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Leukocyte-Derived Interferon (Alpha) in Human Breast Carcinoma

The American Cancer Society Phase II Trial

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A multi-institutional trials program was initiated to define the effects of interferons in disseminated human breast carcinoma. Interferon alpha, prepared from buffy coats, was administered intramuscularly at 3×10^6 U daily for an initial period of 28 days. Of 23 patients who entered the program, five had an objective partial response of 92 days mean duration at diverse sites of involvement. Patients who responded were significantly older ($p = 0.05$) than nonresponders. Dose escalation in eight patients did not result in any clear evidence of additional responses. Major toxicities were fatigue, anorexia with weight loss, and reversible leukopenia (less than 3.5×10^9 leukocytes/L in 16 patients). Natural killer cell and antibody-dependent cell-mediated cytotoxicity were significantly ($p < 0.05$) enhanced 48 hours after interferon administration began but subsequently declined despite continued therapy. Serum β_2-microglobulin concentration increased on day 15 ($p < 0.05$) and remained significantly elevated on day 22 ($p < 0.005$). Peak interferon titers (mean, 62 U) occurred 6 hours after interferon was started, varied widely between patients, and were higher and more persistent with dose escalation. Once an optimal dose is defined, prospectively randomized trials will define what role interferons may have in systemic therapy of breast carcinoma.

INTERFERONS, as vertebrate proteins, are different antitumor compounds from those currently in clinical use. They inhibit viral replication, modulate immunologic responses, inhibit cellular proliferation, alter cellular macromolecular processes, influence the cytoskeleton, and modify the phenotypic expression of cell surface characteristics (1-3). Interferons inhibit tumor growth and prolong survival in experimental tumor models (4, 5). The decision to assess the effects of interferons in breast carcinoma resulted from review of preclinical and clinical data. Female mice of the RIII strain, which have a high spontaneous incidence of mammary carcinoma, had a significant delay in development of tumors when treated weekly from 6 weeks of age with mouse interferon (6). In athymic nude mice carrying human breast carcinoma xenografts, tumor development was delayed and inhibited by human interferon (7). Consistent with this probable antimitotic effect of interferons in nude mice was the inhibition of proliferation of human breast carcinoma cell lines in vitro (8). An antitumor effect of interferons in recurrent human breast carcinoma was reported in a preliminary clinical trial (9).

Based upon these observations, the American Cancer Society initiated a multi-institutional trials program to evaluate the clinical effects of interferons in disseminated human breast carcinoma. The primary objective of the trial was to confirm or refute the effectiveness of interferon alpha as single-agent therapy in inducing tumor regressions. Secondary objectives were to ascertain toxicities, pharmacokinetics, and immunologic effects of interferon alpha (leukocyte interferon).

Methods

INTERFERONS AND TRIAL DESIGN

Interferon alpha, prepared from buffy coats, was obtained from Kari Cantell, M.D. and the Finnish National Red Cross (10). It was partially purified to a specific activity of 10^6 U/mg of protein. Frozen and lyophilized aliquots were packaged 3-million units per vial and were safety tested both in Finland and in the United States. Batch and vial testing confirmed the potency of both frozen and lyophilized preparations. Safety testing

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Table 1. Response of Patients with Metastatic Breast Carcinoma to Human Leukocyte Interferon

	Patients
	n
Partial response*	5
Improvement	4
Stable	8
Progression	6
Total	23

* Durations of responses were 14, 56, 59, 156, and 176 days.

showed the absence of microbial contamination, animal toxicity, unacceptable pyrogenicity after intravenous injection of rabbits and by limulus tests, and hepatitis B virus antigen titers by complement fixation.

The trial consisted of an initial 28-day period of daily intramuscular interferon administration. At the end of this period, any patient with progressive disease was removed from study. Patients whose disease had neither regressed nor progressed were randomized to receive an additional 14 days of treatment at 3×10^6 U or an escalated dose of 9×10^6 U. If no regression of disease was noted after 42 days, the interferon was discontinued and the patient removed from study. Patients who achieved a partial objective response with the first 42 days were randomized either to discontinue interferon or to continue interferon at 3×10^6 U for a period equal to the time to partial response. The maximal duration of interferon administration for any patient regardless of response was 84 days. In treating patients with lymphomas, continued regression of objectively measurable disease had been observed after interferon discontinuation (11). This finding, together with the limited amounts of interferons available, influenced trial design.

PATIENT SELECTION AND EVALUATION

Patients eligible for the trial were those with recurrent disease quantifiable by measurement of soft tissue, lymph node, or pulmonary masses. Good clinical performance status was required and patients could not have received previous cytotoxic chemotherapy for metastatic disease. If patients had received additive hormonal therapy, they must have had response to it and have had no hormones administered for 30 days. Patients with leukocyte counts less than 3.5×10^9/L, platelet counts less than 100×10^9/L, creatinine greater than 1.5 mg/dL, blood urea nitrogen greater than 25 mg/dL, or bilirubin greater than 2.0 mg/dL were excluded from the study.

Twenty-six patients entered the study: nine from the University of Wisconsin Clinical Cancer Center, nine from Roswell Park Memorial Institute, seven from Mt. Sinai Hospital and School of Medicine, and one from M.D. Anderson Hospital and Cancer Center. Three patients were excluded from the final analysis. Two patients were ineligible: one, who failed to respond to interferon, had received cytotoxic chemotherapy for metastatic disease; a second had evaluable, but not objectively measurable, disease that significantly improved. A third patient was eligible, treated, and initially coded as a partial response on the basis of decrease in pulmonary nodules and pleural effusion; the chest roentgenogram could not be found for review, however, and, thus, she was excluded from the final analysis.

Age of the evaluable patients ranged from 37 to 75 years with a median of 55 years. Only four were premenopausal. Disease-free interval after mastectomy ranged from 0 to 96 months (median, 31 months). All but one patient had undergone mastectomy and five had received adjuvant chemotherapy. The dominant metastatic site in 10 patients was soft tissue involvement and in 13 patients, visceral involvement. Of the 17 patients in whom estrogen receptor had been determined, 12 had positive values.

Definitions of response in metastatic breast carcinoma have been developed by the International Union against Cancer and the Breast Cancer Task Force of the U.S. National Cancer Institute (12). These definitions were prospectively incorporated into the planning of this trial. A partial response by these criteria is defined as a 50% or greater reduction in the sum of the product of the maximum perpendicular diameters of the indicator, measurable tumor masses. All other areas of disease are required to remain stable or improve. Progression was defined as a 25% increase in measured lesions or development of new lesions.

PHARMACOLOGIC AND IMMUNOLOGIC METHODS

Mononuclear cell samples from patients were frozen at −170 °C, and stored until therapy on that patient was complete. They were then assayed simultaneously, a method that has proven useful in minimizing inter-assay variability in previous studies from our laboratory (13). After ficoll-hypaque separation, cells were resuspended in RPMI 1640H (Gibco, Grand Island, New York) plus 50% pooled human serum and counted to ascertain number of viable cells. Cell concentration was then adjusted to 2×10^7 mononuclear cells/mL. On ice, 0.5 mL of RPMI plus 20% dimethyl sulfoxide was added dropwise to a 0.5 mL cell suspension in Nunc plastic freezer vials (American Breeder's Service, DeForest, Wisconsin). Vials were sealed and placed in a freezer for cooling at a rate of 1 °C/min. For assay, stored vials were removed from liquid N_2 and immediately placed in a 37 °C water bath. When only a sliver of ice remained, vials were placed on ice where 1.0 mL RPMI 1640H plus 50% pooled human serum was added dropwise. The cells were then gently resuspended and washed with RPMI 1640H plus 50% human serum. Cell viability by trypan blue dye exclusion was 65% to 100% after this procedure.

Natural killer (spontaneous) cell cytotoxicity was measured in a 6-hour chromium release assay in which K562 cells were used as targets. Antibody-dependent cell-mediated cytotoxicity (killer cell activity) was measured in a similar assay with Chang cells as targets and with use of an anti-Chang antiserum at a dilution of 10^{-4} (14). K562 and Chang cells were labeled by incubating 5×10^6 cells in 1 mL of medium with 100 and 200 μCi/mL $Na_2{}^{51}Cr$, respectively, for 40 to 90 minutes. Labeled cells were then resuspended in medium, underlayered with 2 mL fetal bovine serum, and centrifuged at $400 \times g$ for 7 minutes at 24 °C to remove unincorporated isotope. After two such washes, target cells were diluted and added to microtiter wells at 2×10^3 cells per well for the chromium release assay. Mononuclear cells were then added to the wells in quadruplicate at effector:target cell ratios of 100:1, 50:1, 25:1 and 12.5:1. After 6 hours of incubation at 37 °C in a 5% CO_2, humidified atmosphere, supernatants in each well were collected using a Titertek supernatant collection system (Flow Laboratories, Rockville, Maryland). Percent specific ^{51}Cr release was calculated as follows:

$$\frac{\text{cpm test wells} - \text{cpm spontaneous release}}{\text{cpm maximum release} - \text{cpm spontaneous release}} \times 100.$$

Proliferative responses to phytohemagglutinin, concanavalin A, and pokeweed mitogen were determined on Ficoll-hypaque separated fresh cells as described previously (15). Spontaneous sheep erythrocyte rosettes and complement-binding rosettes were ascertained and calculated based on total mononuclear cells by described methods (15).

After separation, collected sera were stored at −70 °C until assay via a viral cytopathic effect reduction assay (16). Human fibroblasts were treated with dilutions of interferon-containing serum for 18 hours before challenge with vesicular stomatitis virus. The reciprocal of that serum dilution, effective in protecting 50% of the monolayer, was considered to be the interferon titer. Titers were expressed in international reference leukocyte units by incorporation of an appropriate internal laboratory standard into each assay and repeated comparison of the geometric mean titer of the unknown with that of the standard and the reference leukocyte standard (MRC 69/19).

Plasma samples, thawed after storage at −70 °C, were analyzed by competition radioimmunoassay. Rabbit anti-β_2-microglobulin covalently bound to sepharose particles was mixed with diluted samples and ^{125}I-β_2-microglobulin. After 3 hours at room temperature, particle bound isotope in each sample was quantified. The β_2-microglobulin concentration was expressed by comparison to a calibration of an internal standard.

Results

DISEASE RESPONSE

Five of the 23 patients achieved objective partial response (Table 1). Sites of response included lymph nodes, chest wall, pleura, and biopsy-proven involvement of the bladder. Dose escalation for 2 weeks in eight patients did not result in any clear evidence of additional responses. Four patients had evidence of regression of measurable or evaluable disease, insufficient to qualify for objective response by the defined criteria. Six patients had progressive disease. Eight patients had neither regression nor progression of their disease during the period of treatment and observation. They were considered stable.

Mean time to response was 42 days. Duration of partial responses ranged from 14 to 176 days with a median of 59 days. Study design required that interferon be discontinued in patients with partial responses either immediately on achieving partial response or after a 4- to-6-week period of consolidation therapy. The responses of shortest duration (14 and 56 days) were in women randomized to discontinue interferon after achieving partial response. The shortest response (14 days) was in a woman an whose measurable lymph nodes had regressed by day 42, who was randomized to discontinue interferon, and who by day 56 had progression of disease. The other patient in whom interferon was discontinued on day 42 after achieving a partial response had a response of 56-days duration. The three responses of longest duration (59, 156, and 176 days) were in patients randomized to continue receiving interferon therapy for an additional period of up to 42 days after achieving a partial response by day 42.

SIDE EFFECTS

Major toxicities of interferon were fatigue, anorexia with weight loss, and leukopenia (Table 2). These symptoms were noted in approximately three fourths of patients and were somewhat more frequent in patients receiving the escalated dose. Anorexia resulted in a mean weight loss of 1.6 kg; 12 patients lost greater than 1.0 kg. The six patients who received an escalated dose for the 2-week period tended to have anorexia and weight loss more frequently. Excess shedding of hair for several weeks after the completion of interferon was noted in seven patients.

Temperature elevation, which occurred in 20 of 23 patients, was not a limiting problem. This symptom, usually most severe on the first day of therapy (mean temperature, 38.2 °C) and subsiding thereafter, was not greater in patients on the escalated dose. One unexpected finding was the development of mucocutaneous crusted labial lesions ("fever blisters") within a few days of initiating interferon therapy in five patients. One of these patients had a fourfold increase in herpes simplex virus complement-fixation titer.

A slight rise in serum aspartate transaminase occurred in approximately half of the treated patients; mean levels increased from 27 U to 57 U (range, 19 to 159 U). This finding was not accompanied by any elevation in lactic dehydrogenase, alkaline phosphatase, or bilirubin. Episodes of diarrhea occurred during treatment in five patients and dry mouth was reported by four patients.

Table 2. Toxicity of Human Leukocyte Interferon

Dose	Fatigue and Malaise	Nausea and Anorexia	Mean Weight Loss	Mean Leukocyte Count on Day 43*
	n patients/n treated		*kg*	*$\times 10^9$/L*
All doses	17/23	17/23	1.6	3.9 (1.7-9.3)
3×10^6 U	11/15	10/15	1.3	4.4 (1.7-9.3)
Escalated to 9×10^6 U	6/8	7/8	2.4	3.0 (2.2-6.6)

* Ranges of values are in parentheses.

Moderate leukopenia was common (Table 2). A leukocyte count of greater than 3.5×10^9/L occurred in 16 patients. The median nadir leukocyte count was on day 15 (3.8×10^9/L). No additional leukocyte count depression was found after 43 days of interferon therapy. Patients receiving the escalated dose of 9×10^6 U of interferon from day 28 to day 43 had somewhat, although not statistically significant, lower leukocyte counts on day 43 than the patients continuing at the dose of 3×10^6 U (Table 2). Granulocyte counts were more depressed than lymphocyte counts (on day 15 the mean granulocyte count decrease was 2.0×10^9/L, whereas the mean lymphocyte count decrease was only 0.2×10^9/L). No appreciable depression in platelet counts resulted. Bone marrow depression was dose-limiting in one patient.

Two deaths occurred within 90 days after interferon administration began. The first was a 64-year-old woman who died 50 days after interferon was discontinued but whose death is considered to be possibly related to interferon. Before treatment she was hypoxic with an arterial Po_2 of 68 mm Hg on the basis of biopsy-proven pleural and pulmonary metastases and biopsy-proven interstitial fibrosis. Within the first 3 weeks after initiation of interferon the patient complained of dry eyes, dry mouth, thick respiratory secretions, and generalized muscle weakness. Decreased size of involved lymph nodes was noted by day 28, but the severity of her fatigue necessitated interferon discontinuation. One week later intubation was needed for respiratory insufficiency and generalized weakness. She was begun on combination chemotherapy. She died of leukopenia and sepsis 41 days into the combination chemotherapy program. The second patient who died was a 47-year-old woman with extensive metastatic involvement including central nervous system, retina, bone, lung, pleura, and liver. She died of progressive disease on day 10 of therapy.

IMMUNOLOGIC FUNCTION

Measurement of lymphocyte proliferative responses to mitogens and quantitation of T cells by sheep erythrocyte rosettes in four patients failed to show any consistent change after interferon administration was begun. Spontaneous and antibody-dependent cell cytotoxicity, ascertained in nine patients, was significantly enhanced ($p < 0.01$) 48 hours after initiation of interferon (Figure 1). However, despite continued daily administration of

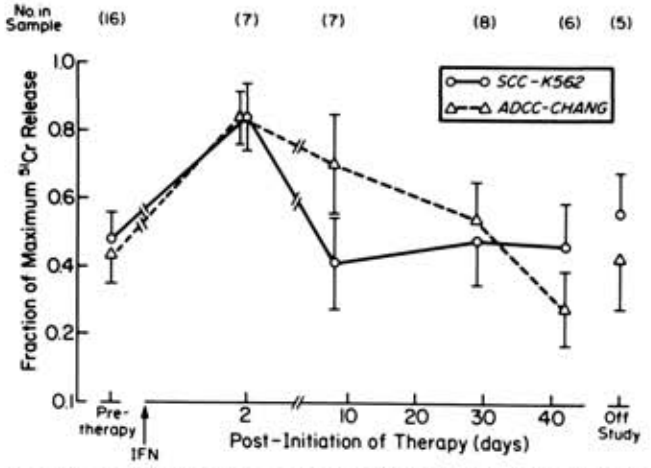

Figure 1. Effects of daily interferon-alpha administration on relative levels of spontaneous (*SCC*) and antibody-dependent (*ADCC*) cellular-cytotoxicity in patients with breast carcinoma. The SCC (0) and ADCC (Δ) levels of peripheral mononuclear cells were assessed on 2 days before initiation of therapy (pretherapy and before treatment on day 1); during therapy (on day 2 and after 2 days of interferon and day 7 after 7 days); and 1 week or more after termination of therapy (off study). Except for the pretreatment values, for which each patient had at least two samples obtained, the number in sample refers to number of patients tested. Results for each patient were normalized by expressing all specific ^{51}Cr-release values relative to the peak ^{51}Cr-release value obtained in that patient during therapy. Pooled results were plotted as the mean ± SE. At 48 hours, both SCC and ADCC were significantly elevated when compared to the values before therapy ($p < 0.01$, Student's *t*-test, two-tailed). Effector to target cell ratio = 50:1.

interferon, spontaneous and antibody-dependent cell cytotoxicity decreased to basal levels over the subsequent weeks. Spontaneous cell cytotoxicity declined somewhat more rapidly than antibody-dependent cell cytotoxicity (Figure 1). After 1 month only a single patient, whose disease improved although not enough to consider a partial response, had persistently elevated spontaneous and antibody-dependent cell cytotoxicity. Although an overall correlation existed between increased spontaneous and antibody-dependent cell cytotoxicity, a clear disassociation occurred in one patient. This woman had basal and enhanced antibody-dependent cell activity with no natural killer cell activity when measured on days 8, 29, and 56 of interferon administration.

The β_2-microglobulin, a protein expressed on all cells as part of the HLA complex, may be important in antigen recognition by lymphocytes (17). It was quantitated by radioimmunoassay in serum of eight patients (Figure 2A). The β_2-microglobulin concentration was increased on day 15 ($p < 0.05$, sign test) and remained significantly augmented on day 22 ($p < 0.005$). To confirm the specificity of this elevation, β_2-microglobulin was measured in a comparable group of women beginning cytotoxic chemotherapy for metastatic breast carcinoma (Figure 2B). No comparable enhancement in β_2-microglobulin concentration occurred either on day 15 or day 22 after therapy initiation in this control group.

PHARMACOKINETICS

All patients who had the assay done had detectable interferon in their serum at some point during the period of administration. Although serum levels were relatively constant in each patient, levels differed widely between patients (Table 3). Peak titers occurred 6 hours after interferon administration and did not correlate with body surface area. Titers (±SE) after 15 days of interferon therapy at two hours after administration (50 ± 10 U) were somewhat higher than on the first day of interferon. However, by 6 hours the mean titer was comparable (60 ± 10 U) to that found on the first day (50 ± 14 U). Dose escalation from 3 to 9×10^6 units resulted in more persistent and at least threefold higher peak serum levels (Table 4). After 24 hours, interferon titers were markedly greater at the higher dose.

PREDICTORS FOR RESPONSE

The median age of patients who responded, 64 years, was significantly greater than that of nonresponders, 55 years ($p = 0.05$ by Mann-Whitney U test). Neither response nor progression correlated with menopausal status, estrogen receptor positivity, previous hormonal therapy, disease-free interval, or body surface area. The severity of weight loss, fever, or leukopenia resulting from interferon administration did not clearly predict for response. Fever blisters developed more frequently in patients who responded (three of five) than those who did not (two of 18). Whether this presumed viral activation represented a true predictive tissue sensitivity to interferons will need confirmation in future trials. Within limitations of the patient sample size, neither baseline spontaneous and antibody-dependent cell cytotoxicity, percent boosting of spontaneous and antibody-dependent cell activity, β_2-microglobulin levels, nor interferon serum levels were predictive of response. However, because of their potential role in mediating tumor regression, these variables should be measured in subsequent trials.

Discussion

This multi-institutional trial has confirmed earlier reports of objective regressions of recurrent neoplasms after administration of buffy-coat-derived interferon (9, 11, 18-19). The objective partial response rate (95% confidence interval for true response frequency: 7% to 44%) was comparable to a previous trial that used a similar dose in metastatic breast carcinoma (9). It is, however, not yet established what role interferons will have in therapy of human breast cancer. Duration of response in this trial, in which interferon was discontinued, was relatively short (mean, 92 days). More durable responses were

Table 3. Interferon Titers After Intramuscular Dose of Leukocyte Interferon*

Hours After Dose	Patients	Serum Interferon Titer Range	Mean Serum Interferon Titers (± SE)
	n	*U*	
0	18	0-5	0 ± 0
2	18	0-135	25 ± 8
6	17	0-208	50 ± 14
24	16	0-29	9 ± 2

* Dose of 3×10^6 U on day 1 of interferon administration.

Table 4. Effect of Interferon Dose on Serum Titers

Hours After Dose	Serum Interferon Titers*	
	After Dose of 3×10^6 U	After Dose of 9×10^6 U
	U	
2	65 ± 25	230 ± 45
6	62 ± 27	150 ± 45
24	6 ± 4	50 ± 50

* Values are expressed as ± SE based on four samples.

achieved in breast carcinoma (mean, 196 days) in the previous trial (9) by continued administration of interferon three times weekly to all responding patients.

Although common side effects of cancer chemotherapy such as vomiting and alopecia were not observed, interferon was not free of toxicities. Fatigue and anorexia with associated weight loss were potentially dose-limiting. Bone marrow depression occurred but was dose-limiting in only one patient. A previously undescribed occurrence of decreased mucosal and glandular secretion resulted in the progressive deterioration of one patient and probably contributed to her eventual death. Fever during the first few days of interferon administration was easily managed clinically. To what extent such toxicities may have resulted from impurities in the current interferon preparation will be defined as purer materials enter clinical trial. However, the antiproliferative effect of pure interferons in vitro (20, 23) and the pyrogenic effect of a pure preparation (24) suggest they will be a property of interferons.

Although estrogen receptor status of patients did not correlate with disease response, older patients were more likely to respond than younger ones. Re-analysis of the earlier trial in metastatic breast carcinoma (9) for this variable verified its predictive significance. In the previous trial, a correlation of response and nadir granulocyte count occurred. This did not reach significance in the current trial. However, pooling the results of the two trials continued to indicate a correlation of granulocyte nadir and disease response in metastatic breast carcinoma (nine of 18 patients with less than 1.5×10^9 granulocytes/L responded versus two of 22 patients with more than 1.5×10^9 granulocytes/L; chi-squared analysis, $p < 0.01$). In both these trials, a threefold dose escalation resulted in no clear increase in response. This finding suggests the possibility that some persons are biologically less sensitive to the effects of interferon alpha.

Fresh allogeneic tumor cells can be effectively lysed by boosting of spontaneous-cell-mediated cytotoxicity activity with interferons (25-26). Interferons have not been shown, however, to augment spontaneous cell cytotoxicity for autologous tumor cells in vitro (26). Thus, although increases in spontaneous and antibody-dependent cell cytotoxicity may be useful parameters for defining a biologically effective dose, clinical studies will be needed to ascertain what role augmentation of immune effector cell activity, when compared to direct antiproliferative effects of interferons, have in tumor regression. It may well be that other parameters, such as activation of 2-5A-synthetase or changes in the cell surface as measured by HLA antigen or β_2-microglobulin expression, may prove a better correlate with the antitumor effects of interferons.

Additional programs for producing naturally produced interferons from buffy coat leukocytes, lymphoblastoid

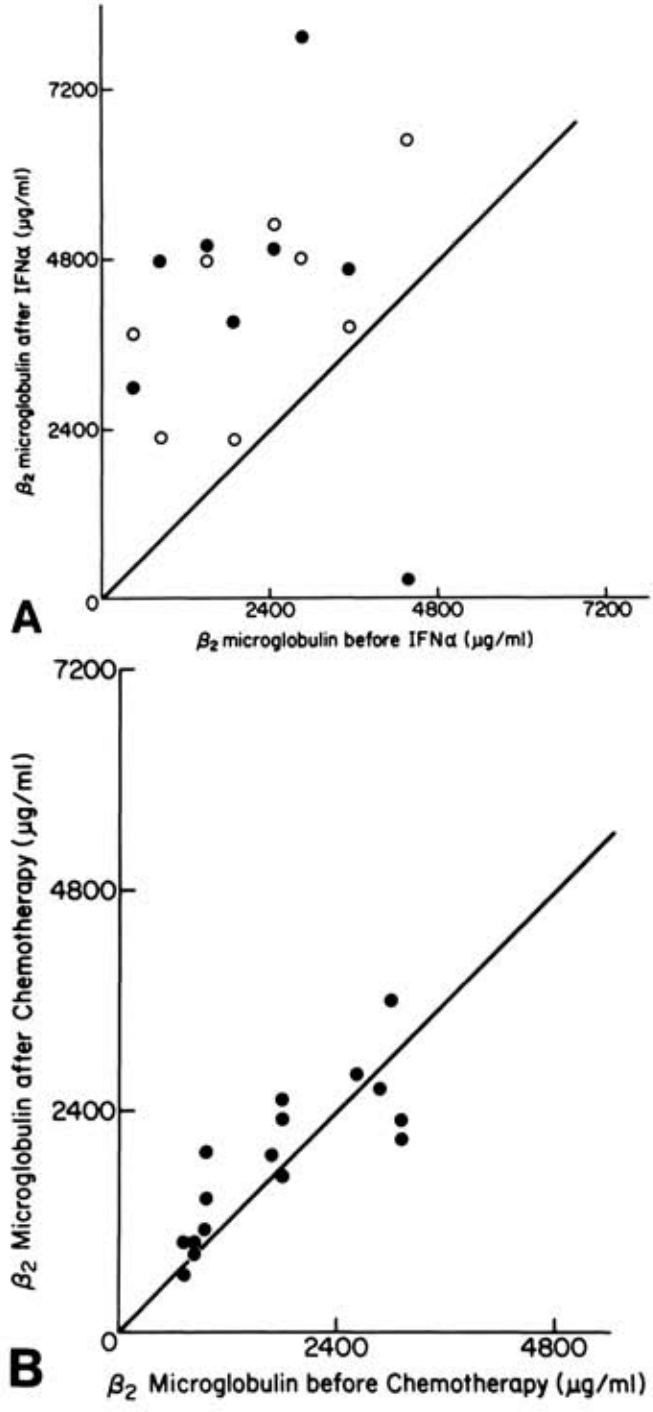

Figure 2. The β_2-microglobulin levels in patients with metastatic breast carcinoma. Each data point represents a patient measurement. Any increase or decrease after initiation of treatment is indicated by relation to the line of equivalence. **A.** Patients treated with 3×10^6 U of interferon alpha. ● = day 15 of interferon. ○ = day 22 of interferon. **B.** Patients treated with cytotoxic chemotherapy. Data obtained 15 days after treatment.

cells, and diploid fibroblasts have been initiated. Recombinant DNA technology will make available large quantities of bacterially-produced, human interferon alpha and beta subspecies. These increased supplies will make possible the definition of biologically effective and maximally tolerated doses. Conventional phase I trials to define optimal dose, route, and schedule are only currently underway. Measurement of pharmacokinetics in such trials should aid design of an optimal administration schedule.

Clinical use of interferons in breast carcinoma should at present remain limited to research programs. At the dose used in this trial, interferon alpha was no more active than other available single agents for metastatic breast cancer. However, optimal dose of interferons has not yet been defined. Interferons probably do differ significantly from other drugs in their mechanism of antitumor action. Data from experimental systems suggest interferons can be beneficially combined with chemotherapy and radiation therapy (27-29). Evaluation of combined modalities should only begin once an optimal dose is identified in Phase I and Phase II studies. At that time, prospectively randomized trials will define what role interferons may have as part of multimodality therapy for primary and metastatic breast carcinomas.

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