) were given leukocyte interferon starting 4 weeks after arrest of fibroblast interferon. The same dosage schedule was used. No changes similar to those observed during fibroblast interferon therapy were seen. In fact, all tumors progressed during therapy.

Biological parameters

Serum levels of interferon were determined in some of the patients at 1, 4 and 24 hr post-injection. As expected from previous studies [11, 15], activities were always below 30 units/ml, which made it difficult to ascertain that the activity measured was indeed interferon.

Hormonal receptor content of tumor tissue was determined in 6 of the 11 treated patients (Table 3). The level of estrogen and/or progesterone binding by the tumor tissue was increased from 2- to 10-fold. Only in one patient (case No. 8) was the binding activity depressed. It should be remarked, however, that in this patient the malignant disease progressed extremely rapidly during interferon therapy. It

*Table 2. Incidence of changes in metastatic lesions during treatment with fibroblast interferon**

Metastatic localization	No. of patients	No. of patients with: Suggestive evidence for tumor regression†	No change	Progression
Skin	11	10	0	1
Lung	3	1	1	1
Pleural cavity	6	1	2	3
Bone	3	0	3	0
Liver	2	0	2	0
Breast	4	1	2	1
Lymph node	6	4	2	0

*Eight intramuscular injections of 6×10^6 units of fibroblast interferon given at 5-day intervals.

†See text for explanation.

*Table 3. Variation in tumor tissue estrogen and progestogen receptor content associated with fibroblast interferon administration**

Patient	Estrogen receptor:† Before treatment	Estrogen receptor:† After treatment	Progestogen receptor:† Before treatment	Progestogen receptor:† After treatment
3	50	330	150	900
4	40	100	20	200
5	–	–	50	70
8	–	–	100	20
9	–	–	<20	120
10	–	–	30	370

*Eight intramuscular injections of 6×10^6 units of fibroblast interferon given at 5-day intervals.
†Expressed in fmol/mg DNA.

is perhaps also worthwhile mentioning that this patient, while receiving interferon injections, inadvertantly continued taking retinol, a therapy which had been instated before the interferon injections were started.

We also determined NK-activity. As can be seen in Table 1, the initial level of this activity varied strongly from one patient to another. In 9 of the patients a sufficient number of samples were obtained to study the effect of the first injection of interferon. In 6 patients sufficient data were available to analyse the effect of continuous treatment. The data are summarized in Table 4. The first injection was associated with a significant increase in activity averaging 46% above pre-treatment levels. Subsequent injections caused increases in NK-activity in some but not all instances. In view of the small number [6] of patients from whom complete data were available, no attempt was done to trace the source of this variability in responses. Rather, the data for the 2nd to 8th injections were pooled per patient in order to obtain an estimate of the average effect of the

*Table 4. Fluctuations in NK-activity associated with fibroblast interferon treatment**

Increment measured	Percentage increase in NK-activity: Mean value	No. of patients tested	Standard error of means	Probability (*P*) of null-hypothesis
After 1st injection (24–72 hr post-injection as compared to pre-treatment level†)	46.2	9	18.8	<0.5
After 1st to 8th injections (average per patient)				
a. base-levels (120 hr after each injection as compared to pre-treatment level†)	11.5	6	14.0	~0.45
b. post-injection levels (24–72 hr after each injection as compared to a)	−3.7	6	3.9	~0.35

*Eight intramuscular injections of 6×10^6 units of fibroblast interferon given at 5-day intervals.
†The mean pre-treatment level was 6.55 lytic units (S.E.: 1.59; *n*: 9).

interferon treatment. The analysis showed that repeated injections were associated with a slight, statistically insignificant increase of 12% in baseline activity (i.e. activity measured 120 hr after each injection). Activities measured 24–72 hr after each injection were not different from the overall baseline activity.

Finally, we also determined serum levels of β_2-microglobulin in some of the patients at times 0, 24, 48 and 72 hr post-injection. As expected from our previous studies [11], the injections of fibroblast interferon were not associated with changes in β_2-microglobulin levels.

DISCUSSION

The purpose of this clinical trial was to evaluate changes in clinical status and in certain biological parameters in advanced breast cancer patients during and subsequent to a relatively short treatment with fibroblast interferon. The dosage schedule (intramuscular injections of 6×10^6 units given at 5-day intervals) was based on a previous study [11] in which it was found that single doses of at least 3×10^6 units were able to bring about increased NK-activity of peripheral blood cells and that a 4- to 5-day interval was necessary and sufficient to allow this activity to return to base-level. The duration of treatment (40 days) was chosen arbitrarily, assuming that any beneficial effect on tumor lesions would have become apparent within this time interval. Patients were chosen on the basis of resistance to conventional forms of treatment, good general condition and presence of tumor localization in the skin, allowing evaluation of changes in size and tissue reaction.

The therapeutic regimen was well tolerated. This is in contrast to findings with earlier preparations of fibroblast interferon from the same source, which were reported to cause high fever [15]. Studies with completely pure fibroblast interferon have suggested that pyrogenicity was due to impurities. Although the purification techniques were not changed [16], it is possible that early preparations contained more impurities due to lower content of interferon in the crude perparations.

Although several types of metastases were monitored, only skin nodules consistently exhibited changes that were suggestive of a therapeutic effect of interferon treatment. All but 1 of the 11 patients showed alterations in some of the skin metastases, either a simple decrease in size or central necrosis without accompanying inflammatory reaction. In 4 out of 5 patients with enlarged lymph nodes, a decrease in size of some of those occurred and infiltrative and ulcerative lesions developed in the overlying skin area. The significance of these changes is not clear. The authors feel that the changes occurred more frequently than would have been the case if no interferon treatment had been given. However, this is a point that needs confirmation in a controlled trial. Assuming that the changes were due to interferon treatment, one can still speculate whether they reflect an increased immunological response of the host, a direct inhibition of tumor cell growth or a phenomenon related to the well-known tumor necrosis effect seen in experimental animals treated with bacterial products which induce the release of tumor necrosis factor [17]. Some of the uncertainties in the interpretation of the findings of the present study are amenable to evaluation.

Receptors for estrogens and progestogens in breast cancer tissue are of particular relevance to the therapeutic effect of hormonal treatment. It has already been reported by Dimitrov *et al.* (personal communication) that *in vitro* exposure of human breast cancer or uterine tissue to leukocyte interferon caused an increase in estrogen-binding activity of the cytosol. Along this line, it is reported in the present study that interferon administration was associated with an increase of progestogen receptor content in tumor tissue of 5 out of 6 patients examined and with an increase of estrogen receptor content in 2 out of 2 patients examined. Since cytosolic and nuclear hormone receptors were determined simultaneously, the increase associated with interferon treatment reflected an alteration of total amount of receptor per cell. Translocation from one cellular compartment to another may have occurred, but could not be revealed by the techniques used. On the basis of *in vitro* observations, it has been proposed that one of the many effects of interferon may consist in shifting cells from the undifferentiated to the differentiated phenotype [18–21]. The increase in hormone receptor content seen in the present study may reflect this action. It would be of particular interest to know whether the changes occurring in hormonal receptors are correlated with the clinical response. In the present study there was only one patient (No. 8) in whom the hormonal receptor content of tumor cells was decreased rather than increased. This was also the only patient in whom no changes in skin metastases were seen. This lends some support to the view that there is a correlation between clinical response and effect

on hormonal receptors. However, larger numbers of observations will be needed to substantiate this.

The effect of single injections of fibroblast interferon on NK-activity of circulating leukocytes, as reported previously [11], was confirmed by the findings in the present study: a 46% increase in NK-activity was noted after administration of the first interferon dose. Subsequent injections of interferon remained without effect on NK-cell levels, indicating that the time interval of 5 days between each injection, although sufficient for NK-levels to return to base-level, was insufficient to allow the relevant cell population to regain its responsiveness to interferon.

In conclusion, the present trial has confirmed good tolerance of patients to fibroblast interferon therapy. It has failed to demonstrate a long-lasting therapeutic effect of the treatment regimen. However, it suggests the occurrence of interferon-mediated, possibly beneficial changes in metastatic nodules which merit further investigation.

REFERENCES

1. GRESSER I. Antitumour effects of interferon. In: BECKER F, ed. *Cancer—A Comprehensive Treatise, Chemotherapy.* New York, Plenum, 1977, Vol. 5, 525–571.
2. RATNER L, NORDLUND JJ, LENGYEL P. Interferon as an inhibitor of cell growth: studies with mouse melanoma cells. *Proc Soc Exp Biol Med* 1980, **163**, 267–272.
3. GRESSER I, BOURALY-MAURY C. Inhibition by interferon preparations of a solid malignant tumor and pulmonary metastases in mice. *Nature New Biol* 1972, **236**, 78–79.
4. GRESSER I, BOURALY C, LEVY JP. Increased survival in mice inoculated with tumor cells and treated with interferon preparations. *Proc Natl Acad Sci USA* 1969, **63**, 51–56.
5. SENIK A, GRESSER I, MAURY C, GIDLUND M, ORN A, WIGZELL H. Enhancement by interferon of natural killer cell activity in mice. *Cell Immunol* 1979, **44**, 186–200.
6. TRINCHIERI G, SANTOLI D. Antiviral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. *J Exp Med* 1978, **147**, 1314–1333.
7. LUCERO MA, FRIDMAN WH, PROVOST MA *et al.* Effect of various interferons on the spontaneous cytotoxicity exerted by lymphocytes from normal and tumor-bearing patients. *Cancer Res* 1981, **41**, 294–299.
8. MERIGAN TC, SIKORA K, BREEDEN JH, LEVY R, ROSENBERG SA. Preliminary observations on the effect of human leukocyte interferon in non-Hodgkin's-lymphoma. *N Engl J Med* 1978, **299**, 1449–1453.
9. GUTTERMAN JU, BLUMENSCHEIN GR, ALEXANIAN RA *et al.* Leukocyte-interferon-induced regression in human metastatic breast cancer, multiple myeloma and malignant lymphoma. *Ann Intern Med* 1980, **93**, 399–406.
10. BILLIAU A. The clinical value of interferons as antitumor agents. *Eur J Cancer Clin Oncol* 1981, **17**, 949–967.
11. LUCERO MA, MAGDELENAT H, FRIDMAN WH *et al.* Comparison of effects of leukocyte and fibroblast interferon on immunological parameters in cancer patients. *Eur J Cancer Clin Oncol* 1982, **18**, 243–251.
12. VAN DAMME J, BILLIAU A. Large-scale production of human fibroblast interferon. *Methods Enzymol* 1981, **78**, 101–119.
13. MAGDELENAT H, TOUBEAU M, PICCO C, BIDRON C. Détermination des récepteurs d'oestrogènes et de progestérone sur forage-biopsie des tumeurs mammaires humaines. In: MARTIN PM, ed. *Récepteurs Hormonaux et Pathologie Mammaire.* Paris, Medsi, 1980, 107–119.
14. FISZER-SZAFARS B, SZAFARS D, GUEVARRA DE MURILLO A. A general, fast and sensitive micromethod for DNA determination. *Anal Biochem* 1981, **110**, 165–170.
15. BILLIAU A, DE SOMER P, EDY VG, DE CLERCQ E, HEREMANS H. Human fibroblast interferon for clinical trials: pharmacokinetics and tolerability in experimental animals and humans. *Antimicrob Agents Chemother* 1979, **16**, 56–63.
16. BILLIAU A, HEINE JW, VAN DAMME J, HEREMANS H, DE SOMER P. Tolerability of pure fibroblast interferon in man. *Ann NY Acad Sci* 1980, **350**, 374–375.
17. CARSWELL EA, OLD LJ, KASSEL RL, GREEN S, FIORE N, WILLIAMSON B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA* 1975, **72**, 3666–3670.

18. GRESSER I. On the varied biological effects of interferon. *Cell Immunol* 1977, **34**, 406–415.
19. CHANY C, VIGNAL M. Effect of prolonged interferon treatment on mouse embryonic fibroblasts transformed by murine sarcoma virus. *J Gen Virol* 1970, **7**, 203–210.
20. GRESSER I, THOMAS M-T, BROUTY-BOYÉ D, MACIERA-COELHO A. Interferon and cell division. IV. Effect of interferon treatment of L1210 cells *in vitro* on tumor and colony formation. *Nature New Biol* 1971, **231**, 20–21.
21. BROUTY-BOYÉ D, GRESSER I. Reversibility of the transformed phenotype. I. Progressive reversion of the phenotype of X-ray-transformed C3H/10T 1/2 cells under prolonged treatment with interferon. *Int J Cancer* 1981, **28**, 165–173.