MAJOR ARTICLE

Recombinant Interferon-γ1b as Adjunctive Therapy for AIDS-Related Acute Cryptococcal Meningitis

Peter G. Pappas,[1] Beatriz Bustamante,[8] Eduardo Ticona,[9] Richard J. Hamill,[2] Philip C. Johnson,[3] Annette Reboli,[4] Judith Aberg,[5] Rodrigo Hasbun,[6] and Henry H. Hsu[7]

[1]University of Alabama School of Medicine, Birmingham; [2]Baylor University School of Medicine and Houston VA Medical Center, and [3]University of Texas at Houston Medical Center, Houston; [4]University of Medicine and Dentistry of New Jersey, Cooper Hospital, Camden; [5]Washington University School of Medicine, St. Louis, Missouri; [6]Tulane University School of Medicine, New Orleans, Louisiana; [7]InterMune Pharmaceuticals, Brisbane, California; [8]Universidad Peruana Cayetano Heredia, and [9]Hospital dos de Mayo, Lima, Peru

We conducted a phase 2, double-blind, placebo-controlled study to evaluate the safety and antifungal activity of adjuvant recombinant interferon (rIFN)–γ1b in patients with acquired immunodeficiency syndrome and acute cryptococcal meningitis. Patients received 100 or 200 μg of rIFN-γ1b or placebo, thrice weekly for 10 weeks, plus standard therapy with intravenous amphotericin B, with or without flucytosine, followed by therapy with fluconazole. End points included conversion of cerebrospinal fluid fungal cultures from positive to negative at 2 weeks, resolution of symptoms, and survival. Among 75 patients, 2-week culture conversion occurred in 13% of placebo recipients, 36% of rIFN-γ1b (100 μg) recipients, and 32% of rIFN-γ1b (200 μg) recipients. There was a trend toward improved combined mycologic and clinical success in rIFN-γ1b recipients (26% vs. 8%; $P = .078$). Therapy with rIFN-γ1b was well tolerated, and there was no apparent influence on serial CD4 cell counts and human immunodeficiency virus load measurements. Adjunctive therapy with rIFN-γ1b holds promise for patients with acute cryptococcal meningitis and warrants further study.

Cryptococcus neoformans is one of the most common causes of central nervous system (CNS) infection among patients with HIV and is the most common cause of fungal meningitis worldwide [1, 2]. Although the incidence of cryptococcal meningitis has declined among patients with AIDS in the developed world, because of ready access to highly active antiretroviral therapy (HAART), acute cryptococcal disease as a presenting manifestation of HIV infection still occurs and can lead to significant morbidity, even with therapy [3, 4]. In the developing world, cryptococcosis continues to cause significant morbidity and mortality and ranks among the 3 most common opportunistic pathogens in HIV-positive patients. Despite advances in antifungal therapy, at least one-third of patients with cryptococcal meningitis who receive appropriate antifungal therapy will have mycologic and/or clinical failure, defined as persistently positive cerebrospinal fluid (CSF) cultures, persistent clinical symptoms and signs, or death due to the infection [5, 6]. New therapeutic approaches could significantly reduce the morbidity and mortality of this potentially devastating infection.

Interferon (IFN)–γ is an endogenous cytokine with diverse biologically beneficial properties, including immunomodulatory activities [7, 8] and enhancement of Th1 responsiveness by stimulating several host effector cells, such as macrophages, monocytes, NK cells, and neutrophils [7–9]. IFN-γ plays a key role in host defense against fungal, viral, parasitic, mycobacterial, and certain intracellular bacterial microorganisms. IFN-γ has been effective as adjunctive therapy for patients with refractory mycobacterial infections, including Hansen disease [10, 11] and leishmaniasis [12, 13].

In 1990, recombinant IFN-γ1b (rIFN-γ1b; Actimmune; InterMune) was approved in the United States for use in patients with chronic granulomatous disease,

Received 20 August 2003; accepted 1 December 2003; electronically published 24 May 2004.

Presented in part: Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 19–21 December 2001 (abstract LB-10).

Potential conflict of interest: H.H.H. is an employee of InterMune, the manufacturer of the study drug, and owns InterMune stock.

Financial support: InterMune.

Reprints or correspondence: Dr. Peter G. Pappas, University of Alabama School of Medicine, 1900 University Blvd., 229 Tinsley Harrison Tower, Birmingham, AL 35294-0006 (pappas@uab.edu).

The Journal of Infectious Diseases 2004; 189:2185–91

0022-1899/2004/18912-0006$15.00

to reduce the frequency and severity of serious infections [14]. More recently, rIFN-γ1b has been studied in vitro and in animal models of cryptococcosis, as adjunctive therapy to conventional antifungal agents, and has yielded promising results [15–18]. In addition, there have been anecdotal reports of efficacy in patients with invasive fungal infection [19–22]. On the basis of these encouraging results, we conducted a phase 2 clinical trial to determine the safety and clinical and mycologic activity of subcutaneously (sc) administered rIFN-γ1b, in conjunction with standard antifungal therapy, for treatment of acute cryptococcal meningitis in patients with AIDS.

PATIENTS AND METHODS

A phase 2, randomized, double-blind, placebo-controlled, dose-ranging pilot study in adult patients with acute cryptococcal meningitis was conducted in 10 sites. Eight sites were in the United States, and 2 were in Lima, Peru.

Eligibility. Patients had to be at least 18 years old, be either HIV positive or HIV negative, and have newly diagnosed or relapsed cryptococcal meningitis. They could not have received >3 days of systemic antifungal therapy for the most recent episode of meningitis, with a cumulative dose of >2.1 mg/kg amphotericin B or 1200 mg of fluconazole or itraconazole. To be eligible for efficacy analyses, patients had to have a CSF culture positive for *C. neoformans* at entry to the study, have received at least 1 dose of study medication, and had at least 1 postbaseline CSF fungal culture. Patients were excluded from the study if they were in a coma, were pregnant or lactating women, or had any of the following laboratory results: serum creatinine level ⩾2 times the upper limit of normal; aspartate transaminase, alanine transaminase, or alkaline phosphatase level >5 times normal; total bilirubin level >2 times normal; absolute neutrophil count <1000 cells/mm^3; hemoglobin level <7 g/dL; or platelet count <50,000 cells/mL. Patients were also excluded if they had received chronic systemic glucocorticosteroids or had been treated for a prior episode of cryptococcosis within 30 days before enrollment. Significant preexisting rheumatologic, cardiac, pulmonary, CNS, or peripheral vascular diseases were additional criteria for exclusion.

Objectives. The primary objective of the present study was to determine the rate of conversion of a CSF fungal culture from positive to negative at week 2 in the 3 study arms. Secondary objectives were (1) to evaluate the safety and tolerability of a thrice-weekly, 10-week course of 100 or 200 μg of sc rIFN-γ1b, (2) to compare the rate of conversion of CSF fungal cultures from positive to negative at weeks 1 and 10, and (3) to assess the effects of therapy on relevant clinical parameters, including resolution of fever, headache, meningeal signs, improvement in neurologic status, and survival. This pilot study was not designed to provide a high probability of detecting statistically significant differences in mycologic or clinical efficacy between therapy arms, but rather to determine whether there was a trend toward better outcome among rIFN-γ1b recipients, compared with placebo recipients. If a trend toward better outcome was demonstrated among rIFN-γ1b recipients, then these data would be used to select the best dose (100 vs. 200 μg) for a phase 3, randomized, placebo-controlled trial with sufficient power to determine a clinically important difference in outcome.

Experimental design. Informed consent was obtained from patients or their parents or guardians, and human-experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions were followed in the conduct of this clinical research. After providing written, informed consent, patients were randomly assigned to receive placebo, 100 μg of rIFN-γ1b, or 200 μg of rIFN-γ, each administered sc thrice weekly for 10 weeks (for a maximum of 30 doses). All patients also received standard antifungal therapy for acute cryptococcal meningitis, which included intravenous amphotericin B (0.7 mg/kg daily), with or without oral flucytosine (100 mg/kg daily in 4 divided doses), both administered for 14 days (induction therapy), followed by oral fluconazole (400 mg daily for 8 weeks). Patients could continue receiving induction therapy at the investigator's discretion, as clinically warranted. After 10 weeks of therapy, per protocol, patients continued to receive fluconazole (at least 200 mg daily). Patients were stratified according to HIV status and CSF opening pressure (⩽250 or >250 mm of H_2O).

Evaluations. At screening or at baseline (before starting any protocol therapy), a complete history was obtained, and patients underwent physical examination; mental assessment with the Mini-Mental Status Examination (MMSE); functional assessment with the Karnofsky Performance Status (KPS) evaluation; and lumbar puncture with culture of CSF and determination of opening pressure, cell count, protein, glucose, India ink, fungal culture, and cryptococcal antigen (CrAg). Laboratory tests included blood and urine fungal cultures, routine blood chemistry analyses, urinalysis, prothrombin time, HIV-1 and -2 antibody tests, complete and differential blood cell counts, HIV load measurement, and CD4 and CD8 cell counts. Lumbar puncture and CSF assessments were repeated at weeks 1, 2, and 10 after enrollment; other clinical and laboratory assessments were repeated at frequent intervals. Patients were queried about adverse events at each visit and were followed-up through 4 weeks after completion of therapy with the study drug (total duration of study, 14 weeks). Important clinical parameters evaluated included fever, headache, meningeal signs, and neurologic findings, including MMSE. Patients were withdrawn from the study for serious toxicity judged to be related to the study medication, intercurrent illness that could affect assessment of clinical status, a baseline CSF culture negative

for *C. neoformans,* noncompliance, withdrawal of consent, or pregnancy occurring during the therapy period.

Outcome measurements. Mycologic, clinical, and combined outcomes were assessed in patients for whom data could be evaluated. Mycologic success was defined as a negative CSF fungal culture at 2 weeks. Secondary measures of mycologic success included rates of conversion of CSF fungal cultures at weeks 1 and 10. Patients with missing CSF fungal culture results were considered to have a positive culture if the most recent culture was positive. Clinical success at 2 weeks was defined as stable or improved clinical and neurologic parameters, compared with baseline values. Combined mycologic-clinical success was defined as a negative CSF fungal culture at 2 weeks and clinical stability or improvement, compared with baseline values, as described above. Patients with insufficient clinical data to determine outcome were considered to have experienced therapy failure.

Statistical analyses. The population analyzed for mycologic and clinical end points included patients who had a positive CSF fungal culture at baseline, had received at least 1 dose of study medication, and had at least 1 postbaseline CSF fungal culture during the 10-week therapy period. All patients randomly assigned to therapy who received any study medication and had at least 1 follow-up safety assessment were evaluated for safety end points. Conversion of CSF fungal culture from positive to negative and clinical status were analyzed by use of the Cochran Mantel-Haenszel test. CrAg titers in CSF were assessed by analysis of covariance, using baseline values as covariates. No adjustments were made for multiple comparisons, and patients with missing CSF fungal culture results were considered to have experienced therapy failure.

RESULTS

Enrollment and demographics. Seventy-nine patients were randomized to the study. Three patients who did not receive any study drug and 1 patient who had a negative baseline CSF fungal culture were excluded from the safety and efficacy analyses. Thus, 75 patients were analyzed for safety and clinical end points. Five additional patients with no postbaseline CSF fungal cultures were excluded from analyses of mycologic end points. Accordingly, 70 patients were analyzed for mycologic end points, including 57 men (81%) and 13 women (19%) (table 1). The mean age of the patients was 34.7 years (range, 21–80 years); 6 patients were white, 5 were black, and 59 were Hispanic. All patients were HIV positive. Among the 70 patients with mycologic end points, 12 (17%) were enrolled at US sites, and 58 (83%) were enrolled in Peru. In general, these 2 subpopulations were comparable with respect to demographic characteristics.

Baseline clinical and laboratory findings. At baseline, most patients had clinical signs and symptoms of CNS cryptococcosis, including headache (95%), fever (74%), and meningismus (73%). The median MMSE score was 22 (maximum score, 30), and the mean KPS score was 70 (maximum score, 100). Twenty-two patients (31%) had CSF opening pressures >250 mm of H_2O. Mean CSF protein, glucose, and white and red blood cell counts were similar among the therapy groups.

All patients had positive CSF CrAg titers, with median baseline titers of 1:4096, 1:2048, and 1:4096 in the placebo, rIFN-γ1b (100 μg), and rIFN-γ1b (200 μg) therapy groups, respectively. Blood and urine fungal cultures were positive for *C. neoformans* in 59% and 56% of patients, respectively. At baseline, the median CD4 cell count was 16 cells/mm^3 (range, 2–429 cells/mm^3), and the median HIV load was 244,817 copies/mL (range, 650 to >750,000 copies/mL). Although the difference was not statistically significant, Peruvian patients tended to have findings indicative of more-advanced cryptococcosis at initial presentation, compared with their US counterparts: they had higher median CSF CrAg titers (1:4096 vs. 1:1280) and higher median CSF opening pressures (183 vs. 153 mm of H_2O), and a higher proportion of Peruvian patients had positive fungal blood cultures (62% vs. 36%), positive urine cultures (66% vs. 33%), and greater frequency of headache (98% vs. 75%).

Study therapy. Compliance with the study regimen was excellent and was better among Peruvian patients (98% of doses taken) than among US patients (83% of doses taken). Physician-directed dose modifications were necessary for 46% of US patients and for 20% of Peruvian patients. Cumulative doses of amphotericin B were similar in both geographic subgroups (10.1 mg/kg for US patients and 9.7 mg/kg for Peruvian patients), as were median number of days receiving amphotericin B (13 days [range, 7–40 days] for US patients and 15 days [range, 3–22 days] for Peruvian patients). No Peruvian patients received 5-flucytosine, whereas all 12 US patients received concomitant therapy with 5-flucytosine during the first 2 weeks of therapy. Exposure to fluconazole varied among US and Peruvian patients, but most patients received 6 mg/kg/day (400 mg daily for most adults). Compliance with the antifungal therapy regimen was similar across the 3 therapy arms. No patient began receiving HAART during the first 3 weeks of receiving the study drug.

Mycologic and clinical outcome. Among the 70 patients with data for mycologic status, 23 were in the placebo group, 25 were in the rIFN-γ1b (100 μg) group, and 22 were in the rIFN-γ1b (200 μg) group. CSF fungal cultures were negative at 2 weeks in 13%, 36%, and 32% of patients in the placebo, rIFN-γ1b (100 μg), and rIFN-γ1b (200 μg) groups, respectively, with a trend toward more-rapid sterilization of CSF among rIFN-γ1b versus placebo recipients (placebo vs. rIFN-γ1b [100 μg], $P = .072$; placebo vs. rIFN-γ1b [200 μg], $P = .139$) (table 2). Among 58 Peruvian patients, who did not receive 5-flucytosine, CSF fungal cultures were negative at 2 weeks for 19%,

Table 1. Demographic and baseline characteristics of HIV-positive patients with *Cryptococcus neoformans* infection, by therapy group.

Characteristic	Placebo (n = 23)	rIFN-γ1b 100 μg (n = 25)	rIFN-γ1b 200 μg (n = 22)
Age, mean (range), years	35 (21–61)	37 (22–80)	33 (23–73)
Male, no. (%)	17 (74)	23 (92)	17 (77)
Race, no. (%)			
White, non-Hispanic	2 (9)	3 (12)	1 (5)
Black	1 (4)	3 (12)	1 (5)
Hispanic	20 (87)	19 (76)	20 (91)
Weight, mean, kg	52.0	54.7	52.6
Blood culture positive for *C. neoformans,* no. (%)	12 (52)	14 (56)	14 (64)
Urine culture positive for *C. neoformans,* no. (%)	11 (48)	12 (48)	16 (73)
Signs or symptoms, no. (%)			
Headache	21 (91)	24 (96)	21 (95)
Fever	20 (87)	15 (60)	17 (77)
Meningismus	18 (78)	16 (64)	15 (68)
MMSE score, median	22	22	22
KPS score, mean (range)	70 (20–90)	60 (20–80)	70 (30–90)
CD4 cell count, median (range), cells/mm^3	12 (3–349)	22 (2–429)	16 (2–212)
HIV RNA load, median (range), copies/mL	333,854 (19,871 to >750,000)	226,137 (657 to >750,000)	217,014 (19,635 to >750,000)
CSF opening pressure, median (range), mm of H_2O	200 (26–650)	170 (60–600)	155 (60–550)
CSF CrAg titer			
Geometric mean	1:6199	1:2702	1:6169
Median	1:4096	1:2048	1:4096
Range	1:128–1,048,526	1:4–4,194,354	1:32–1,048,596
Protein level in CSF, mean, mg/dL	76	91	75
Glucose level in CSF, mean, mg/dL	30	37	32

NOTE. CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; KPS, Karnofsky Performance Status; MMSE, Mini-Mental Status Examination; rIFN-γ1b, recombinant interferon-γ1b.

33%, and 32% of placebo, rIFN-γ1b (100 μg), and rIFN-γ1b (200 μg) recipients, respectively. In the combined rIFN-γ1b therapy groups, CSF fungal cultures were negative at 2 weeks for 16 (34%) of 47 patients, compared with 3 (13%) of 23 placebo recipients ($P = .064$). Among US patients, 4 (33%) of 12 had documented CSF fungal culture conversion at 2 weeks, compared with 15 (26%) of 58 Peruvian patients. CSF fungal cultures were negative at 10 weeks for 51 (73%) of 70 patients. CSF opening pressure decreased with therapy in all 3 cohorts, and there were no differences between therapy groups.

CSF CrAg titers were measured at baseline and during therapy. The relative reduction in titer was assessed on the basis of the mean fold-change in titer, compared with the baseline titer, for patients for whom paired data were available. The change in titer showed a trend toward a more-rapid decrease in rIFN-γ1b recipients, compared with placebo recipients: at 1 week, there was a 5-fold decrease in CrAg titers among all groups; at 2 weeks, there was a 24-fold, 12-fold, and 8-fold decrease in the rIFN-γ1b (100 μg), rIFN-γ1b (200 μg), and placebo groups, respectively; at 10 weeks, there was a 69-fold, 54-fold, and 24-fold decrease in the rIFN-γ1b (100 μg), rIFN-γ1b (200 μg), and placebo groups, respectively.

There were no statistically significant differences or meaningful trends among the 3 study arms, with regard to resolution of clinical signs or symptoms, when these variables were considered individually. A global composite outcome measure (combined mycologic-clinical end point) was analyzed, and these results are shown in table 3. This analysis was performed among the 75 patients who received at least 1 dose of study drug and were culture positive for *C. neoformans* at baseline. Patients with missing culture data were considered to have experienced therapy failure. At week 2, 27% and 25% of the rIFN-γ1b (100 μg) and rIFN-γ1b (200 μg) recipients, respectively, met the criteria for combined mycologic-clinical success, compared with 8% of placebo recipients. At week 10, the combined response rates were 65%, 58%, and 48% in the rIFN-γ1b (100 μg), rIFN-γ1b (200 μg), and placebo groups, respectively.

Safety. Seventy-six patients randomly assigned to therapy

Table 2. Cerebrospinal fluid (CSF) fungal culture results at baseline and at 1, 2, and 10 weeks in HIV-positive patients with *Cryptococcus neoformans* infection, by therapy group.

Time, result	Placebo (n = 23/4)[a]	rIFN-γ1b 100 μg (n = 25/6)[a]	rIFN-γ1b 200 μg (n = 22/2)[a]
Baseline, positive	23 (100)	25 (100)	22 (100)
Week 1			
Negative	3 (13)	8 (32)	2 (9)
Positive	17 (74)	17 (68)	18 (82)
NA[b]	3 (13)	0 (0)	2 (9)
Week 2			
Negative	3 (13)	9 (36)	7 (32)
Positive	14 (61)	13 (52)	13 (59)
NA[b]	6 (26)	3 (12)	2 (9)
Week 10			
Negative	17 (74)	18 (72)	16 (73)
Positive	1 (4)	1 (4)	1 (5)
NA[b]	5 (22)	6 (24)	5 (22)

NOTE. Data are no. (%) of patients. For data analysis, patients with missing cultures were considered to have positive culture results if the prior CSF fungal culture was positive. NA, not available; rIFN-γ1b, recombinant interferon-γ1b.

[a] Total no. of patients/no. of patients who received 5-flucytosine.

[b] CSF fungal cultures were not obtained at the specified time.

received at least 1 dose of study drug and were included in the safety analysis. There were no notable differences in the number of doses administered to the 3 groups (mean range, 22.3–24.6 doses across the groups). One or more adverse events judged by the investigators to be therapy related were reported for 56% of placebo recipients, 70% of rIFN-γ1b (100 μg) recipients, and 88% of rIFN-γ1b (200 μg) recipients. The most common therapy-associated adverse events were those known to occur with administration of IFNs, such as fever, rigors, malaise, headache, and fatigue, which tended to occur more commonly among rIFN-γ1b recipients than among placebo recipients (table 4). Anemia and neutropenia occurred more commonly among rIFN-γ1b (200 μg) recipients. Eleven patients discontinued study medication because of an adverse event, including 4, 5, and 2 in the placebo, rIFN-γ1b (100 μg), and rIFN-γ1b (200 μg) groups, respectively. Eight of the 76 treated patients died (2 in the placebo group and 3 in each of the rIFN-γ1b groups) 9–95 days after initiation of study therapy; an additional 6 patients died within 30 days after the end of the study period (after 98 days).

Therapy with rIFN-γ1b did not adversely affect key parameters of HIV infection. There was no significant change in CD4 cell count or the CD4:CD8 ratio at week 10 (end of therapy). Therapy with rIFN-γ1b did not adversely influence HIV load. Indeed, the mean HIV load decreased by 17% at 10 weeks, compared with baseline, in the rIFN-γ1b groups, whereas there was an increase of 13% in HIV load in the placebo group (P, not significant).

DISCUSSION

To our knowledge, the present study is the first randomized, controlled clinical trial to examine the use of rIFN-γ1b as adjunctive therapy for a serious invasive fungal infection. Although the number of patients in this pilot study was too small to demonstrate a statistically significant difference in the primary efficacy end point (i.e., clearance of *C. neoformans* from CSF at 2 weeks), the results did show a trend toward more-rapid sterilization of CSF in the rIFN-γ1b recipients, compared with placebo recipients. Furthermore, at the end of the study, CSF CrAg titers had decreased by 69-fold and 54-fold in the rIFN-γ1b (100 μg) and rIFN-γ1b (200 μg) recipients, respectively, versus 24-fold in the placebo recipients.

There was no dose response of lower-dose versus higher-dose rIFN-γ1b, with respect to CSF fungal culture conversion (36% in the 100 μg group and 32% in the 200 μg group). At week 10, the culture conversion rate and survival were similar across the 3 therapy groups. However, there was a trend toward a greater proportion of patients showing improved combined mycologic-clinical outcome at both weeks 2 and 10 in the rIFN-γ1b therapy arms, compared with the placebo arm.

The clinical significance of early culture conversion among patients with cryptococcosis and AIDS is not fully delineated, but some investigators have observed that persistently positive CSF fungal cultures at 2 weeks predict culture positivity at 10 weeks and poorer clinical outcome [6, 23]. Among HIV-negative patients with CNS cryptococcosis, persistently positive CSF fungal cultures are associated with a significantly worse clinical out-

Table 3. Combined mycologic-clinical response rates among HIV-positive patients with *Cryptococcus neoformans* infection, by therapy group.

Study week, outcome	Placebo (n = 25)	rIFN-γ1b 100 μg (n = 26)	rIFN-γ1b 200 μg (n = 24)
Week 2			
Success	2 (8)	7 (27)	6 (25)
Failure	23 (92)	19 (73)	18 (75)
P[a]		.078	.116
Week 10			
Success	12 (48)	17 (65)	14 (58)
Failure	13 (52)	9 (35)	10 (42)
P[a]		.222	.466

NOTE. Data are no. (%) of patients. rIFN-γ1b, recombinant interferon-γ1b.

[a] Active therapy groups vs. placebo group (Cochran Mantel-Haenszel test).

Table 4. Summary of therapy-associated adverse events considered to be related to study therapy and that occurred in ⩾10% of HIV-positive patients with *Cryptococcus neoformans* infection, by therapy group.

Category	Placebo (n = 25)	rIFN-γ1b 100 μg (n = 27)	rIFN-γ1b 200 μg (n = 24)
Fever	5 (20)	10 (37)	10 (42)
Rigors	1 (4)	4 (15)	8 (33)
Headache	2 (8)	5 (19)	2 (8)
Malaise	1 (4)	3 (11)	1 (4)
Anemia	3 (12)	1 (4)	5 (21)
Neutropenia	0 (0)	1 (4)	3 (13)
Nausea	0 (0)	0 (0)	4 (17)
Patients with at least 1 adverse event	14 (56)	19 (70)	21 (88)

NOTE. Data are no. (%) of patients. Drug-related events include events deemed by the investigator to be associated with or of unknown association to study medication. rIFN-γ1b, recombinant interferon-γ1b.

come [24, 25]. Thus, in both HIV-positive and -negative patients, early aggressive therapy leading to more-rapid CSF fungal culture conversion is prudent and could have a beneficial effect on the long-term clinical course of CNS cryptococcosis.

It is unclear why overall rates of culture negativity at 2 weeks in the present study (13% for placebo, 36% for rIFN-γ1b [100 μg], and 32% for rIFN-γ1b [200 μg] recipients) were lower, compared with results from other published clinical trials. Culture negativity rates of 51%–60% after 2 weeks of induction therapy with amphotericin B, with or without flucytosine, were noted among US patients participating in a randomized trial of therapy for AIDS-associated acute cryptococcal meningitis [6]. In the present study, only 26% of Peruvian patients were culture negative at 2 weeks, and all of these patients were treated with amphotericin B alone. This low conversion rate could reflect differences in therapeutic approach (i.e., no exposure to 5-flucytosine). However, in the present study, only 33% of a small number of US patients were culture negative at 2 weeks. A more likely explanation is that Peruvian patients had more-advanced cryptococcosis, as suggested by significantly higher CSF CrAg titers, twice the rate of cryptococcemia, higher initial CSF opening pressures, and more-frequent headaches. Each of these characteristics suggests more-advanced disease at the time of presentation. Access to HAART in the 2 populations probably had no effect on overall mycologic and clinical outcome in the present study, because patients began receiving HAART only several weeks after initiation of study therapy, and there were no observed differences in outcome between patients who did and those who did not receive HAART during the study period. Moreover, when assessed at 10 weeks, the Peruvian patients showed responses to therapy that were comparable to those expected for a US population.

rIFN-γ1b was well tolerated and led to few dose-limiting adverse events, consistent with the results of previous studies [10, 11, 14]. Only 11 patients were withdrawn from the study because of significant adverse events, and those prematurely withdrawn were distributed evenly among the 3 study arms. The most commonly reported adverse events were fever, malaise, myalgias, and a flulike illness, each of which appeared to increase in frequency with the higher dose of rIFN-γ1b. Hematologic disorders, especially anemia, were also more common in the rIFN-γ1b (200 μg) group. Concerns about the potential negative influence of rIFN-γ1b on the clinical course of HIV disease were not supported by evidence from the present study; indeed, quantitative HIV loads were generally lower in the rIFN-γ recipients at 10 weeks, compared with baseline, and total CD4 cell counts and CD4:CD8 ratios did not vary between therapy groups. Efficacy measures were similar between the 2 rIFN-γ1b arms, but, because of the trend toward more adverse events with the higher dose, 100 μg of rIFN-γ1b appears to be the most reasonable dose for further study.

Data from the present trial suggest that rIFN-γ1b may induce more-rapid early sterilization of CSF among patients with HIV-associated cryptococcal meningitis and may result in better combined mycologic and clinical outcome. rIFN-γ1b is well tolerated and usually not associated with dose-limiting toxicity in this setting. No adverse events of therapy on key parameters of HIV infection were seen. These data support the concept that, in patients with invasive fungal infection, augmentation of the host immune response through the administration of adjunctive immunotherapy may have therapeutic potential. This approach may be especially important as the population of individuals at risk for invasive fungal infection expands to include not only patients with AIDS but also patients with hematologic malignancies, long-term glucocorticosteroid recipients, and others with underlying disorders associated with significant immune dysfunction.

Acknowledgments

We thank William E. Dismukes, for his careful review of the manuscript, and Windell Ross, for manuscript preparation.

References

1. Dismukes W. Cryptococcal meningitis in patients with AIDS. J Infect Dis **1988**; 157:624–8.
2. Chuck SL, Sande MA. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. N Engl J Med **1989**; 321:794–9.
3. Kaplan JE, Hanson D, Dworkin MS, et al. Epidemiology of human immunodeficiency virus–associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. Clin Infect Dis **2000**; 30(Suppl 1):S5–14.

4. Sacktor N, Lyles RH, Skolasky R, et al. HIV-associated neurologic disease incidence changes: Multicenter AIDS Cohort Study, 1990–1998. Neurology **2001**; 56:257–60.
5. Saag MS, Graybill RJ, Larsen RA, et al. Practice guidelines for the management of cryptococcal disease. Clin Infect Dis **2000**; 30:710–8.
6. van der Horst CM, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. N Engl J Med **1997**; 337:15–21.
7. Gallin JI, Farber JM, Holland SM, Nutman TB. Interferon-γ in the management of infectious diseases. Ann Int Med **1995**; 123:216–24.
8. Murray HW. Interferon-gamma and host antimicrobial defense: current and future clinical applications. Am J Med **1994**; 97:459–67.
9. Torrico F, Heremans H, Rivera MT, Van Marck E, Billiau A, Carlier Y. Endogenous IFN-γ is required for resistance to acute *Trypanosoma cruzii* infection in mice. J Immunol **1991**; 146:3626–32.
10. Holland SM, Einstein EM, Kuhns DB, et al. Treatment of refractory disseminated nontuberculous mycobacterial infection with interferon gamma: a preliminary report. N Engl J Med **1994**; 330:1348–55.
11. Nathan CF, Kaplan G, Levis WR, et al. Local and systemic effects of intradermal recombinant interferon-γ in patients with lepromatous leprosy. N Engl J Med **1986**; 315:6–15.
12. Arana BA, Navin TR, Arana FE, Berman JD, Rosenkaimer F. Efficacy of a short course (10 days) of high-dose meglumine antimonite with or without interferon-γ in treating cutaneous leishmaniasis in Guatemala. Clin Infect Dis **1994**; 18:381–4.
13. Harms G, Chehade AK, Douba M, et al. A randomized trial comparing a pentavalent antimonial drug and recombinant interferon-gamma in the local treatment of cutaneous leishmaniasis. Trans R Soc Trop Med Hyg **1991**; 85:214–6.
14. The International Chronic Granulomatous Disease Cooperative Study Group. A controlled trial of interferon-gamma to prevent infection in chronic granulomatous disease. N Engl J Med **1991**; 324:509–16.
15. Lutz JE, Clemons KV, Stevens DA. Enhancement of antifungal chemotherapy by interferon-gamma in experimental systemic cryptococcosis. J Antimicrob Chemother **2000**; 46:437–42.
16. Kawakami K, Tohyama M, Teruya K, Kudeken N, Xie Q, Saito A. Contribution of interferon-γ in protecting mice during pulmonary and disseminated infection with *Cryptococcus neoformans*. FEMS Immunol Med Microbiol **1996**; 13:123–30.
17. Joly V, Saint-Julien L, Carbon C, Yeni P. In vivo activity of interferon-γ in combination with amphotericin B in the treatment of experimental cryptococcosis. J Infect Dis **1994**; 170:1331–4.
18. Herrmann JL, Dubois N, Fourgeaud M, Basset D, Lagrange PH. Synergic inhibitory activity of amphotericin-B and γ interferon against intracellular *Cryptococcus neoformans* in murine macrophages. J Antimicrob Chemother **1994**; 34:1051–8.
19. Gallin JI, Malech HL. Update on chronic granulomatous diseases of childhood: immunology and potential for gene therapy. JAMA **1990**; 263:1533–7.
20. Bernhisel-Broadbent J, Camargo ED, Jaffe HS, Lederman HM. Recombinant human interferon-γ as adjunct therapy for *Aspergillus* infection in a patient with chronic granulomatous disease. J Infect Dis **1991**; 163:908–11.
21. Mulliez P, Jaggi F, Darras A, Demory JL, Smith M. Pulmonary aspergillosis complicating chronic septic granulomatosis: treatment with itraconazole and gamma interferon. Rev Mal Respir **2000**; 17:975–8.
22. Phillips P, Forbes JC, Speert DP. Disseminated infection with *Pseudoallescheria boydii* in a patient with chronic granulomatous disease: response to gamma-interferon plus antifungal chemotherapy. Pediatr Infect Dis J **1991**; 10:536–9.
23. Robinson PA, Bauer M, Leal ME, et al. Early mycological treatment failure in AIDS-associated cryptococcal meningitis. Clin Infect Dis **1999**; 28:82–92.
24. Dismukes WE, Cloud G, Gallis HA, et al. Treatment of cryptococcal meningitis with combination amphotericin B and flucytosine for four as compared with six weeks. N Engl J Med **1987**; 317:334–41.
25. Bennett JE, Dismukes WE, Duma RJ, et al. A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptococcal meningitis. N Engl J Med **1979**; 301:126–31.