CONCISE COMMUNICATION

A Randomized, Placebo-Controlled Trial of Granulocyte-Macrophage Colony-Stimulating Factor and Nucleoside Analogue Therapy in AIDS

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Preliminary preclinical and clinical data suggest that granulocyte-macrophage colony-stimulating factor (GM-CSF) may decrease viral replication. Therefore, 105 individuals with AIDS who were receiving nucleoside analogue therapy were enrolled in a placebo-controlled, double-blind study and were randomized to receive either 125 μg/m^2 of yeast-derived, GM-CSF (sargramostim) or placebo subcutaneously twice weekly for 6 months. Subjects were evaluated for toxicity and disease progression. A significant decrease in mean virus load (VL) was observed for the GM-CSF treatment group at 6 months (-0.07 $\log_{10}$ vs. -0.60 $\log_{10}$; $P = .02$). More subjects achieved human immunodeficiency virus (HIV)–RNA levels <500 copies/mL at ⩾2 evaluations (2% on placebo vs. 11% on GM-CSF; $P = .04$). Genotypic analysis of 46 subjects demonstrated a lower frequency of zidovudine-resistant mutations among those receiving GM-CSF (80% vs. 50%; $P = .04$). No difference was observed in the incidence of opportunistic infections (OIs) through 6 months or survival, despite a higher risk for OI among GM-CSF recipients. GM-CSF reduced VL and limited the evolution of zidovudine-resistant genotypes, potentially providing adjunctive therapy in HIV disease.

Evidence of multiple drug–resistant mutations in human immunodeficiency virus (HIV) type 1 strains isolated from individuals receiving highly active antiretroviral therapy (HAART) has led to a renewed focus on therapeutic strategies that block viral entry or augment host immunity [1]. One such approach involves correcting the cytokine disregulation that directly contributes to the immunopathogenesis of AIDS. Specifically, HIV-infected individuals have been shown to have deficient production of granulocyte-macrophage colony-stimulating factor (GM-CSF) [2], a hematopoietic growth factor that augments the number and function of a wide variety of immune cells, including neutrophils, macrophages, lymphocytes, and dendritic cells.

Recent studies have also suggested that GM-CSF may provide clinical benefit to HIV-infected individuals. Pilot clinical trials with GM-CSF have demonstrated increases in neutrophils, monocytes, and CD4 cells in HIV-positive individuals [3, 4]. In vitro studies have demonstrated that GM-CSF activation of monocytes increases the resistance of these cells to HIV by both impairing viral entry and enhancing the activity of some antiretroviral agents [5–9]. Preliminary clinical trials have supported these findings, demonstrating reductions in virus load in some individuals receiving GM-CSF therapy [10, 11]. Therefore, GM-CSF therapy was evaluated to maintain or improve host defenses in HIV-infected individuals.

Methods

Subjects. Between August 1995 and July 1997, 105 HIV-seropositive individuals 18–55 years old were enrolled at 2 Brazilian centers (Salvador-Bahia and Curitiba-Parana). Subjects were required to have had an AIDS-defining diagnosis based on 1993 Centers for Disease Control and Prevention (CDC) criteria within the last 3 months or CD4 cell count <300 cells/μL at entry, and <6 months exposure to zidovudine. Subjects were excluded for the presence of active AIDS-defining diagnosis, with the exception of stage I Kaposi's sarcoma.

Study design. A randomized, placebo-controlled, double-blind study design was utilized to evaluate the safety and efficacy of

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This study was approved by the institutional review boards of all participating institutions, and all subjects gave written informed consent.

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Table 1. Baseline characteristics and demographics.

Characteristic	Placebo (n = 52)	GM-CSF (n = 53)	P
Median age, years (range)	35 (23–54)	32 (23–48)	NS
Sex			
Male	41 (79)	40 (75)	NS
Female	11 (21)	13 (25)	
Karnofsky performance status			NS
60%	0	1 (2)	
70%	2 (4)	4 (8)	
80%	2 (4)	3 (6)	
90%	8 (15)	8 (15)	
100%	40 (77)	37 (70)	
Median virus load, copies/mL (range)	93,000 (40–3,600,000)	155,000 (400–2,000,000)	.21
HIV-RNA level <500 copies/mL	3 (6)	1 (2)	
Median CD4 cell count, cells/μL (range)	136 (4–347)	80 (3–284)	.04
CD4 cell count <50 cells/μL	9/52 (17)	21/52 (40)	.01
CD4 cell count <100 cells/μL	21/52 (40)	30/53 (58)	.08
Median CD8 cell count, cells/μL (range)	723 (95–2955)	691 (35–1986)	NS
Prior opportunistic infection	29/52 (56)	37/53 (70)	.14
Antiretroviral therapy			
Mean AZT duration before study, days	21	19	NS
AZT only	18 (35)	17 (32)	NS
AZT + 2d agent during study	16 (31)	18 (34)	
AZT + 2d agent before study	18 (35)	18 (34)	
Receiving any 2d agent	34 (65)	36 (68)	NS
ddI	32 (62)	26 (49)	
DDC	4 (8)	8 (15)	
3TC	4 (8)	5 (9)	
Saquinavir	1 (2)		

NOTE. Data are no. (%), except where noted. 3TC, lamivudine; AZT, zidovudine; DDC, zalcitabine; ddI, didanosine; GM-CSF, granulocyte-macrophage colony-stimulating factor; HIV, human immunodeficiency virus; NS, not significant.

treatment with zidovudine (300 mg/day [12, 13]) and either 125 μg/mm of yeast-derived recombinant human GM-CSF (sargramostim; Immunex, Seattle, WA) or placebo administered subcutaneously twice weekly for 24 weeks. The use of additional nucleoside analogues was permitted, as agents became available in Brazil. Subjects were withdrawn from the study because of discontinuation of zidovudine, severe adverse event, or 2 opportunistic infections (OIs) during the study. One subject, randomized to placebo, received saquinavir during the last 8 weeks of study. The treatment was considered to have failed at the 6-month time point in the intent-to-treat analysis. Prophylactic trimethoprim-sulfamethoxazole was administered when CD4 cell counts were <200 cells/μL.

Study evaluations. Clinical and hematology evaluations were performed at baseline and weekly during the first 4 weeks and monthly through month 6. Assessment of serum chemistries and liver and renal function were performed at baseline and monthly throughout the study. Follow-up data for survival at 1 year was collected retrospectively by review of medical records.

Heparinized blood and plasma were collected at baseline and at months 1, 3, and 6 during study for lymphocyte subset analysis by flow cytometry and virological assays. Plasma was cryopreserved at a central laboratory and batch analyzed to quantify HIV-RNA concentration by nucleic acid sequence–based assay (NASBA, Organon-Teknika, Boxtel, The Netherlands). Samples collected at baseline and 6 months were also evaluated for HIV genotype variation at codons 41, 69, 70, 74, 184, 214, and 215 of the reverse transcriptase, using specific probe sequence hybridization (LiPA HIV-1 RT, Murex Innogenetics, Dartford, United Kingdom). This subset analysis was conducted on samples from 46 of 77 subjects (39 placebo and 38 GM-CSF) who had completed the treatment phase with a minimum of 95% study drug compliance, as assessed by directly observed therapy. The clinical characteristics of patients whose samples were used in the genotype analysis were not different from the overall groups.

Statistical analysis. Prospectively defined study objectives included comparing groups for infections, survival, and surrogate markers of disease. Data were analyzed by using likelihood ratio χ^2 tests (Fisher's exact test in the case of 0 frequencies) and Wilcoxon rank-sum tests. All tests were 2-sided. Differences between treatment groups in mean change from baseline in $\log_{10}$ HIV RNA levels were summarized by using 95% confidence intervals (CIs) based on the t distribution. Analyses for $\log_{10}$ HIV-RNA levels included data from all subjects at each time point, using last observation carried forward for missing data values. The proportions of subjects experiencing an OI or any infection during the study period were compared between treatment groups by use of the likelihood ratio χ^2 test. Analyses of all clinical variables (infections, survival, and hospitalization) were performed on an intention-to-treat basis, using all available data. For all other variables (hematology, serum biochemistry, and drug-resistance testing), comparisons between the treatment groups were made using the likelihood ratio χ^2 test for categorical data (Fisher's exact test in

Table 2. Frequency of opportunistic infections (OIs), by type and hospitalizations.

Clinical event	Placebo	GM-CSF	*P*
OI during study[a]	14 (27)	17 (32)	NS
History of prior OI[a]	7 (50)	17 (100)	<.01
Baseline CD4 cell count <50 cells/μL[a]	6 (42)	7 (41)	NS
Category of OI[b]			
Toxoplasmosis	1	5	
Cytomegalovirus	2	1	
Candida	5	5	
Histoplasmosis	1	1	
Tuberculosis	3	2	
Pneumocystis carinii pneumonia/pneumonia	5	4	
Herpes	2	1	
Cryptosporidia/isospora	0	3	
Withdrawal for 2 OIs	3	3	
Any infection during study[a]	37 (71)	36 (68)	NS

NOTE. GM-CSF, granulocyte-macrophage colony-stimulating factor; NS, not significant.

[a] Data are no. (%) of patients.

[b] Data are no. of hospitalizations.

the case of 0 frequencies) and the Wilcoxon rank-sum test for continuous data.

Results

Baseline characteristics. One hundred five subjects were randomized to receive either GM-CSF (53 subjects) or placebo (52 subjects). Groups were balanced for demographics, Karnofsky performance score, and the type and duration of antiretroviral therapy. There were significant differences between groups in baseline CD4 cell count and the proportion of subjects with CD4 cell count <50 cells/μL that suggested an increased risk of OIs in the active treatment group (table 1).

Virus load. Mean HIV RNA levels steadily declined in the GM-CSF cohort throughout the 6 months of treatment, with mean change from baseline ($\pm$SE) of -0.24 (± 0.11) $\log_{10}$ at 1 month, -0.51 (± 0.15) $\log_{10}$ at 3 months, and -0.60 (± 0.14) $\log_{10}$ at 6 months. This was not observed in the placebo group: $+0.04$ (± 0.11) $\log_{10}$ at 1 month, -0.10 (± 0.16) $\log_{10}$ at 3 months, or -0.07 ($+0.14$) $\log_{10}$ at 6 months. The decline in virus load at 6 months was significantly greater for the GM-CSF group than the placebo group ($P = .02$; [95% CI, -0.94–0.12]), with trends toward statistical significance at months 1 and 3 ($P = .17$; [95% CI, -0.58–0.02] and $P = .06$; [95% CI, -0.83–0.01], respectively). A 1 $\log_{10}$ or greater decrease in virus load was demonstrated in 20 (38%) of 53 GM-CSF–treated subjects versus 9 (17%) of 52 controls ($P = .02$). A significantly greater number of subjects receiving GM-CSF had HIV-RNA levels <500 copies/mL at $\geq$2 evaluations than did subjects receiving placebo (11% vs. 2%, $P = .04$).

A decrease in median plasma virus load was observed for GM-CSF–treated subjects versus placebo receiving each of the 3 antiretroviral regimens (monotherapy [-0.18 $\log_{10}$ vs. $+0.24$ $\log_{10}$; $P = .05$], mono $\rightarrow$ dual therapy [-0.73 $\log_{10}$ vs. -0.30 $\log_{10}$; $P = .23$], and dual therapy [-0.79 $\log_{10}$ vs. -0.29 $\log_{10}$; $P = .27$]), although small patient numbers in each subgroup limited statistical significance.

Genotype analysis. Genotype analysis was performed for 43 subjects selected before study unblinding, and evidence of genotypic resistance to zidovudine at baseline was identified in 26% of placebo subjects and 25% of GM-CSF subjects. After 6 months of therapy, viral sequences from 80% of placebo subjects and 50% of GM-CSF subjects demonstrated new resistance mutations ($P = .04$). Mutation M184V was observed in 3 of 9 subjects receiving lamivudine at 6 months (2 placebo and 1 GM-CSF). Genotypic mutations associated with resistance to didanosine (ddI) and zalcitabine were not detected.

Immunological parameters. The increase in CD4 cell count was greater in the GM-CSF group relative to the control group at each time point, although this did not achieve statistical significance (month 1, +57 vs. +22, $P = .36$; month, 3 +64 vs. -2, $P = .06$; month 6, +35 vs. +12, $P = .42$). More subjects in the GM-CSF group than in the placebo group had a $\geq$30% increase in CD4 cell count at any visit during the study (59% vs. 80%, respectively, $P = .03$). The mean percent increase from baseline in CD8 cell count at 6 months was similar between groups: $-2\% \pm 8\%$ for placebo versus $30\% \pm 17\%$ for GM-CSF ($P = .16$). GM-CSF at this dose did not result in a significant increase in absolute neutrophil count.

Infections, hospitalizations, and survival. No difference was observed between treatment groups in the proportion of subjects developing any infection or OI (14/52 placebo vs. 17/53 GM-CSF; *P,* not significant; table 2), the incidence of specific OIs (table 2), or median CD4 cell count at the onset of OIs (data not shown), despite the increased risk of OI among the GM-CSF–treated subjects. All 17 subjects in the GM-CSF arm who developed an OI on study had a history of $\geq$1 prior OI, compared to only 50% of 14 placebo subjects ($P < .01$).

The number of patients hospitalized was similar between

Table 3. Safety and toxicity evaluations.

Evaluation	Placebo	GM-CSF	*P*
Withdrawal for adverse event	2/52 (4)	5/53 (9)	NS
Biochemistry			
AST >2.5× NL	8/48 (17)	8/49 (16)	
ALT >2.5× NL	6/48 (13)	7/49 (14)	
ALP >5× NL	4/48 (8)	6/49 (12)	
Cr >1.5 mg/dL	1/48 (2)	0/49 (0)	
Plts <50 K/μL	2/49 (4)	1/52 (2)	
Hb <8 gm/dL	5/49 (10)	6/52 (12)	
Fever	24/51 (47)	25/53 (47)	NS
Injection site reaction	1/51 (2)	16/53 (30)	.001
Flu syndrome	2/51 (4)	8/53 (15)	.05

NOTE. Data are no. of patients who experienced an adverse event/total no. of patients in group (%). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; GM-CSF, granulocyte-macrophage colony-stimulating factor; Hb, hemoglobin; NL, normal limit; NS, not significant; Plts, platelets.

groups (21% for placebo and 32% for GM-CSF; $P = .20$). Admission diagnoses included infections (8 for placebo and 9 for GM-CSF), adverse events (5 for placebo and 7 for GM-CSF), and other (2 for placebo and 2 for GM-CSF).

Two deaths (1 suicide and 1 disease progression) occurred during the 6 months on study, both in the GM-CSF group. Survival for both groups was also similar at 1 year (36 [69%] of 52 placebo vs. 36 [68%] of 53 GM-CSF). Kaplan-Meier analysis demonstrated no differences in survival between groups.

Safety. No difference was observed in the incidence of fever; hematologic, hepatic, or renal function toxicities; or withdrawals because of adverse events (table 3). Flulike syndrome (15% vs. 4%) and injection site reactions (30% vs. 2%) were more frequent with GM-CSF, though nearly all were mild (grade 1–2).

Subject withdrawal was similar between groups (13/52 placebo and 15/53 GM-CSF). Adverse events accounted for only 7 subject withdrawals: 2 in the placebo group (1 anemia and 1 thrombocytopenia) and 5 in the GM-CSF group (1 confusion and 4 anemia). The remaining subjects were withdrawn because of disease progression or death (4 per group), noncompliance (3 per group), loss to follow-up (2 per group), and reasons listed as "other" (2 placebo and 1 GM-CSF).

Discussion

This is the first clinical study to demonstrate conclusively that GM-CSF can significantly suppress plasma viremia in adults with AIDS receiving nucleoside analogues. Virus load fell to <500 copies/mL in a greater number of GM-CSF-treated subjects, which suggests that the reduction in virus load with GM-CSF may be clinically significant. Also, significantly fewer zidovudine-resistant mutations developed during treatment, which suggests that GM-CSF may extend the effectiveness of antiretroviral therapy by delaying viral breakthrough. This effect is likely a direct consequence of the greater decrease in virus load observed in the treatment group.

The anti-HIV effect of GM-CSF may be distinct from antiretroviral agents. Typically, antiretroviral agents produce a rapid fall in virus load during the first 3–10 weeks of therapy, followed by an increase in plasma viral RNA due to the development of resistant phenotypes or difficulty in regimen adherence [14–17]. In contrast, GM-CSF therapy produced a steady decline in virus load that did not appear to have reached a plateau by 6 months of treatment. These data raise the possibility that longer courses of therapy may produce further decreases in viral burden or permit reductions in antiretroviral therapy.

In vitro studies have demonstrated several mechanisms by which GM-CSF may reduce virus production. First, GM-CSF has been reported to increase the intracellular concentration of azido-nucleoside active metabolites and thus augment the antiviral activity of zidovudine and stavudine [5, 6]. Second, macrophages activated with GM-CSF, but not M-CSF or G-CSF, have been shown to resist infection with macrophage-tropic strains of virus both by down-regulating of CCR5 and CXCR4 chemokine receptor expression on monocyte-derived macrophages and by inducing the monocyte/macrophages to secrete β-chemokines that competitively inhibit HIV entry in bystander CD4 T cells [7–9]. Finally, GM-CSF has been shown to augment host defenses. Further studies are needed to determine the mechanism by which GM-CSF exerts an antiviral effect.

Although the incidence of OIs and survival at 6 months and 1 year were similar between groups, it is important to note that the GM-CSF group had a greater risk of infections and mortality based on baseline CD4 cell count, proportion of subjects with CD4 cell counts <50 cells/μL, and history of OIs before the study. In fact, GM-CSF subjects who did not have a history of OIs before the study did not develop OIs during the study, unlike placebo subjects.

This study demonstrates that GM-CSF may be a novel biological agent for treating HIV disease. Studies are needed to evaluate GM-CSF in combination with HAART and longer courses of therapy and to determine the effects of GM-CSF on viral reservoirs and immune recovery.

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References

1. Cohen OJ, Fauci AS. Transmission of multidrug-resistant human immunodeficiency virus: the wake-up call. N Engl J Med **1998**;339:341–3.
2. Esser R, Glienke W, Andreesen R, et al. Individual cell analysis of the cytokine repertoire in human immunodeficiency virus–1-infected monocytes/macrophages by a combination of immunocytochemistry and in situ hybridization. Blood **1998**;91:4752–60.
3. Barbaro G, Di Lorenzo G, Grisorio B, Soldini M, Barbarini G. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on HIV-related leukopenia: a randomized, controlled clinical study. AIDS **1997**;11:1453–61.
4. Bernstein ZP, Brooks S, Hayes FA, Gould M, Jacob S, Tomasi TB. A pilot study in the use of GM-CSF in human immunodeficiency virus infected individuals. Blood **1997**;90(Suppl 1):133a [abstract 584].
5. Perno CF, Yarchoan R, Cooney DA, et al. Replication of human immunodeficiency virus in monocytes: granulocyte-macrophage colony-stimulating factor (GM-CSF) potentiates viral production yet enhances the antiviral effect mediated by 3'-azido-2'3'-dideoxythymidine (AZT) and

other dideoxynucleoside congeners of thymidine. J Exp Med **1989**;169: 933–51.

6. Perno CF, Cooney DA, Gao WY, et al. Effects of bone marrow stimulatory cytokines on human immunodeficiency virus replication and the antiviral activity of dideoxynucleosides in cultures of monocyte/macrophages. Blood **1992**;80:995–1003.
7. Di Marzio P, Tse J, Landau NR. Chemokine receptor regulation and HIV type 1 tropism in monocyte-macrophages. AIDS Res Hum Retroviruses **1998**;14:129–38.
8. Di Marzio P, Mariani R, Tse J, Thomas EK, Landau NR. GM-CSF or CD40L suppresses chemokine receptor expression and HIV-1 entry in human monocytes and macrophages [abstract 37]. In: Program and abstracts of the 5th Conference on Retroviruses and Opportunistic Infections (Chicago). Alexandria, VA: Foundation for Retrovirology and Human Health, **1998**:86.
9. Matsuda S, Akagawa K, Honda M, Yokota Y, Takebe Y, Takemori T. Suppression of HIV replication in human monocyte–derived macrophages induced by granulocyte-macrophage colony-stimulating factor. AIDS Res Hum Retroviruses **1995**;11:1031–8.
10. Skowron G, Stein D, Drusano G, et al. Safety and anti-HIV effect of GM-CSF in patients on highly active antiretroviral therapy. J Infect Dis **1999**;180:1064–71.
11. Hewitt RG, Morse GD, Lawrence WD, et al. Pharmacokinetics and pharmacodynamics of granulocyte-macrophage colony-stimulating factor and zidovudine in patients with AIDS and severe AIDS-related complex. Antimicrob Agents Chemother **1993**;37:512–22.
12. Collier AC, Bozzette S, Coombs RW, et al. Pilot study of low dose zidovudine in human immunodeficiency virus infection. N Engl J Med **1990**;323: 1015–21.
13. Fischl MA, Parker CB, Pettinelli C, et al. Randomized controlled trial of a reduced dose of zidovudine in patients with the acquired immunodeficiency syndrome. The AIDS Clinical Trials Group. N Engl J Med **1990**;323:1009–14.
14. Schooley RT, Ramirez-Ronda C, Lange JM, et al. Virologic and immunologic benefits of initial combination therapy with zidovudine and zalcitabine or didanosine compared with zidovudine monotherapy. Wellcome Resistance Study Collaborative Group. J Infect Dis **1996**;173:1354–66.
15. Katzenstein DA, Hammer SM, Hughes MD, et al. The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 CD4 cells per cubic millimeter. AIDS Clinical Trials Group Study 175 Virology Study Team. N Engl J Med **1996**;335:1091–8.
16. Gulick RM, Mellors JW, Havlir D, et al. Simultaneous vs. sequential initiation of therapy with indinavir, zidovudine, and lamivudine for HIV-1 infection: 100-week follow-up. JAMA **1998**;280:35–41.
17. Anonymous. Report of the NIH Panel to Define Principles of Therapy of HIV Infection. Ann Intern Med **1998**;128:1057–78.