

Safety and Effectiveness of Long-term Interferon Gamma Therapy in Patients with Chronic Granulomatous Disease

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ABSTRACT: In chronic granulomatous disease (CGD), diminished or absent neutrophil NADPH oxidase function leads to recurrent pyogenic infections and granuloma formation. In a recent randomized, placebo-controlled trial, short-term prophylactic use of recombinant human interferon gamma (rIFN- γ 1b) reduced the risk of serious infection in CGD patients by 67%. The current study evaluated the safety and effectiveness of long-term rIFN- γ therapy in CGD patients. Patients were treated three times weekly with rIFN- γ and evaluated semiannually. Serious infections (requiring hospitalization and parenteral antibiotic therapy), adverse clinical events, and measures of growth and development were noted. Thirty patients were evaluated for 12 months. The total average duration of rIFN- γ therapy was 2.5 years. Three patients developed a total of four serious infections (0.13 infections per patient year). This rate compares favorably with rates of 1.10 and 0.38 infections per patient year found in the placebo and rIFN- γ groups, respectively, during a previous study. Common adverse events were fever (23%), diarrhea (13%), and flu-like illness (13%). No serious adverse event was attributable to rIFN- γ therapy and no obvious effects on growth and development were observed. rIFN- γ is a safe and effective adjunctive therapy for reducing the frequency and severity of serious infections in CGD patients.

Keywords: chronic granulomatous disease, interferon gamma, interferons, neutrophils, phagocytes, NADPH oxidase.

INTRODUCTION

Neutrophils constitute the first line of defense against invading pathogens. Their effectiveness requires quick and carefully controlled generation of toxic oxygen compounds, which are lethal to the majority of pathogenic invaders. When exposed to suitably opsonized microbes, phagocytes undergo a respiratory burst, and increase their oxygen consumption as much as

100-fold (1). This response can be further augmented, particularly in monocytes, by pre-exposure to lipopolysaccharides or certain cytokines (2,3). The phagocyte NADPH oxidase, assembled on the interior of the cell membrane, reduces molecular oxygen to the reactive species superoxide, which in turn can form hydrogen peroxide, hypochlorous acid, and perhaps other microbicidal molecules (1).

The NADPH oxidase is a complex enzyme

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composed of four separate protein subunits, each of which is critical to oxidase function. Two of these proteins, designated gp91-*phox* (phagocyte oxidase) and p22-*phox*, are components of the membrane protein cytochrome b_{558} (4). Catalytic activity requires two cytosolic components, p47-*phox* and p67-*phox*, to be translocated to the membrane surface. Proper oxidase regulation and function also absolutely require GTP as well as *ras*-like proteins *rac*-2 or the highly homologous *rac*-1 and possibly *rac*-1a (4).

The oxidase components are encoded by a series of unrelated genes, each located on a different chromosome. Defects in any of the four genes can result in the loss of oxidase activity. Defects in p22-*phox*, p47-*phox*, and p67-*phox* are inherited in an autosomal recessive fashion, while gp91-*phox* deficiency is transmitted in X-linked fashion (4). A variety of mutations has been described for each of the involved genes (4).

Until recently, the mainstays of clinical management of CGD patients have been infection avoidance measure, prophylactic antibiotic therapy (with trimethoprim-sulfamethoxazole and others), and aggressive management of documented infections (5). While this approach has improved survival for many patients, the disease still carries substantial morbidity, with rates of severe infections on the order of 1.1 per patient year (6).

In 1988, clinical trials with recombinant human interferon gamma (rIFN- γ) were begun, based on in vitro experiments which showed that the immunostimulatory effects of rIFN- γ might be able to overcome the defect in CGD neutrophils. The results of a randomized, double-blind, placebo-controlled Phase III trial with an average treatment duration of nine months were published in early 1991 (6). In that multicenter trial, 63 patients receiving rIFN- γ had a 67% reduction in the risk of serious infection (defined as infection requiring hospital admission and parenteral antibiotic therapy) and approximately one-half as many serious infections as the 65 patients receiving placebo.

Early evidence from experiments conducted on neutrophils and monocytes from one pair of brothers with X-linked CGD suggested that

interferon gamma directly increased the production of superoxide by neutrophils, thereby conferring improved microbicidal activity and reducing clinical infections (7). Several recent studies, however, have shown that in the vast majority of patients interferon gamma does not reverse this intrinsic biochemical defect (6, 8, 9). As yet, it is not known how rIFN- γ works in CGD, although rIFN- γ 's mechanism of action in decreasing severe infections is currently under investigation.

This study was conducted to further evaluate the long-term efficacy of rIFN- γ and to monitor patients for the development of any unforeseen adverse effects resulting from prolonged therapy.

METHODS

Patients

Enrollment in this study was offered to all patients with a confirmed diagnosis of CGD who were previously enrolled at the Scripps Research Institute in either the rIFN- γ Phase III or compassionate-use studies. Enrollment was also offered to CGD patients from other study centers who were able to travel to Scripps for the required periodic evaluations.

Eligibility criteria included the following: diagnosis of CGD confirmed by both abnormal stimulated neutrophil NBT slide test and neutrophil superoxide production less than 20% of normal; preserved renal (creatinine <2.0mg/dL, proteinuria <2+), hepatic (bilirubin <1.5mg/dL, prothrombin time <1.3 x control), and hematologic (WBC>3,000/mm³, neutrophil >1500/mm³, platelets >100,000/mm³) function; minimum life expectancy of 3 months; and full recovery from previous surgery or serious infection. Patients who were pregnant, lactating, or not using adequate contraception were excluded.

Informed consent was obtained from adult patients or from the parents of minor children.

Study Medication

Recombinant human interferon gamma

(Actimmune® Interferon gamma-1b; Genentech, Inc., South San Francisco) was supplied in 0.5 mL vials containing 100 µg of drug in sterile diluent.

Study Design

All enrolled patients received the study medication subcutaneously three times per week. Body surface area was used to determine dose; those patients ≥ 0.5 m² received 50 µg/m² per dose, and those < 0.5 m² received 1.5 µg/kg per dose.

All patients were interviewed and examined at the Scripps Research Institute every 6 months for 1 year. These visits included medical history, physical examination (including height and weight), review of medication diary, complete blood count, chemistry profile (electrolytes, BUN, creatinine, albumin, liver enzymes, iron, cholesterol, triglycerides), quantitative immunoglobulins, urinalysis, endocrine function tests (FSH, LH, testosterone or estradiol, T₄, TSH), developmental evaluation (Tanner staging), and assay for IFN-γ antibodies. IFN-γ antibody assay was performed at Genentech, using a radioimmunoassay method (10). All other laboratory testing was done in the clinical pathology laboratory at Scripps Clinic using standard assays.

Data were recorded on case report forms for all serious infectious events, those requiring both hospitalization and parenteral antibiotics. Notation was made of the date of diagnosis, hospitalization dates, type of infection, and type and route of medication prescribed. All adverse clinical events were also recorded, noting type, severity, and duration of symptoms. A subjective assessment of their relationship to rIFN-γ therapy was made by the principal investigator (JTC).

Data Analysis

Baseline characteristics, serious infections and adverse events were compared with data from both the placebo- and the rIFN-γ treated groups of the Phase III study. Measurements of height and weight were compared with standardized national percentiles.

RESULTS

Study Population

From March through October of 1991, 40 consecutive patients evaluated at the Scripps Research Institute were eligible for this study. Of this group, 30 patients (25 males and 5 females) were enrolled, and all 30 completed the first year of follow-up in this multi-year study. These patients included 10 of the previous 16 Phase III clinical trial participants from Scripps, 1 Phase III patient from another center, all 5 of the patients receiving rIFN-γ on a compassionate-use basis at Scripps, and 8 patients on compassionate use at other centers in the U.S. and Canada. In addition, 6 new patients were enrolled.

Six patients enrolled in the Phase III trial at Scripps were not enrolled on this Phase IV study: 2 were lost to follow-up, 1 declined enrollment because of prior severe headaches from rIFN-γ, 1 declined for personal reasons, 1 patient died of *Pseudomonas cepacia* sepsis after conclusion of the Phase III study, and 1 patient transferred to another center during the Phase III study. Four new patients declined enrollment: 1 because of previous side effects (headache and malaise) from rIFN-γ and 3 because their CGD was clinically mild.

Patient characteristics in this group were not significantly different from the Phase III study groups with regard to age, sex, pattern of inheritance, and use of prophylactic antibiotics (see Table 1). Patients ranged from 0.8 to 34.7 years of age. Moreover, the distribution of patients with the four major types of CGD was comparable to that observed in two large series (Table 1) (4).

The duration of rIFN-γ therapy ranges from 1.0 to 4.3 years (including previous therapy during Phase III and compassionate-use studies), with an average duration of therapy of 2.5 years per patient. Therefore, the cumulative treatment experience with rIFN-γ was 75 patient years overall and 31 patient years in this study.

During this study the median number of missed doses per patient was 14.5. Six patients received all doses, 13 patients missed < 20 doses, 8 patients missed between 20 and 49 doses, and 3 patients missed 50-185 doses. Nine patients had

treatment temporarily held or reduced because of adverse events, which included: abnormal liver function tests (2 patients), neutropenia (3 patients, ages 1.6, 5.8, and 28.9 years), hallucinations, headache with joint stiffness, rule-out sepsis episode, and chills with fever. All patients resumed full schedules of therapy and all events were resolved except abnormal liver function tests in a 23-year-old male (described below),

headaches with joint stiffness in a 14-year-old male, and chills with fever in a 17-year-old male.

Twenty-eight of the 30 patients under observation received concomitant prophylactic antibiotic therapy according to a predetermined regimen based on age and history of medication allergy (Table 1). Twenty-five patients received trimethoprim-sulfamethoxazole.

Table 1. Characteristics of Patients with CGD Compared with Phase III Study Patients

	Phase III Placebo ^a	Phase III rIFN- γ ^a	Phase IV rIFN- γ	p Value ^b
Number of patients	65	63	30	
Age at enrollment (years)	15.0 \pm 9.6	14.3 \pm 10.1	12.8 \pm 9.8	0.26
Sex:				
Male	53 (82%)	51 (81%)	25 (83%)	0.79
Female	12 (18%)	12 (19%)	5 (17%)	
Inheritance:				
X-linked	41 (63%)	45 (71%)	23 (77%)	0.31
Autosomal ^c	24 (37%)	18 (29%)	7 (23%)	
Prophylactic antibiotics	55 (85%)	56 (89%)	28 (93%)	0.32

^aPhase III patients have been described previously (6).

^bAll Phase IV patients (n = 30) compared with all Phase III patients (n = 128).

^cOf the seven Phase IV patients with autosomal inheritance, one had p22-*phox* deficiency (3% of all Phase IV patients), three had p47-*phox* deficiency (10%), and three had p67-*phox* deficiency (10%). The autosomal recessive patients in the Phase III study were not similarly subclassified.

Infection Analysis

During the 12 months of evaluation during this study, 3 (all of whom have X-linked CGD) of the 30 patients were hospitalized for a total of four serious infections (see Table 2). All 3 patients were treated by their primary physicians for these serious infections. All 3 patients received empiric broad spectrum antibiotic therapy, and 2 required surgical intervention as well (lymphadenectomy; incision and drainage). In two of these infections, rIFN- γ was continued through the acute episode. Each patient recovered fully. It is interesting to note that the responsible pathogen, presumably bacterial, could not be identified in any of these episodes.

The overall rate of serious infection was 0.13

infections per patient year. This rate is lower than that seen in the placebo arm of the published Phase III study (rate = 1.10) and compares favorably with the Phase III rIFN- γ arm (rate = 0.38) (Table 3). In addition to having fewer infections, these patients required shorter hospital stays. Hospitalizations for the 3 patients with infections totaled 68 days, for an average stay of 23 days per patient. This is better than average stays of 48 and 32 days, respectively, in the placebo and rIFN- γ arms of the previous study (Table 3).

Postpubertal and adult patients showed no decrement in sexual function historically, or in measured sex hormone levels. Both postpubertal females reported normal menses. One patient fathered a normal child during the course of this study.

Table 2. Serious Infections

Age/Sex	Type of CGD	Infection ^a	Days in Hospital	Months on Study	Months on rIFN- γ
31 yo Male	X-linked	Pneumonia	13	0.4	0.0
		Perigastric Lymphadenitis	24	4.9	4.9
8 yo Male	X-linked	Sepsis-like illness	22	1.9	12.1
9 yo Male	X-linked	Cutaneous abscess	9	4.6	30.2

^aSerious infections were defined as an episode requiring both hospitalization and parenteral antibiotics.

Table 3. Efficacy of Recombinant Human Interferon in Preventing Serious Infections in CGD

Variable	Clinical Study		
	Phase III Placebo (n = 65)	Phase III rIFN- γ (n = 63)	Phase IV rIFN- γ (n = 30)
Average duration of therapy (yrs)		0.74	1.03 (2.5) ^b
Patient years of observation on study ^a	50.9	52.1	31.0
Total serious infections	56	20	4
Serious infections per patient year	1.10	0.38	0.13
Number of patients with at least one serious infection	30 (46%)	14 (22%)	3 (10%)
Average hospital stay (days)	48	32	23

^aPatient years of observation for the Phase III study were computed as follows: the observation period was computed as the number of days from randomization to the study termination date. However, if a patient was still hospitalized at the time of study termination, the observation period was extended up to discharge.

^bPhase IV study plus previous use.

Adverse Events

The adverse events reported during this study by more than 10% of patients were mild to moderate in degree and included fever (23%, n=7), diarrhea (13%, n=4), and flu-like illness (13%, n=4). Fever occurred less frequently than in the previous Phase III study (52%), suggesting that tolerance to this side effect may develop overtime. Diarrhea was reported in 14% of the patients in the Phase III study; this was similar to the rate reported in the placebo group. Asthma was also reported by 4 patients, but in 2 of these the symptoms antedated rIFN- γ therapy and in the other 2 the symptoms were mild and judged to be unrelated to the drug. One patient experienced a new severe adverse event, a left diaphragmatic hernia, which was judged to be unrelated to rIFN- γ therapy.

Twenty-one patients (0.68/patient year) experienced non-serious infections or CGD-related illnesses. This rate compares favorably with that in the Phase III study of 0.60 and 0.65, respectively, in the rIFN- γ and placebo groups (11). Seven events were severe and included esophageal granuloma, duodenitis, gastric outlet obstruction, pharyngitis, ulcerative colitis (two episodes in one patient), and granulomatous dermatitis. Mild and moderate events included balanitis, bronchitis, conjunctivitis, cutaneous and rectal abscesses, fungal dermatitis, gastric outlet obstruction, lymphadenopathy, otitis media, pneumonia, presence of hepatitis B surface antigen, stomatitis, tuberculosis skin test reactivity, upper respiratory infection, and varicella zoster infection.

Four patients (0.13/patient year) developed neutropenia (<1500 cells/mm³). The patients were male with ages 1.6, 2.2, 5.8, and 8.4 years. The rate for the placebo group in the Phase III study was 0.10 (11). One patient, age 5.8 years, developed an absolute neutrophil count of less than 1,000/mm³. This occurred after 7 months of treatment; the neutropenia (970 cells/mm³) resolved after rIFN- γ was temporarily withheld.

Five patients developed both SGOT and SGPT elevations and 2 patients had at least one measurement greater than three times the upper limit of normal. One, an 0.8 year old infant who

previous to this study developed cholestasis while on rIFN- γ and who was on a reduced dose of rIFN- γ , was treated with a full dose as the enzyme elevations resolved. The other patient, an adult male with two years of prior interferon therapy, was noted to have moderate, asymptomatic elevation of transaminases at the time of enrollment on this study and was started on a reduced schedule. He later developed an elevation five times that of normal, requiring temporary cessation of therapy. His liver enzyme abnormalities improved but did not resolve with dose modification; he continued to have mild to moderate transaminasemia after resumption of full-dose rIFN- γ therapy. In the Phase III study, no patient has hepatic enzyme elevation ≥ 3 times that of normal.

Growth and Development

Twenty-three of the 30 patients were actively growing and developing at the time of the study; the other 7 were physiologically adult. The majority of patients maintained steady growth and development during the 1-year observation period.

Children with CGD tend to be shorter than their peers, with 84% of the patients in this study measuring below the 50th percentile for age and 67% measuring below the 25th percentile. The majority of children remained within 10 percentile points of their baseline height during the study. Two children exceeded this: one changed from the 10th to the 27th percentile and one from the 22nd to the 76th percentile. Four children experienced decreasing height percentiles as follows: 21 to 3, 50 to 32, 26 to 13 and 28 to 14.

Weight percentiles standardized for height were used for patients with heights ≥ 55 cm and <139 cm (females) and <149 cm (males). Nine (53%) of the 17 patients who met these height parameters exceeded the 50th weight percentile at study entry. After 12 months 6 patients had increased their weight by 10 or more percentile points and 2 patients had decreased their weight by 10 or more percentile points. Of the 13 patients exceeding 138 cm (females) and 148 cm (males) in height, a weight increase of >2 kg was seen in 9 patients and a weight loss of >2 kg was seen in 1 patient.

Of the 3 patients ages 15 to 17 enrolled in the

study, 3 demonstrated Tanner Stage 4 or 5 development and had normal testosterone or estrogen levels for their age. The one exception suffered from severe granulomatous colitis, requiring enteral hyperalimentation during part of the study. By the end of the year, however, his testosterone level had risen to normal for his age. No patients below age 11 showed evidence of puberty ($n = 17$), and 3 patients between ages 11 and 14 were progressing appropriately.

Other findings of interest included one prepubertal male patient who was discovered to have thyroid stimulating hormone levels (TSH) elevated less than two-fold. He remained clinically euthyroid throughout the year of observation. Also, an adolescent male patient (who had previously received rIFN- γ therapy for 2 years without event) developed psychotic depression accompanied by visual hallucinations. His symptoms did not improve following withdrawal of rIFN- γ therapy, so treatment was resumed without change in symptoms. Finally, no antibodies to rIFN- γ were detected in any of the 84 samples collected during the course of this study.

Discussion

In the past few years, several cytokines have been introduced into clinical practice, most often as brief therapies for acute medical conditions. As yet, cumulative clinical experience with chronic use of these agents remains limited. Before committing patients, particularly children, to chronic cytokine therapy, one must be satisfied that the clinical effectiveness of the proposed treatment does not wane over time, and that there is no late or cumulative toxicity. In this regard, the present study indicates that the use of rIFN- γ in CGD is safe and has measurable benefit in reducing the frequency and severity of serious infections, even after two and one-half years of continuous treatment. Studies like this one are therefore important in guiding ongoing clinical management.

In this study, all 3 patients who developed serious infections had X-linked CGD. It has been observed that patients with cytochrome b_{558} -

negative CGD (gp91-*phox* deficient and p22-*phox* deficient) have a more severe clinical course than those patients with cytochrome b_{558} -positive disease (p47-*phox* deficient and p67-*phox* deficient) for reasons as yet unclear (5, 12, 13). The low infection rate for autosomal patients in this study is likely due to characteristics of the underlying disease, and not to a differential effect of rIFN- γ in this subgroup. Nevertheless, the low serious infection rate observed in this study group compared with that of the Phase III rIFN- γ treatment group is reassuring.

Factors independent of rIFN- γ may contribute to the improved serious infection rate seen. For example, unlike the Phase III study, the current study involved only one research center. It is possible that the data reflect some element of single institution case management bias, although all of the infectious episodes were managed by patients' primary physicians, with telephone consultation with the principal investigator if needed. While inclusion of nontreated controls in the study might have ruled out this bias, it would not have been ethical to include such a group. It is also possible that the perception of medication benefit by patients and their parents may have enhanced compliance with all aspects of the treatment plan.

Of some concern is the theoretical possibility that cytokine therapy may enhance some components of the immune system while suppressing others, leading to an increase in opportunistic infections. However, the spectrum of infections encountered by patients receiving rIFN- γ appears to be similar to those experienced by untreated CGD patients. The similar rates of these non-serious illnesses for patients in this study and those in the Phase III study suggest that no significant immune suppression is present.

The most common adverse effects encountered during this study have been previously noted (6). Only 1 patient experienced a severe adverse event (diaphragmatic hernia) and this was judged to be unrelated to rIFN- γ therapy. It is interesting to note that the distribution of fever varied with the age of the patient. Forty-four percent of patients between ages 1 and 5 reported fever compared with 38% of patients between 6 and 11 and only 8% of patients 12 years and older.

Slightly higher rates of neutropenia and transaminasemia >3 times that of normal were seen in this study compared with the Phase III placebo patients; however, these did not differ significantly from the Phase III rates. Of note is the observation that all three children under 3 years of age experienced either neutropenia or hepatic transaminasemia. In a compassionate-use study of patients with CGD treated with rIFN- γ , six of nine patients less than 1 year of age reported abnormal liver function tests; in four of these infants the abnormalities were considered severe and therapy was discontinued in two patients.

One of the nine infants experienced neutropenia <1,000 cells/mm³ (11). Although data from the placebo-controlled study (6) showed no difference between treatment groups in the rate of abnormal liver function tests or neutropenia, only six children under 5 years of age included in that study were treated with rIFN- γ . Also, higher doses of rIFN- γ have been noted to result in both (14). These young patients, as do all patients, require careful monitoring, for neutropenia and transaminasemia. Although there still may be late toxicities that will not be apparent for many more years, the present data do not reveal any worrisome trends or clues.

Of interest was the finding of elevated TSH in one patient. Thyroid abnormalities have been seen in breast cancer patients treated with IFN- α , but previous thyroid function abnormalities have not been seen in CGD patients receiving rIFN- γ (6, 15, 16). An idiosyncratic reaction of rIFN- γ therapy cannot be excluded; however, this may also represent early hypothyroidism.

This study does not attempt to elucidate the underlying mechanism by which rIFN- γ modulates the immune response in CGD patients. Additional research will be needed to provide further insight into this more difficult and potentially far-reaching question. However, it is clear that the drug does not reverse the specific biochemical defect in CGD. This suggests that rIFN- γ 's immunostimulatory effects should be studied in other clinical situations. In particular, one might envision a role for rIFN- γ in other hereditary or acquired neutrophil function disorders, in systemic illnesses where immune function is compromised, or in augmenting the normal host's immune response to recalcitrant or

multidrug resistant microbial infections.

In conclusion, the clinical effectiveness of interferon gamma appears to extend beyond short-term usage when used for infection prophylaxis in patients with chronic granulomatous disease. Its long-term use does not appear to delay growth or maturation in children with this disorder, nor has any serious toxicity developed over time. Its use should be considered an integral element in the treatment of patients with chronic granulomatous disease.

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