

Randomized, Double-Blind, Placebo-Controlled, Dose-Escalating Study of Aerosolized Interferon Gamma-1b in Patients With Mild to Moderate Cystic Fibrosis Lung Disease

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Summary. Interferon gamma-1b (IFN- γ 1b) is a pleiotropic cytokine with immunomodulatory activities that could decrease bacterial burden, inflammation, and obstruction in patients with CF. Patients with CF (≥ 12 years old, FEV₁ $\geq 40\%$ predicted) were randomly assigned to sequential dose cohorts inhaling 500 μ g IFN- γ 1b, 1,000 μ g IFN- γ 1b, or placebo by Respigard II[®] nebulizer thrice weekly for 12 weeks. Sputum bacterial density and spirometry were measured. Safety, antibiotic use, hospitalization, and sputum neutrophils, elastase, DNA, IL-8, and myeloperoxidase were also evaluated. Sixty-six patients (mean age, 24 years, with mean baseline FEV₁ of 74 ± 20 (SD) percent predicted) were studied. One patient had bronchospasm after the first dose of IFN- γ 1b; the overall withdrawal rate was 15% (5 in the placebo group, 2 in the 500- μ g IFN- γ 1b group, and 3 in the 1,000 μ g IFN- γ 1b group). The 500- μ g IFN- γ 1b dose was well-tolerated, but the 1,000- μ g dose cohort, who had a higher baseline bacterial density than placebo patients (mean difference, 1.2 log₁₀ CFU/g sputum, 95% confidence interval (CI), 0.1,2.8, $P=0.04$), had 24% more hospitalizations for exacerbation than placebo patients (95% CI, 2,45%, $P=0.05$). There was a 0.12-l difference between the 500- μ g IFN- γ 1b and placebo groups with respect to the

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12-week change in FEV₁ (active group minus placebo group, 95% CI, -0.03,0.26, $P=0.11$), as compared to a 0.01-l difference between the 1,000- μ g IFN- γ 1b and placebo groups (95% CI, -0.16,0.17, $P=0.96$). No effects of IFN- γ 1b were seen in sputum bacterial density or inflammatory biomarkers at 12 weeks. Aerosolized IFN- γ 1b did not improve pulmonary function, reduce sputum bacterial density, or affect inflammatory sputum markers in patients with mild-moderate lung disease. **Pediatr Pulmonol.** 2005; 39:209–218. © 2004 Wiley-Liss, Inc.

Key words: cystic fibrosis; interferon; inflammation; aerosol; immunomodulation; *Pseudomonas aeruginosa*.

INTRODUCTION

Cystic fibrosis (CF) is a hereditary disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator gene, which codes for a cAMP-activated apical chloride channel and regulatory protein. CF is characterized by chronic endobronchial infection and inflammation, destruction of lung tissue, and respiratory failure.¹ Median life expectancy approaches 33 years, and approximately 90% of patients die of respiratory failure.^{2,3} Chronic bacterial infection in the airways of CF patients, particularly by *Pseudomonas aeruginosa* (PA), is a result of inefficient pulmonary clearance of thick secretions as well as other potential factors, including deficiencies in the nitric oxide (NO) defense system and the development of a bacterial biofilm.^{4,5} Bacterial biofilm, an unfavorable prognostic factor, resists opsonins, phagocytes, and antibiotics, and thus perpetuates polymorphonuclear neutrophil (PMN) inflammation.⁶ It is thought that the persistence of free PMN elastase contributes to bronchiectasis by multiple mechanisms, including directly damaging elastin.⁷

Interferon gamma (IFN- γ) is a pleiotropic cytokine with immunomodulatory antimicrobial, antiproliferative, and antifibrotic activities that also modulates the production or activities of several cytokines and chemokines.^{8,9} The rationale for studying IFN- γ in CF is based on several of its properties, including the ability to activate macrophages,¹⁰ correct decreased NO production in vitro,¹¹ and inhibit proliferation of murine T-helper type 2 (TH₂)

but not T-helper type 1 (TH₁) lymphocyte clones.¹² Animal model data show that C3H/HeN (TH₁-type responder) mice as compared with BALB/c (TH₂-type responder) mice with PA-induced pulmonary disease have milder inflammation, more rapid clearance of bacteria, and lower mortality.^{13,14} Animal models of chronic PA endobronchial infection indicate that lung inflammation, PMN, and microabscesses are reduced in animals treated with IFN- γ compared with controls.¹⁵ Clinical data from CF patients show deficient IFN- γ production by peripheral blood mononuclear cells, as well as a positive correlation between PA-stimulated IFN- γ production by these cells and pulmonary status.^{16–18}

Based on these data suggesting a possible beneficial effect of IFN- γ on lung infection and inflammation in patients with CF, we conducted a multicenter, randomized, double-blind, placebo-controlled, cohort dose-escalation study of aerosolized IFN- γ 1b (Actimmune[®], InterMune, Inc., Brisbane, CA) to examine its safety and efficacy in patients with CF. IFN- γ 1b is a purified recombinant protein that is licensed in the United States as a subcutaneous treatment for chronic granulomatous disease and severe malignant osteopetrosis. The most common adverse effects with subcutaneous IFN- γ 1b therapy are flu-like symptoms such as fever, headache, chills, myalgia, or fatigue that may decrease in severity as treatment continues or be minimized by pretreatment with acetaminophen. Less frequently, dose-related leukopenia, neutropenia, and abnormal liver function tests occur. IFN- γ 1b was also studied in other diseases, including renal-cell carcinoma, pulmonary fibrosis, and infections caused by bacteria, fungi, viruses, and parasites. We assessed IFN- γ 1b treatment administered by inhalation thrice weekly over 12 weeks in patients with CF with mild-moderate obstructive lung disease.

METHODS

Objectives

The primary objectives of this study were to determine 1) the safety and tolerability, 2) the effect on pulmonary function as measured by mean absolute change in FEV₁ from baseline to week 12, and 3) the effect on infection as measured by mean change in sputum bacterial density (log₁₀ CFU/g), from baseline to week 12, of inhaled IFN- γ 1b over 12 weeks in CF patients with mild-moderate

ABBREVIATIONS

CF	Cystic fibrosis
CFU	Colony-forming units
CI	Confidence interval
DSMB	Data and Safety Monitoring Board
FEV ₁	Forced expiratory volume in 1 sec
FVC	Forced vital capacity
HLA	Human leukocyte antigen
IFN- γ	Interferon-gamma
IL-8	Interleukin-8
IP-10	Interferon-inducible protein 10
MDR-TB	Multidrug-resistant <i>Mycobacterium tuberculosis</i>
NO	Nitric oxide
PA	<i>Pseudomonas aeruginosa</i>
PMN	Polymorphonuclear neutrophils
TDN	Therapeutics Development Network
TH ₁	T-helper type 1
TH ₂	T-helper type 2

obstructive lung disease. The secondary objectives were to determine: 1) the proportion of patients receiving treatment with systemic and/or anti-PA antibiotics at home or in the hospital; 2) the proportion of patients hospitalized for pulmonary exacerbation; and 3) the levels of IL-8, elastase, myeloperoxidase, DNA, and PMN in sputum.

Eligibility Criteria

Inclusion criteria were as follows: ≥ 12 years of age; informed consent; diagnosis of CF (sweat chloride ≥ 60 meq/l by quantitative pilocarpine iontophoresis test or two CFTR mutations and one or more clinical findings consistent with CF); FEV₁ $\geq 40\%$ predicted for patients ≥ 18 years of age and $\geq 45\%$ predicted for patients < 18 years of age; ability to expectorate ≥ 1 g sputum; baseline room air oximetry $\geq 90\%$ saturation; for females, negative pregnancy test and agreement to use contraception during the study period; stable clinical status (no exacerbation during the 14 days before study drug administration); no hemoptysis ≥ 60 cc during the 30 days before study drug administration or prior history of massive hemoptysis (> 240 cc at one time); no isolation of *Burkholderia cepacia* within 6 months before study drug administration; and no severe abnormalities in liver function tests and hematology parameters. Pregnant or breast-feeding women were excluded. Patients receiving dornase alfa (Pulmozyme[®], Genentech, South San Francisco, CA) or bronchodilators (inhaled or systemic) on a stable dose for ≥ 14 days before study drug administration were eligible, as were those either on chronic suppressive alternate-month inhalational tobramycin (Tobi[®], Chiron, Emeryville, CA), or those not on chronic treatment with Tobi[®] who also had not used Tobi[®] or other inhaled antibiotics (e.g., colistimethate) within 4 weeks before study drug administration. Patients continued their prestudy Tobi[®] treatments throughout the study. Finally, patients were excluded if they had received parenteral or oral anti-PA β -lactam, quinolone, or macrolide antibiotic ≤ 14 days before study drug administration or initiated systemic corticosteroid therapy ≤ 14 days before study drug administration. A stable systemic corticosteroid regimen was acceptable if started > 14 days before the first study treatment.

Study Design

Cohort 1 of this double-blind, placebo-controlled, sequential dose-escalating study received 500 μ g IFN- γ 1b or placebo, while cohort 2 received 1,000 μ g IFN- γ 1b or placebo in a 2:1 active:placebo randomization ratio. Randomization was stratified according to the presence or absence of chronic treatment with Tobi[®]. Study drug was delivered by a Respigard II[®] jet nebulizer three times a week for 12 weeks. The protocol was approved by all participants' Institutional Review Boards, and patients

were enrolled after providing informed consent. Study visits occurred on screening (day -28 to day -3), week 0 (baseline/day 1), week 1 (days 6–12), week 4 (days 22–28), week 6 (days 36–42), week 9 (days 57–63), week 12 (days 78–84), and week 14 (follow-up, days 92–98). Predosing spirometry and laboratory evaluations were performed at screening, baseline, and the week 1, 4, 6, 9, 12, and 14 visits. Sputum for culture was collected at baseline and at week 4, 6, 12, and 14 visits. Sputum for measuring IL-8, elastase, myeloperoxidase, DNA, and PMN were collected at baseline and at week 1, 4, 6, 12, and 14 visits. Sputum collection, handling, shipping, and centralized blinded analyses were performed according to standard operating procedures developed by the CF Therapeutics Development Network (TDN), with assays performed in the Microbiology, Cytology, and Inflammatory Mediator TDN Core Laboratories at the University of Washington, Case Western Reserve University, and University of Colorado, respectively, as described.¹⁹ For patients on chronic treatment with Tobi[®], sputum was collected ≥ 3 hr after a Tobi[®] dose. Pregnancy tests were obtained at baseline before study drug administration, and after all doses of study drug were administered, at week 12 or 14. Serum samples were collected at baseline and week 12 for IFN- γ levels, and at baseline and week 14 to assess antibody development; samples were analyzed centrally (Covance Laboratories, Chantilly, VA).

IFN- γ 1b and placebo (vehicle) were supplied as sterile, clear, colorless pH 5.0 liquid in 0.5-ml vials. Cohort 1 received 2.5 ml and cohort 2 received 5 ml of study drug, using a new Respigard II[®] nebulizer for each dose. For patients receiving other inhaled treatments (e.g., Pulmozyme[®], Tobi[®]), with the exception of bronchodilators, study drug was given ≥ 1 hr after morning treatments or ≥ 1 hr before or after midday or evening treatments. Patients receiving ongoing Tobi[®] were enrolled at the end of an "on" (≥ 24 days) month, and received their first dose of study drug 8–72 hr after the last Tobi[®] dose.

The first dose of study drug in each cohort was administered by study personnel. Patients using an inhaled bronchodilator on an ongoing basis self-administered the bronchodilator before the first dose of study drug. Predose FEV₁ was measured 20 min after the use of inhaled bronchodilator, and administration of the study drug followed. FEV₁ was measured 30 min after administration of study drug. If percent predicted FEV₁ decreased by $\geq 15\%$ below predose baseline at 30 min, the patient received no further study drug and was observed for ≥ 6 hr, receiving inhaled bronchodilators as necessary until deemed clinically stable before discharge home, and returned in 2 weeks for a final visit. For patients not on chronic bronchodilators, if percent predicted FEV₁ decreased by $\geq 15\%$ 30 min after study drug administration, an inhaled bronchodilator was given, and the patient was

observed and returned to the clinic for administration of the second dose of study drug under observation within 3 days. FEV₁ was measured 20 min after the use of inhaled bronchodilator, and study drug administration followed. If percent predicted FEV₁ decreased by $\geq 15\%$ of the predose baseline 30 min after the second study dose, the patient received no further study drug, was observed and received inhaled bronchodilators as necessary, and returned in 2 weeks for a final visit.

Concomitant Medications

Treatment of acute exacerbation of CF during the study period was permitted as deemed necessary by the primary physician, but initiation of chronic therapies during the 14-week study period was not permitted. Thus, initiation of chronic Pulmozyme[®], Tobii[®], or other inhaled antibiotics within 4 weeks prior to study drug administration was not permitted, but patients were allowed to receive Tobii[®] or other inhaled antibiotics during the 14-week study period for short-term treatment of exacerbations. Initiation of inhaled or systemic bronchodilator, corticosteroid, or systemic antibiotic was permitted as deemed necessary by the primary physician. Investigational or off-label therapies, including chronic azithromycin, were not permitted.

Data and Safety Monitoring Board

An independent Cystic Fibrosis Foundation-designated Data and Safety Monitoring Board (DSMB) reviewed serious adverse events and study withdrawals as they occurred, and conducted the planned safety and pulmonary function data reviews after the first 8 patients in each cohort had been treated for 4 weeks. The DSMB reviewed safety data after all patients in cohort 1 had been treated, and allowed initiation of enrollment in cohort 2.

Statistical Methods

Descriptive statistics were used to summarize the data for each time point and for changes from baseline. The primary FEV₁ analysis used a repeated-measures regression model approach to estimate the average 12-week change in FEV₁ in each treatment group.²⁰ The model-based estimates of the 12-week change and corresponding robust standard deviations from the model were then used to compare the primary endpoint between each active group and the placebo group, using a two-sample *t*-test. Comparisons between treatment groups for sputum bacterial density outcomes, as well as other continuous secondary outcome measures, were performed using two-sample *t*-tests. Categorical secondary outcome measures were assessed using a chi-square or Fisher's exact test. All statistical tests were evaluated at a two-sided 0.05 significance level. With 20 patients in each group, there was 80% power to detect a ≥ 0.16 -l difference between one

active treatment group and the placebo group with respect to the 12-week absolute change from baseline, and a ≥ 1.6 log₁₀ CFU/g difference between one active treatment group and the placebo group with respect to the 12-week change in sputum bacterial density. Additional exploratory analyses were performed to evaluate changes from baseline to other timepoints in the primary endpoints, and to investigate the 12-week change in FEV₁ after adjusting for baseline sputum bacterial density, as well as to look for specific interactions with treatment including tobramycin usage. Linear regression models were used to adjust for applicable baseline characteristics and to investigate potential interactions.

RESULTS

Study Conduct

Twelve sites participated in the study, with 2–10 patients enrolling at each site, and a total of 66 patients received at least one dose of study drug (1,000- μ g IFN- γ 1b group *n* = 24, 500 μ g IFN- γ 1b group *n* = 21, placebo, *n* = 21). One patient intolerant of the first dose (decreased FEV₁; see Methods) was excluded from efficacy analyses. Baseline demographics and clinical characteristics were generally balanced between groups (Table 1). Mean age was 24 years (range, 12–45), and 50% of patients were homozygous for the δ F508 mutation. For those with baseline sputum cultures available, 15/19 (79%) placebo, 17/19 (89%) 500- μ g IFN- γ 1b dose level and 21/23 (91%) 1,000- μ g IFN- γ 1b dose level patients had *P. aeruginosa* infection. Overall, 33% of patients were on chronic Tobii[®], 53% on Pulmozyme[®], and 23% on both. There was a spectrum of pulmonary obstruction (% predicted FEV₁ <50 in 11% of patients, 50–59 in 24%, 60–69 in 15%, 70–79 in 11%, and ≥ 80 in 39%). Study randomization resulted in higher baseline bacterial density in the 1,000- μ g IFN- γ 1b group than in the placebo group (mean difference, 1.2 log₁₀ CFU/g; 95% CI, 0.1,2.8; *P* = 0.04). Also, more patients in the 1,000- μ g IFN- γ 1b group as compared to the other groups used chronic Tobii[®], Pulmozyme[®], and systemic antibiotics (Table 1).

Safety Measures

Ten patients (15%) withdrew early. Five patients (24%) on placebo withdrew due to one episode each of erythema nodosum, pseudotumor cerebri (in a patient withdrawn from prednisone shortly before enrollment), hemoptysis, vomiting, and decreased FEV₁. Two patients (9.5%) in the 500- μ g IFN- γ 1b group withdrew, one due to an increase in liver transaminases and another due to a 17% decrease in first-dose FEV₁. Three patients (12.5%) in the 1,000- μ g IFN- γ 1b group withdrew, one due to PA pneumonia, and two withdrew consent. No violations or deviation patterns that would have substantially affected study results occurred. Compliance (average % returned empty vials)

TABLE 1—Baseline Characteristics of Study Patients

Demographics and baseline characteristics ¹	IFN- γ 1b		
	1000 μ g (n = 24)	500 μ g (n = 21)	Placebo (n = 21)
Mean age, years (range)	24 (13–37)	24 (12–43)	23 (12–45)
Sex, N (%)			
Male	10 (42)	6 (29)	9 (43)
Female	14 (58)	15 (71)	12 (57)
Race, N (%)			
Caucasian	23 (96)	21 (100)	19 (90)
American Indian or Alaskan Native	1 (4)	0 (0)	0 (0)
African-American	0 (0)	0 (0)	2 (10)
Genotype, N (%)			
Delta F508 homozygous	9 (38)	14 (67)	10 (48)
Delta F508 heterozygous	11 (46)	7 (33)	8 (38)
Unknown	4 (17)	0 (0)	3 (14)
FEV ₁ (liters)			
Mean \pm SD	2.5 \pm 1.0	2.2 \pm 0.6	2.7 \pm 0.8
Range	1.2–4.6	1.4–3.4	1.5–4.5
P value ²	0.47	0.08	
FEV ₁ , % predicted, N (%)			
<50%	4 (17)	1 (5)	2 (10)
50–59%	6 (25)	7 (33)	3 (14)
60–69%	2 (8)	4 (19)	4 (19)
70–79%	5 (21)	1 (5)	1 (5)
\geq 80%	7 (29)	8 (38)	11 (52)
Sputum bacterial density (log ₁₀ CFU/g) ³			
Mean \pm SD	7.8 \pm 0.9	6.9 \pm 1.7	6.6 \pm 2.3
Range	5.8–8.7	2.7–9.0	0–9.1
P value ²	0.04	0.66	
Use of concomitant medications, N (%) ⁴			
Chronic tobramycin	11 (46)	4 (19)	7 (33)
Dornase alfa	16 (67)	9 (43)	10 (48)
Chronic tobramycin and dornase alfa	9 (38)	2 (10)	4 (19)
Systemic antibiotics	6 (25)	2 (10)	3 (14)
Systemic corticosteroids	1 (4)	0 (0)	2 (10)

¹There were no statistically significant differences between each treatment group vs. placebo in any of these demographics and baseline characteristics, except where noted.

²Compared to placebo using a two-sided, two-sample *t*-test at alpha = 0.05 significance level.

³n = 22 for 1,000 μ g IFN- γ 1b, n = 19 for 500 μ g IFN- γ 1b, n = 19 for placebo.

⁴Each patient could be counted multiple times.

was 91%, 86%, and 76% in the 500- μ g IFN- γ 1b, 1,000- μ g IFN- γ 1b, and placebo groups, respectively.

Adverse events occurring in \geq 20% of patients in either active treatment group are shown in Table 2. The greatest differences in adverse event rates between the 1,000- μ g IFN- γ 1b group and placebo groups were with the occurrence of dyspnea (28% higher occurrence in the 1,000- μ g group; 95% CI, 3%,49%), and with the occurrence of hemoptysis (28% higher in the 1,000- μ g group; 95% CI, 3%,49%). The greatest difference in adverse event rates between the 500- μ g IFN- γ 1b group and placebo groups was with the occurrence of pulmonary congestion (24% lower occurrence in the 500- μ g group; 95% CI, -46%, -1%). Serious adverse events as defined by the FDA, including all hospitalizations, occurred in no placebo patients in cohort 1, and 3 placebo patients in cohort 2 (one admission each for hematuria, benign intracranial hypertension, and pulmonary exacerbation). One patient in the

500- μ g IFN- γ 1b group was hospitalized for exacerbation, while 8 patients (33%) in the 1,000- μ g IFN- γ 1b group were hospitalized for exacerbation (n = 7) or acute sinusitis (n = 1). No differences were observed between either treatment group and the placebo group in clinical laboratory values. No treatment-associated serum antibodies to IFN- γ were detected. Serum IFN- γ levels were below the detection limit of 15.6 pg/ml except for 3 patients: one in the 500- μ g IFN- γ 1b group with a low baseline level, one in the 1,000- μ g IFN- γ 1b group with low levels at baseline and week 12, and one in the placebo group with a low level at week 12.

Efficacy

The primary efficacy endpoints were the mean changes from baseline to week 12 in FEV₁ and sputum bacterial density. As shown in Table 3 and Figure 1, there were no

TABLE 2—Adverse Events That Occurred in $\geq 20\%$ of Patients in at Least One Active Treatment Group¹

Adverse event, n (%)	IFN- γ 1b		Placebo (n = 21)
	1,000 μ g (n = 24)	500 μ g (n = 21)	
Chest pain	5 (21)	6 (29)	3 (14)
Fatigue	12 (50)	6 (29)	11 (52)
Pyrexia	3 (13)	5 (24)	4 (19)
Lung function decreased	5 (21)	5 (24)	5 (24)
Chest tightness	4 (17)	6 (29)	4 (19)
Cough	17 (71)	16 (76)	17 (81)
Dyspnea ²	9 (38)	3 (14)	2 (10)
Hemoptysis ²	9 (38)	3 (14)	2 (10)
Nasal congestion	5 (21)	7 (33)	5 (24)
Pulmonary congestion	8 (33)	1 (5)	6 (29)
Sore throat NOS	8 (33)	6 (29)	5 (24)
Sputum increased	8 (33)	10 (48)	8 (38)
Headache	8 (33)	8 (38)	7 (33)

¹Results are numbers of patients (percentages).

²All dyspnea and hemoptysis events were mild or moderate, except for one severe dyspnea event in 1,000- μ g IFN- γ 1b group.

statistically significant differences in FEV₁ from baseline to week 12, although notably there was a 0.12-l improvement in the 500- μ g IFN- γ 1b group compared to placebo at week 12 (95% CI, $-0.03, 0.26$; $P = 0.11$) which was much larger than the 0.01-l difference in the 12-week change that was observed between the 1,000- μ g IFN- γ 1b group and the placebo group (95% CI, $-0.15, 0.17$; $P = 0.95$). Of note, there were no significant interactions between treatment and tobramycin usage upon additional exploratory analyses. For sputum bacterial density, no statistically significant difference between active and placebo groups was seen from baseline to week 12 (Table 3). Use of either systemic antibiotic or anti-PA systemic antibiotic was not significantly different between groups, but there was a higher percentage of hospitaliza-

tions for exacerbations in the 1,000- μ g IFN- γ 1b group than in the placebo group (24% more in the 1,000- μ g IFN- γ 1b group; 95% CI, 2%, 45%; $P = 0.05$, Table 4). Significant differences were not observed between active treatment groups and placebo for sputum PMN, IL-8, elastase, myeloperoxidase (Fig. 2), or DNA (not shown). Further exploratory analyses found significant differences between the 500- μ g IFN- γ 1b group and the placebo group with respect to the 4-week change in FEV₁ (mean difference = 0.20 l; 95% CI, 0.06, 0.35; $P = 0.01$) and in the 1,000- μ g IFN- γ 1b group compared to placebo for reduction in bacterial density (mean difference = $-1.1 \log_{10}$ CFU/g; 95% CI, $-1.89, -0.23$; $P = 0.02$), but these changes were not sustained through the remainder of the study (Fig. 1).

DISCUSSION

Standard therapies for CF lung disease include ongoing airway clearance techniques to facilitate the removal of inspissated secretions, nutritional support, antibiotics, bronchodilators, anti-inflammatory agents, and mucolytics. Despite this panoply, CF patients generally lose 2–3% lung function annually.²¹ New therapies are clearly needed to further increase longevity and quality of life.

IFN- γ is a potent immunomodulatory cytokine that is one of the principal T-lymphocyte products mediating macrophage activation.¹⁰ Activities of IFN- γ include antimicrobial, antiproliferative, and antifibrotic effects, as well as complex cytokine and chemokine modulation.^{8–10} The ability of IFN- γ to activate macrophages, increase bacterial removal and killing, and modulate the cytokine response and other effector molecules (e.g., chemokines, NO) favoring a TH₁-type immune response provided the basis to study its potential use in CF. In vitro, IFN- γ enhances macrophage activation by inducing production of reactive oxygen species, resulting in increased killing of

TABLE 3—Primary Endpoints of 12-Week Change in FEV₁ and Sputum Bacterial Density

Week 12 change	IFN- γ 1b		
	1,000 μ g (n = 24)	500 μ g (n = 21)	Placebo (n = 21)
FEV ₁ (liters)			
n	24	20	21
Mean (SD)	-0.09 (0.298)	0.02 (0.212)	-0.10 (0.243)
Difference ¹	0.01	0.12	
95% CI	$-0.16, 0.17$	$-0.03, 0.26$	
P -value ²	0.96	0.11	
Sputum bacterial density (\log_{10} CFU/g)			
n	16	15	13
Mean (SD)	-0.14 (1.539)	0.64 (0.649)	0.77 (2.759)
Difference ¹	-0.91	-0.13	
95% CI	$-2.57, 0.75$	$-1.64, 1.38$	
P -value ²	0.31	0.87	

¹Computed as active group minus placebo group.

²Compared to placebo using a two-sided, two-sample t -test at $\alpha = 0.05$ significance level.

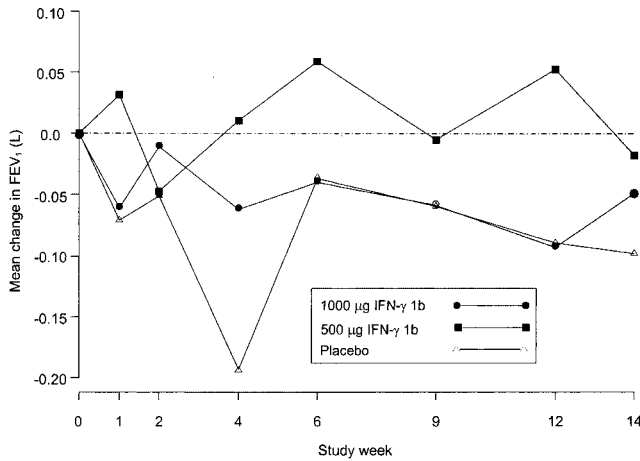


Fig. 1. Mean change from baseline in FEV₁ for all patients tolerating initial dose, by treatment group. ●-●-● signifies starting values (baseline).

ingested bacteria, including PA.^{22,23} IFN- γ was also shown to reverse the repression of inducible NO synthase, thereby increasing the production of NO and potentially facilitating bacterial clearance.¹¹ Respiratory epithelial cells derived from CF patients as well as genetically engineered CF mice demonstrated decreased production of NO;^{5,24,25} this can potentially be corrected by IFN- γ , as demonstrated in vitro and in CF mice.²⁶ Induction of IFN-inducible protein-10 (IP-10), a mononuclear cell chemotactic factor, is reduced in mice deficient for IFN- γ or the IFN- γ receptor.²⁷ Also, IFN- γ downregulates production of IL-8, a neutrophil chemotactic factor, in human

monocytes.²⁸ The net result of these actions of IFN- γ is to induce a more macrophage-dominated TH₁-type inflammatory response and to downregulate the predominantly granulocytic TH₂-type inflammatory response. In murine models, IFN- γ also decreases TH₂-type inflammatory responses by suppressing the proliferation of TH₂ lymphocyte clones.²⁹ Nebulizing IFN- γ to ovalbumin-sensitized BALB/c mice decreases the capacity of their systemic T cells to produce TH₂-type cytokines and shifts their T-cell response toward a TH₁-like cytokine pattern.¹²

A chronic infection produced by PA embedded in agar or alginate beads instilled into rodent airways is considered the best animal model of the chronic exaggerated neutrophilic inflammatory response that occurs in lungs of infected CF patients.³⁰ Moser et al.^{13,14} compared the pulmonary disease induced by intratracheal challenge with PA embedded in alginate beads in TH₁-type responder (C3H/HeN) and TH₂-type responder (BALB/c) mice. They found that compared to BALB/c mice, C3H/HeN mice had milder lung inflammation, more rapid clearance of bacteria, and lower mortality, as well as lower levels of IL-4 and TNF- α and higher levels of IFN- γ .^{13,14} Johansen et al.¹⁵ examined the effects of exogenous IFN- γ on pulmonary disease induced by PA embedded in alginate beads in rats: those treated with IFN- γ showed a reduction in severity of lung inflammation, decreased PMN infiltration, and fewer microabscesses compared with untreated animals.¹⁵

Deficient in vitro production of IFN- γ was observed in activated peripheral blood CF mononuclear cells as well as in bronchial mucosal biopsies during acute exacerbation, whereas bronchial cells from patients with mild stable CF produced substantially higher levels of IFN- γ .^{17,18,31} A positive correlation between PA antigen-stimulated IFN- γ production in CF peripheral blood mononuclear cells and parameters of lung function was also observed.¹⁶ Thus, data from in vitro, animal, and clinical studies suggest that IFN- γ may facilitate the removal and killing of organisms from the chronically infected CF lung, modify the inflammatory response, reduce the severity and frequency of pulmonary infections, and enhance lung function.

For treatment of CF, we felt that aerosol administration of IFN- γ 1b offered the best potential therapeutic index. In healthy human volunteers, IFN- γ 1b was inhaled in single doses of up to 2,000 μ g and multiple doses of 500 μ g.³²⁻³⁴ Adverse events were limited to uncommon transient cough following administration; one subject had a transient decrease in white blood cell count.³²⁻³⁴ Inhaled IFN- γ 1b produced a dose-dependent increase in lung epithelial lining fluid IFN- γ levels without detection in serum. Using the Respigard II[®] nebulizer, I¹²³-labeled IFN- γ 1b aerosol was uniformly deposited in the lungs, with maximum deposition occurring within 10 min and 12-32% of the nominal dose delivered to the airways.

TABLE 4—Secondary Endpoints of Systemic Antibiotic Use (Total and Antipseudomonal) and Hospitalizations for Pulmonary Exacerbation

	IFN- γ 1b		Placebo (n = 21)
	1,000 μ g (n = 24)	500 μ g (n = 20)	
Systemic antibiotic use			
N (%)	13 (54)	11 (55)	10 (48)
Difference	6	7	
95% CI	-21,33	-21,35	
P value ¹	0.66	0.76	
Antipseudomonal systemic antibiotic use			
N (%)	8 (33)	5 (24)	4 (19)
Difference	14	6	
95% CI	-12,37	-20,31	
P value ²	0.33	>0.99	
Hospitalization for a pulmonary exacerbation of CF			
N (%)	7 (29)	1 (5)	1 (5)
Difference	24	0	
95% CI	2,45	-19,19	
P value ²	0.05	>0.99	

¹Compared to placebo, using two-sided chi-square test at alpha = 0.05 significance level.

²Compared to placebo, using two-sided Fisher's exact test at alpha = 0.05 significance level.

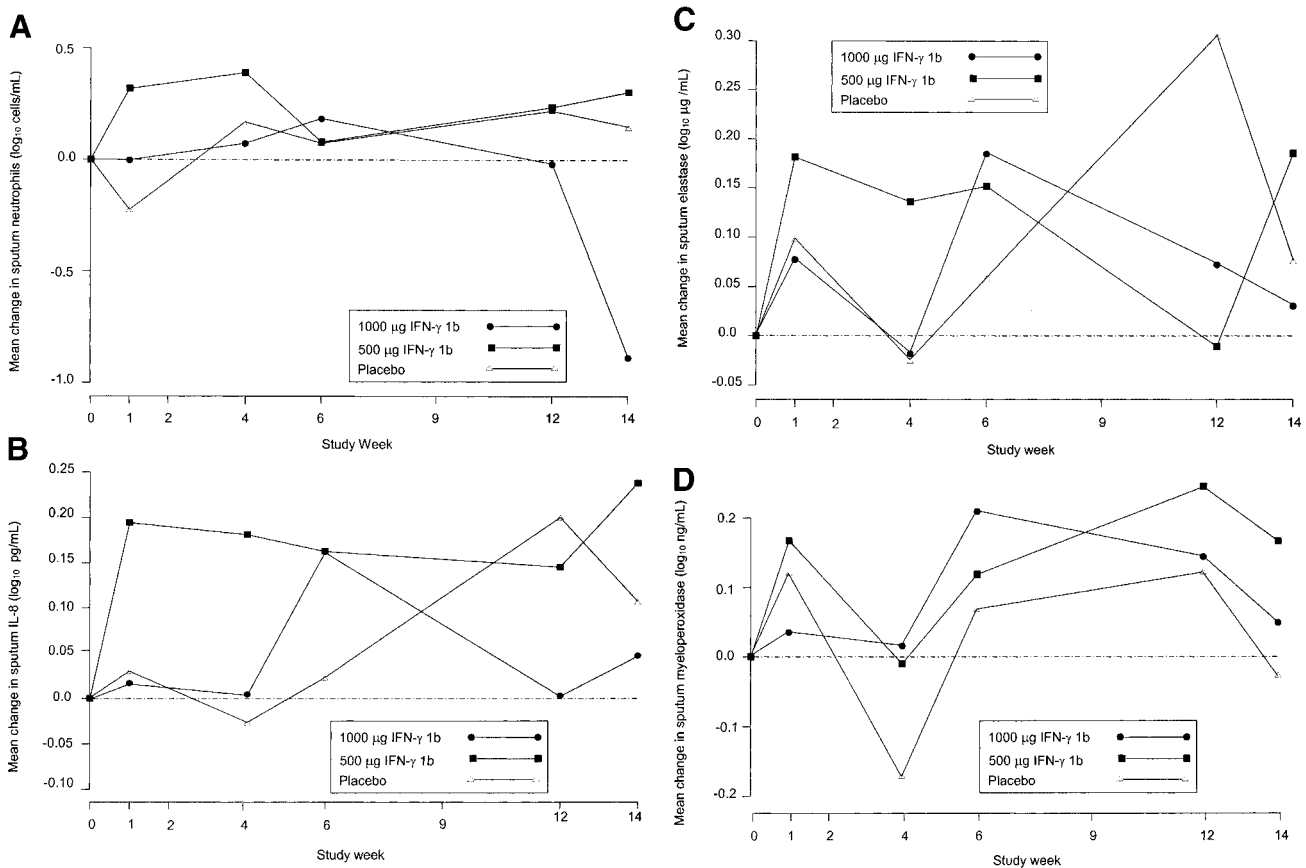


Fig. 2. Mean change from baseline in sputum biomarkers, by treatment group. **A: Neutrophils.** **B: Interleukin-8.** **C: Elastase.** **D: Myeloperoxidase.** ●—●—● signifies starting values (baseline).

Alveolar macrophages but not blood monocytes were activated as measured by transcription of IP-10, a gene induced by IFN- γ 1b, and no significant HLA-DR induction in peripheral blood cells was detected.^{32–34} In patients with asthma, lung cancer, multidrug-resistant *Mycobacterium tuberculosis* (MDR TB), and *Mycobacterium avium* complex infection, aerosols of up to 5,400 μ g and multiple doses of 500 μ g for up to 10 weeks produced no significant intolerance, but some patients had transient fever, mild cough, myalgias, and reversible reduction in peak flow rate.^{35–39} In patients with lung cancer, aerosolized IFN- γ 1b (200–5,400 μ g) resulted in measurable IFN- γ in epithelial lining fluid and activation of lung macrophages, with minimal systemic side effects.³⁶ In contrast, 250 μ g subcutaneous IFN- γ 1b did not result in IFN- γ in epithelial lining fluid or lung macrophage activation.³² In patients with MDR TB, 7–13% of the 500- μ g nebulized dose was deposited to normal lung areas, while cavitory areas had much less deposition. Bioactivity was evidenced by increased levels of alveolar macrophage IP-10 transcripts after 1 month of treatment. A reduction in size of cavities with clearing of sputum acid-fast smears implied that biologic activity was induced in these regions, despite poor ventilation.^{37,38} These studies suggest that inhalation of 500–1,000 μ g

IFN- γ 1b offers advantages of local macrophage activation with minimal toxicity, and these were the doses employed in our study.

Inhaled IFN- γ 1b was well-tolerated at the 500- μ g dose level in our study, but significantly more pulmonary exacerbations, as well as more dyspnea and hemoptysis, were seen in the 1,000- μ g IFN- γ 1b group compared to the 500- μ g IFN- γ 1b or placebo groups. While this did not affect the withdrawal rate from the study, the higher rate of these adverse events in the 1,000- μ g IFN- γ 1b subjects may have been partly due to enrollment of somewhat sicker patients, as suggested by significantly greater baseline sputum bacterial density, use of chronic Tobin[®] and Pulmozyme[®] and systemic antibiotics, and the proportion of patients with lower FEV₁. It is also possible that the adverse effects were related to longer nebulization times (25–35 min to inhale 1,000 μ g study drug vs. 10–20 min to inhale 500 μ g) or the direct adverse pulmonary effects of 1,000 μ g inhaled IFN- γ 1b.

IFN- γ 1b treatment did not achieve the desired primary efficacy endpoints of significant improvement in FEV₁ or reduction in bacterial density at week 12. Due to randomization, the 500- μ g IFN- γ 1b group started with generally lower FEV₁ values ($P = 0.08$) that remained stable over the trial period, while those of the placebo and

1,000- μg IFN- γ 1b groups declined. A significant FEV₁ difference in favor of 500 μg of IFN- γ 1b at 4 weeks ($P = 0.01$) was likely related to an unexpectedly large but transient early decline in the placebo group (Fig. 1). No effect of IFN- γ 1b at either dose was seen in reducing sputum bacterial density, or in the secondary endpoints of reducing systemic antibiotic usage, hospitalizations, or inflammatory biomarkers in sputum.

Recently, a study of 15 healthy adults nebulizing 500 μg IFN- γ 1b labeled with ^{99m}Tc-DTPA resulted in total lung deposition of only 28 ± 2.6 (SD) μg IFN- γ 1b.⁴⁰ Given the extensive sputum secretion and coughing in CF patients, it is plausible that delivery could have been even lower in our patient population. This raises the question of adequacy of dosing, given that the usual dose of IFN- γ 1b in patients with chronic granulomatous disease is ~ 100 μg subcutaneously. To our knowledge, there is no evidence that IFN- γ 1b is vulnerable to the proteolytic activity of human neutrophil elastase, but it is also possible that *Pseudomonas* proteases may have reduced IFN- γ 1b bioactivity, since this organism was present in 87% of patients in this study.⁴¹ Similarly, the adverse events observed in patients receiving 1,000 μg IFN- γ 1b may have been due to other factors, as discussed above.

Analysis of sputum PMN, IL-8, elastase, myeloperoxidase, and DNA did not reveal suppression by IFN- γ 1b at either dose. Interestingly, results in the placebo group suggest that despite the current treatment, sputum markers of inflammation increased over time (here, 3 months) in CF. The magnitude of progressive sputum inflammation we observed is in line with that seen in other placebo groups in controlled clinical CF trials.^{19,42} These sputum data on inflammation provide an important benchmark for future studies of therapeutic agents in CF.

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