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Safety, Activity, and Pharmacokinetics of GLQ223 in Patients with AIDS and AIDS-Related Complex

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GLQ223 is a highly purified single-chain ribosome-inactivating protein with selective effects against a variety of cells, including macrophages infected with human immunodeficiency virus. We evaluated the safety, pharmacokinetics, and immunologic effects of multiple doses of GLQ223 in 22 patients with AIDS or AIDS-related complex; $CD4^+$ T-cell counts were between 100 and 350/mm^3. GLQ223 was administered intravenously at doses of 8, 16, 24, 36, and 50 μg/kg of body weight; the drug was administered by constant infusion over 3 h to achieve a concentration in serum of 50 ng/ml; this concentration is known to be associated with anti-HIV effects in vitro. All patients reported a flu-like syndrome characterized by muscle and joint aches and an increase in creatinine kinase levels; symptoms were controlled easily. For patients who received 36 and 50 μg/kg, target concentrations in serum were achieved and an increase in $CD4^+$ and $CD8^+$ T cells was sustained; this sustained increase persisted for at least 28 days after the last infusion. β_2-Microglobulin levels increased during the infusions and then declined when the infusions ended. Repeat infusions of GLQ223 were safe and relatively well tolerated. The target concentration of GLQ223 in serum was achieved and sustained. Our results suggest that GLQ223 may have activity in treating patients with human immunodeficiency virus infection.

GLQ223 is a highly purified formulation of the single-chain ribosome-inactivating protein (RIP) trichosanthin (GLQ223), which is extracted from the root tubers of *Trichosanthes kirilowii* Maximowicz (17). The selective antiviral activities of RIPs have been demonstrated against a number of different viruses (1, 12, 26, 27). While the mechanism of antiviral action of this class of compounds is unknown, its activity may relate to a preferential uptake of RIP, resulting in selective cytotoxicity for virally infected cells but with relative sparing of noninfected cells (4, 8).

GLQ223 was the first RIP shown to preferentially inhibit replication of human immunodeficiency virus (HIV) type 1 in vitro (17, 19). Selective anti-HIV activity has been confirmed for GLQ223 as well as other RIPs (9, 12, 14, 15, 22, 27). In vitro studies demonstrated that GLQ223 treatment selectively decreases HIV viral protein levels under conditions that do not comparably inhibit the synthesis of host cell proteins (17, 18). The drug inhibited HIV replication in acutely infected T-lymphoid cells and chronically infected monocyte-derived macrophages (17–19). In contrast, drugs like zidovudine, which act by inhibiting the HIV-encoded enzyme reverse transcriptase, do not demonstrate antiviral activity in chronically infected cells (6, 24). Since macrophages may serve as a reservoir of viral replication, the activity of GLQ223 in this cell population is of particular interest in the development of anti-HIV compounds.

A preliminary study to evaluate the safety, tolerability, and pharmacokinetics of GLQ223 was conducted in 18 HIV-infected patients (14). A single bolus infusion was reasonably well tolerated by all but one patient, who experienced a severe neurocortical reaction. At the highest dose administered (36 μg/kg of body weight), the peak concentration of drug achieved in serum was comparable to the in vitro levels that mediate antiretroviral effects. However, concentrations of GLQ223 were not sustained at this level over a 3-h period, the minimal exposure time associated with anti-HIV activity in vitro. Another clinical trial of GLQ223, which was conducted in community physicians' offices, suggested that it has anti-HIV activity (3).

Subsequently, pharmacokinetic data from previous studies and computer modeling were used to calculate the doses and the duration of infusion of GLQ223 required to maintain a level in serum of at least 50 ng/ml for a minimum 3 h. The calculated dose and infusion scheme of GLQ223 would thus mimic the minimum GLQ223 exposure associated with anti-HIV effects in vitro. The safety study reported here was designed to assess the effects of GLQ223 by using this modified mode of administration.

MATERIALS AND METHODS

Study design. The study described here was a dose-escalated, open-label evaluation of the safety, tolerability, and pharmacokinetics of GLQ223 in patients with HIV infection. Twenty-two patients were enrolled in one of five dose groups. The dose levels were 8, 16, 24, 36, and 50 μg/kg. Prior to intravenous infusion, each patient received a 50-μg test dose of GLQ223 to assess hypersensitivity. The first three patients received the drug at 8 μg/kg over 10 min with no loading dose and no continuous infusion. The next three patients received the drug at 16 to 8 μg/kg over 10 min as a loading dose, and the other patients received the drug at 8 μg/kg by constant infusion over 2 h. Subsequent patients who were administered 24, 36, or

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50 μg/kg received a 4-μg/kg loading dose over 10 min, with the balance of the dose (20, 32, and 46 μg/kg, respectively) administered as a 3-h infusion. GLQ223 was administered weekly for 4 consecutive weeks to assess the effects of multiple dosings. Patients were observed for continued drug effects for up to 4 weeks after the last infusion. The total length of the study was 49 days.

Patients. The study included ambulatory adults who met the diagnostic criteria established by the Centers for Disease Control for AIDS or had symptomatic AIDS-related complex (ARC) (5). To be eligible for the study, all patients needed to have serologic evidence of anti-HIV antibody on enzyme-linked immunosorbent assay that was confirmed by Western blotting (immunoblotting), and their $CD4^+$ T-cell counts had to be between 100 and 350/mm^3. Additional entry requirements included granulocytes level of $\geq$1,000/mm^3, bilirubin level of $\leq$1.5 mg/dl, aspartate aminotransferase (AST) level of <1.5 times the upper limit of normal, prothrombin time of <1.5 times the control, and calculated creatinine clearance of $\geq$80 ml/min (20).

Patients were not allowed into the study if they had an active opportunistic infection or required ongoing maintenance therapy for a prior opportunistic infection other than *Pneumocystis carinii*. They were permitted to take aerosolized pentamidine, 300 mg every 30 days, but no other prophylaxis was allowed. None of the patients had received immunomodulating therapy for at least 3 weeks prior to the study or antineoplastic chemotherapy for 6 weeks prior to the study. The Committee for Human Research at the University of California, San Francisco, approved the study, and all patients gave written informed consent.

Clinical and laboratory monitoring. Patients were treated and monitored in the General Clinical Research Center at San Francisco General Hospital for at least 24 h after each infusion. Physical examinations and laboratory evaluations were performed before each infusion and again at 1, 2, and 4 weeks after the last infusion. Patients who discontinued the study prematurely were evaluated by physical examination and laboratory testing 4 weeks after their last infusion. Laboratory tests, which were performed at regular intervals, included complete blood count with differential, platelet and reticulocyte counts; chemistry profile, including albumin, alkaline phosphatase, total bilirubin, blood urea nitrogen, calcium, cholesterol, triglycerides, glucose, lactate dehydrogenase, AST, alanine aminotransferase (ALT), sodium, potassium, and uric acid; routine urinalysis; prothrombin time; activated partial thromboplastin time; chest radiograph; and electrocardiogram. Assessment of surrogate activity parameters included enumeration of T-cell subset populations, measurement of β_2-microglobulin levels, and determination of quantitative serum HIV p24 antigen.

Concomitant medications. Zidovudine treatment was not permitted, and subjects discontinued zidovudine treatment 2 weeks prior to the first infusion and during the 49-day study period. To determine the effects of zidovudine on the pharmacokinetic profile of GLQ223 and to assess the safety of GLQ223 and zidovudine in combination, two patients in the 50-μg/kg dose group were given zidovudine orally at 500 mg/day, concomitantly with GLQ223. Topical medications and drugs for symptomatic relief were permitted as needed; these included acetaminophen, nonsteroidal anti-inflammatory agents, diphenhydramine, codeine, meperidine, oral antifungal agents, benzodiazepines, oral antacids, astemizole, and corticosteroids.

Study drug. GLQ223 was provided by Genelabs Technologies, Inc. (Redwood City, Calif.). Its purity was greater than 98%, as determined by laser densitometric scanning of Coomassie blue-stained gels and by size-exclusion, high-performance liquid chromatography.

Pharmacokinetic evaluation of GLQ223 and detection of GLQ223-reactive antibody. Plasma GLQ223 concentrations were determined at Genelabs by a sensitive capture sandwich enzyme-linked immunosorbent assay (10). Intraday and interday variabilities were 6.3 and 16.8%, respectively, at a plasma GLQ223 concentration of 2 ng/ml, 8.9 and 10.4%, respectively, at 20 ng/ml, and 4.3 and 11.8%, respectively, at 200 ng/ml. Each plasma sample was measured at least in duplicate, and the mean value was used for pharmacokinetic analysis. Blood samples (2 ml) for GLQ223 analysis were obtained from the arm contralateral to that used for the drug infusion at the following times: 0, 5, 10, 20, 30, 45, 60, and 90 min and 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, and 24 h postinfusion. Plasma samples containing the anticoagulant EDTA were immediately placed on ice and centrifuged, and the plasma was removed and frozen at −20°C until it was assayed.

Pharmacokinetic parameter estimates were obtained by using noncompartmental methods. Following construction of semilogarithmic concentration-versus-time plots, the terminal elimination rate constant was estimated by exponential regression by using at least the last three datum points. The terminal half-life was calculated by dividing this rate constant into ln 2. The area under the plasma concentration-time curve (AUC) from time zero to infinity ($AUC_{0-\infty}$) was determined by using the linear trapezoidal rule for ascending concentration and the logarithmic trapezoidal rule for descending concentrations, with extrapolation to infinity determined by dividing the last concentration by the terminal elimination rate constant. The following parameters were also determined: plasma clearance (CL) = dose/$AUC_{0-\infty}$ and volume of distribution at steady-state (V_{ss}) = dose (AUMC)/$(AUC)^2$-($T/2$ × dose/AUC), where AUMC is the area under the moment curve from time zero to infinity, and T is the duration of the infusion.

Statistical methods. Clinical adverse events were recorded at each visit and were coded by using COSTART terminology. Additionally, reports of hypotension, rash, pruritus, or urticaria were combined and reported as "allergic reactions"; fever, chills, myalgia, arthralgia, or headache were combined and reported as "flu-like syndrome." Analysis of surrogate markers was performed by determining absolute changes from the baseline and mean changes over time. Changes from the baseline in each parameter at each dose were evaluated by using a paired t test. Pharmacokinetic modeling predicted that target exposure levels of GLQ223 would be obtained in patients who received at least 36 μg/kg. Therefore, analysis of immunologic markers was conducted in the following two groups: patients who received $\geq$36 μg of GLQ223 per kg and those who received <36 μg/kg at each infusion.

Mean changes in T-cell and β_2-microglobulin levels over time were evaluated by using the normalized AUC for $CD4^+$ and $CD8^+$ T cells. To determine the normalized AUC, the AUC was calculated by summing the areas of a series of trapezoids, where the height of each trapezoid was defined by two sequential values of the variable of interest and the width was equal to the duration (in days) between measurements. The AUC was then normalized to the baseline by dividing it by the mean of two baseline values multiplied by the total study duration. The correlation of normalized AUC with the total dose administered was evaluated by using Pearson's correlation (7). No corrections were made for repeated significance testing.

TABLE 1. Baseline demographic characteristics of patients[a]

Characteristic	Mean ± SEM	Range
Wt (kg)	74.2 ± 2.0	59.8–92.8
Age (yr)	38.4 ± 1.6	25.6–55.2
Age at diagnosis (yr)	36.9 ± 1.7	24.6–54.4
Duration on zidovudine (days)	347 ± 104	7–1,155
Karnofsky (%)	98.2 ± 0.9	85–100
β_2-Microglobulin concn (mg/dl)	3.5 ± 0.2	2.0–6.2
No. of:		
$CD3^+$ T cells/mm^3	1,112 ± 96	494–2,498
$CD4^+$ T cells/mm^3	204 ± 20	17–369
$CD8^+$ T cells/mm^3	924 ± 99	354–2246
$CD4^+$ cell:$CD8^+$ cell ratio	0.25 ± 0.03	0.03–0.58

[a] A total of 22 patients were studied.

TABLE 2. Pharmacokinetics of GLQ223 at different doses

Total dose (μg/kg)	CL (liters/h)	V_{ss} (liters)	$t_{1/2}$ (h)
8 (n = 2)	21.1 ± 8.2	4.3 ± 13.7	2.3 ± 0.4
16 (n = 3)	13.4 ± 2.5	19.1 ± 1.9	2.5 ± 1.0
24 (n = 5)	16.2 ± 3.5	33.0 ± 21.7	2.5 ± 0.5
36 (n = 6)	12.2 ± 3.7	25.1 ± 11.9	4.2 ± 1.4
50 (n = 3)	14.3 ± 2.5	29.1 ± 10.3	4.1 ± 0.9
Overall	14.7 ± 4.4	28.9 ± 14.6	3.3 ± 1.3

[a] CL, clearance from plasma; V_{ss}, volume of distribution at steady state; $t_{1/2}$, terminal half-life.

RESULTS

Demographics. Twenty-two patients entered the study; 13 had a prestudy diagnosis of ARC and 9 had AIDS. Demographic characteristics, symptoms, and laboratory test results were comparable between patients with AIDS and those with ARC. At the time of entry into the study, 13 patients had lymphadenopathy, 10 had hairy leukoplakia, 11 had oral thrush, and 6 had Kaposi's sarcoma. Less common prestudy conditions included weight loss, fatigue, diarrhea, dementia, neuropathy, and a history of tuberculosis. Other demographic characteristics are presented in Table 1. Sixteen patients had previously been treated with zidovudine; the median duration of zidovudine treatment prior to entry into the study was about 347 days. Patients were relatively healthy, with median $CD4^+$ and $CD8^+$ T lymphocyte counts of 204 ± 20 and 924 ± 99 cells per mm^3, respectively. Baseline values of T-cell subsets and β_2-microglobulin were comparable between patients treated with <36 or ≥36 μg of GLQ223 per kg. Concurrent medications for HIV-related conditions included acyclovir (n = 11 patients), aerosolized pentamidine (n = 10), dapsone (n = 3), ketoconazole (n = 3), trimethoprim-sulfamethoxazole (n = 1), and alpha interferon (n = 1).

Pharmacokinetics. A dose-dependent increase in the mean concentrations of GLQ223 in plasma was observed (Fig. 1). At the two highest doses, GLQ223 concentrations approached or exceeded the target level of 50 ng/ml, and this level was maintained for at least 3 h. The overall mean value for V_{ss} was 28.9 ± 14.6 liters. The overall mean values for CL and terminal half-life were 14.7 ± 4.4 liters/h and 3.3 ± 1.3 h, respectively. The average values observed for patients within each dose group are provided in Table 2. The effects of orally administered zidovudine on the pharmacokinetic profile of GLQ223 were evaluated in two of the subjects who received the highest dose of GLQ223. Each subject received zidovudine at a dose of 500 mg/day. In these two subjects, to whom 50 μg of GLQ223 per kg was administered, the observed pharmacokinetic profile of GLQ223 was not affected by zidovudine treatment (data not shown).

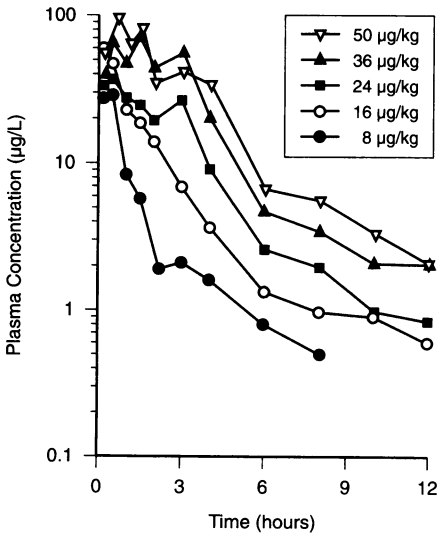

FIG. 1. Time-concentration profile of GLQ223.

Safety and clinical evaluations. In general, administration of GLQ223 was well tolerated and side effects were evaluated to be predominantly mild or moderate. No patient developed a new or recurrent life-threatening opportunistic infection or disabling neurologic complication during the study. Four patients failed to complete the study. One patient treated with 24 μg/kg discontinued the study after the first infusion because of an acute allergic reaction characterized by erythema and urticaria. Another patient, who was treated with 36 μg/kg, discontinued the study after the first infusion because of an episode of transient confusion, which resolved after 24 h. He had been taking psychoactive medications, and it was not clear whether the confusion was attributable to GLQ223; however, he was not rechallenged with GLQ223. Two patients withdrew their consent to continue with the study for unstated reasons.

Five additional patients interrupted treatment because of adverse events or other reasons. One patient, who received a dose of 24 μg/kg, had a 1-week delay in treatment between the third second and third infusions secondary to grade 3 myalgia and flu-like syndrome. Another patient, who also received 24 μg/kg, had the third infusion withheld at his request; this was unrelated to any toxicity. Three patients treated with GLQ223 at 36 μg/kg had an interruption of treatment; one patient had his fourth infusion held after nausea, vomiting, hypotension, diarrhea, and diaphoresis occurred immediately after the challenge dose; a fourth infusion was not administered. Another patient developed hypotension and periorbital edema after the third infusion and did not receive a fourth infusion. A final patient who received the 36-μg/kg dose had a 6-week hiatus between his third and fourth doses for personal reasons.

All patients in the study complained of a flu-like syndrome characterized by fevers, myalgias (particularly in the neck and shoulder areas), arthralgia, and/or headache (Table 3). This symptom complex generally began 8 to 12 h after drug infusion and usually diminished slowly over the next 24 to 72 h. Acetaminophen, diphenhydramine, and nonsteroidal anti-inflammatory agents were administered routinely, usually resulting in amelioration or complete resolution of the symptoms.

TABLE 3. Adverse events reported by patients

Dosage	No. of infusions (no. of patients)	No. of patients											
		By body system							By specific event				
		Body as a whole	Digestive	Central nervous system	Respiratory	Skin	Allergic reaction	Flu-like syndrome	Asthenia	Anorexia	Nausea	Pharyngitis	Herpes simplex virus
<36 μg/kg	1 (12)	10	5	4	3	1	5	10	4	2	1	3	0
	2 (12)	10	5	4	1	4	4	8	2	3	0	1	2
	3 (11)	11	3	4	4	2	4	10	5	2	2	4	1
	4 (11)	11	6	1	2	2	3	10	2	3	1	2	0
	All (12)	12	8	8	7	7	8	12	5	5	3	7	3
≥36 μg/kg	1 (10)	10	5	0	5	1	6	9	5	1	2	5	0
	2 (9)	9	3	1	7	3	5	7	2	0	2	7	2
	3 (9)	9	4	1	3	4	5	6	2	3	2	3	2
	4 (9)	8	3	1	0	1	3	4	4	2	2	0	0
	All (10)	10	8	4	9	6	10	10	10	4	5	9	3

[a] Adverse events that occurred between infusions were attributed to prior infusions.

There was no difference in the development of flu-like syndrome in patients treated with GLQ223 at <36 or ≥36 μg/kg. Of all the adverse events, 98% were rated as mild to moderate (grade 1 or 2), and only three events were considered to be both severe and related to the study drug. These included one episode of hypotension as well as the reports of urticaria and transient confusion mentioned above. All events resolved within 24 h.

The flu-like syndrome was associated with transient but significant elevations of creatinine kinase for patients treated with either <36 or ≥36 μg of GLQ223 per kg (Table 4). Isoenzyme analysis revealed that increases in creatinine kinase levels were restricted to the MM fraction (data not shown). The changes in AST and ALT levels mirrored those seen in creatinine kinase, with increases observed after the first infusion, peaking after the third infusion, and returning to normal values 28 days after the GLQ223 infusions were discontinued. Over the period of analysis, increases in creatinine kinase, AST, and ALT levels in patients treated with ≥36 μg/kg were not significantly different from the increases observed in those treated with <36 μg/kg. Laboratory parameters returned toward baseline values after the cessation of treatment. There was no change in serum creatinine levels during the study.

Hemoglobin levels decreased initially in the group treated with <36 μg of GLQ223 per kg, while patients treated with ≥36 μg of GLQ223 per kg showed slowly declining hemoglobin levels during the study period. Twenty-eight days after the cessation of study drug, both groups had modest but significant decreases in hemoglobin levels compared with those at the baseline; the group treated with ≥36 μg/kg had greater decreases in hemoglobin levels. However, this was not a reflection of generalized bone marrow suppression, because platelet levels increased in both groups after the second infusion and continued to increase, returning to normal values 28 days after the last study drug treatment. In addition, the leukocyte count increased following the first infusion and remained elevated only in the group receiving ≥36 μg of GLQ223 per kg.

After 4 weeks of treatment, immunoglobulin G antibody to GLQ223 was found in most patients. Antibodies directed to GLQ223 developed more frequently in patients receiving the high dose than in patients receiving the low dose (Table 5). Patients treated with ≥36 μg of GLQ223 per kg developed GLQ223 antibodies after fewer infusions of GLQ223 than did those treated with <36 μg of study drug per kg.

Surrogate markers. There was an increase in $CD4^+$ T lymphocytes in patients treated with ≥36 μg of GLQ223 per kg (Table 6 and Fig. 2); the increase was statistically significant after the second infusion. Patients treated with <36 μg/kg had an initial small decrease in $CD4^+$ T lymphocytes; this was followed by modest increases after subsequent infusions. Twenty-eight days after the last infusion, $CD4^+$ T lymphocytes remained elevated compared with those at the baseline. A significant decrease in $CD8^+$ T cells was seen 1 week after the first infusion in patients taking doses of <36 μg/kg. In those infused with ≥36 μg of GLQ223 per kg, the mean $CD8^+$ T-cell count increased progressively after each infusion following the second infusion, although the changes were not statistically significant.

At doses of ≥36 μg/kg, statistically significant increases in β_2-microglobulin levels from the baseline were seen at all visits, including a time point 28 days after the last infusion ($P < 0.01$). Patients treated with <36 μg of GLQ223 per kg also had significant increases in β_2-microglobulin levels after the second and third infusions and at 28 days after the last infusion ($P < 0.05$). The magnitude of the increase in β_2-microglobulin levels was greater in patients who received ≥36 μg of GLQ223 per kg than in those who received <36 μg/kg.

Eleven patients had detectable p24 antigen in their sera. Of these, no baseline value was recorded for one patient and baseline values only were recorded for three patients. Two of the remaining seven patients received ≥36 μg of GLQ223 per kg at each infusion and the other five patients received <36 μg/kg. Two patients, one treated with GLQ223 at 8 μg/kg and the other treated with GLQ223 at 24 μg/kg, had at least a 50% decline in serum p24 antigen levels at some time during the study. The remaining patients had smaller changes. Patients without detectable p24 antigen at the baseline did not develop p24 antigenemia during the study.

DISCUSSION

Our primary objective was to evaluate the safety and pharmacokinetics of GLQ223 in patients with ARC and AIDS. The results indicate that repeated infusions of GLQ223 can be given safely to these patients. Target concentrations in plasma that were predicted by computer modeling techniques were achieved. At the two highest doses administered, we were able to achieve (and sustain for 3 h) levels in plasma similar to those associated with anti-HIV effects in vitro. The concentrations of

TABLE 4. Changes in laboratory values from baseline

Time value was determined	Creatine kinase (U/liter)		AST (IU/liter)		ALT (IU/liter)		Creatinine (mg/dl)		Hemoglobin (g/dl)		Platelets (10^3/mm^3)		Leukocytes (10^9/liter)	
	<36 μg/kg	≥36 μg/kg	<36 μg/kg	≥36 μg/kg	<36 μg/kg	≥36 μg/kg	<36 μg/kg	≥36 μg/kg	<36 μg/kg	≥36 μg/kg	<36 μg/kg	≥36 μg/kg	<36 μg/kg	≥36 μg/kg
Baseline	99 ± 32	98 ± 38	42 ± 4	36 ± 4	50 ± 7	31 ± 3	1.0 ± 0.05	1.1 ± 0.01	13.3 ± 0.4	12.9 ± 0.2	164 ± 13	170 ± 13	4.4 ± 0.4	4.4 ± 0.3
Infusion[a]:														
1	128 ± 28	183 ± 36[b]	51 ± 3	57 ± 6[b]	62 ± 12	45 ± 4[b]	1.0 ± 0.05	1.1 ± 0.02	12.7 ± 0.4[c]	12.7 ± 0.3	161 ± 9	182 ± 15	4.6 ± 0.4	5.4 ± 0.5[c]
2	149 ± 25[c]	211 ± 36[c]	61 ± 7[c]	70 ± 10[b]	87 ± 17[c]	62 ± 8[b]	1.0 ± 0.05	1.1 ± 0.05	13.0 ± 0.3	12.2 ± 0.3[c]	185 ± 13[c]	235 ± 22[c]	5.1 ± 0.5	6.7 ± 0.8[b]
3	168 ± 35[c]	219 ± 39[c]	62 ± 9	72 ± 11[b]	70 ± 11	59 ± 8[b]	1.0 ± 0.05	1.1 ± 0.04	13.0 ± 0.4	11.8 ± 0.4[c]	198 ± 19[b]	257 ± 25[b]	4.7 ± 0.4	7.3 ± 1.2[c]
4	131 ± 29	171 ± 36	50 ± 5	53 ± 8	62 ± 14	46 ± 7[c]	1.0 ± 0.04	1.1 ± 0.04	12.9 ± 0.4	12.1 ± 0.3[c]	188 ± 20	201 ± 13[c]	4.5 ± 0.3	6.0 ± 0.6[b]
4-wk follow-up	108 ± 32	152 ± 36	34 ± 6	48 ± 6	38 ± 10	42 ± 4[b]	1.0 ± 0.06	1.1 ± 0.04	12.9 ± 0.5[c]	11.6 ± 0.4[c]	168 ± 27	199 ± 28	4.5 ± 0.5	6.2 ± 1.1

[a] Test performed 1 week after each infusion, immediately prior to the next infusion.
[b] Statistically significant, $P < 0.01$.
[c] Statistically significant, $P < 0.05$.

TABLE 5. Development of anti-GLQ223 antibodies

Dose (μg/kg)	No. of patients with positive tests/total no. tested				
	After infusion[a]:				At 4-wk follow-up
	1	2	3	4	
8	0/3	0/3	0/3	1/3	1/3
16	0/3	0/3	1/3	1/3	2/3
24	1/5	2/6	3/5	2/4	4/5
36	0/6	3/5	4/5	3/3	4/5
50	0/4	1/4	4/4	4/4	3/4

[a] Test performed 1 week after each infusion, immediately prior to the next infusion.

GLQ223 in plasma declined in a biexponential fashion following the infusion of the drug. The different concentrations achieved were proportional to the administered dose, as illustrated in Fig. 1. The average terminal half-life for GLQ223 was 3.3 ± 1.3 h, with a mean CL of 14.7 ± 4.4 liters/h and a V_{ss} of 28.9 ± 4.6 liters. These values describing the disposition of GLQ223 appeared to be independent of the dose. The half-life was virtually identical to that observed previously in a group of patients with AIDS and ARC determined in a phase 1 study (10). Values for CL and V_{ss} in that study were lower, 9.2 ± 4.2 liters/h and 12.8 ± 5.0 liters, respectively, compared with those obtained in the current study. The exact reasons for these differences are unknown; however, in the current study the patients were in better general health and the drug was administered as an infusion, whereas in the earlier study the drug was administered as a bolus. Zidovudine did not affect the pharmacokinetic profile of GLQ223 in two subjects infused with GLQ223 and treated with 500 mg of zidovudine. Zidovudine did not affect the pharmacokinetic profile of GLQ223 in the two subjects infused with GLQ223 and treated with 500 mg of zidovudine. One must be extremely careful in interpreting a data set limited to two subjects; only larger clinical trials of the combination of GLQ223 and zidovudine will identify pharmacokinetic and biologically important interactions between GLQ223 and zidovudine. Such a trial has been initiated and fully accrued.

Clinical toxicities, characterized primarily by a flu-like syndrome, with fever, myalgia associated with elevated creatinine kinase levels in serum, arthralgia, and headache, were treated with acetaminophen, nonsteroidal anti-inflammatory agents, and diphenhydramine. In general, the frequency and severity of adverse events diminished over time. Allergic reactions in two patients necessitated their early withdrawal from the study. Laboratory abnormalities, primarily characterized by elevations in creatinine kinase, AST, and ALT levels, returned toward the baseline after the cessation of the study drug. There was a progressive decline in hemoglobin levels and an increase in leukocytes and platelets in patients who received ≥36 μg of GLQ223 per kg. The increase in platelets was surprising. There is no evidence that GLQ223 directly affects thrombopoiesis; however, other anti-HIV therapies have also been shown to increase platelet levels (21, 25). Therefore, an increase in platelet levels may reflect an anti-HIV effect of GLQ223. Alternatively, the increase in platelet levels may have resulted from the immunostimulation induced by GLQ223 and may not reflect an antiviral effect.

Increases in $CD4^+$ T-lymphocyte levels were evident after the first dose of GLQ223 and persisted for at least 4 weeks after the last infusion. $CD4^+$ T lymphocytes were observed in patients treated with doses of ≥36 μg/kg. The increase in the number of $CD4^+$ T cells was significantly increased from the

TABLE 6. Changes in surrogate markers from baseline

Time value was determined	$CD4^+$ T cells		$CD8^+$ T cells		β_2-microglobulin (mg/l)	
	<36 μg/kg	≥36 μg/kg	<36 μg/kg	≥36 μg/kg	<36 μg/kg	≥36 μg/kg
Baseline	228 ± 28	221 ± 29	959 ± 142	882 ± 142	3.7 ± 0.3	3.4 ± 0.2
Infusion[a]						
1	−13 ± 13	+11 ± 12	−182 ± 48[b]	−131 ± 92	+0.0 ± 0.2	+0.7 ± 0.2[b]
2	+1 ± 9	+71 ± 23[c]	−55 ± 67	+60 ± 149	+0.4 ± 1.0[c]	+1.5 ± 0.3[b]
3	+11 ± 13	+66 ± 25[c]	−55 ± 56	+92 ± 89	+0.4 ± 0.1[c]	+1.7 ± 0.2[b]
4	+20 ± 92	+66 ± 41	+10 ± 48	+95 ± 1,126	+0.3 ± 1.0	+1.8 ± 0.2[b]
4-wk follow-up	+39 ± 15[c]	+45 ± 32	+205 ± 95	+92 ± 616	+0.5 ± 0.2[c]	+1.0 ± 0.2[b]

[a] Test performed 1 week after each infusion, immediately prior to the next infusion.
[b] Statistically significant, $P < 0.01$.
[c] Statistically significant, $P < 0.05$.

baseline in patients treated with <36 μg of GLQ223 per kg; however, the numerical increase was greater in those treated with ≥36 μg/kg. Patients in both groups had decreases in $CD8^+$ T-lymphocyte levels after the first infusion, and the higher-dose group showed a persistent increase in $CD8^+$ T-lymphocyte levels. While increases in $CD4^+$ T-lymphocyte levels have been interpreted as a surrogate marker of antiretroviral effects, the concomitant increases observed in $CD8^+$ T-lymphocyte levels might also be suggestive of immunomodulatory effects of GLQ223 in vivo. Ongoing clinical trials with GLQ223 will provide a comparative analysis of T-cell subsets and viral load, which we hope will clarify this issue.

Elevated β_2-microglobulin levels have been proposed as a surrogate marker of HIV disease activity, and decreases in β_2-microglobulin levels reported after treatment with nucleoside analogs (2, 13) may confirm this hypothesis. The dose-

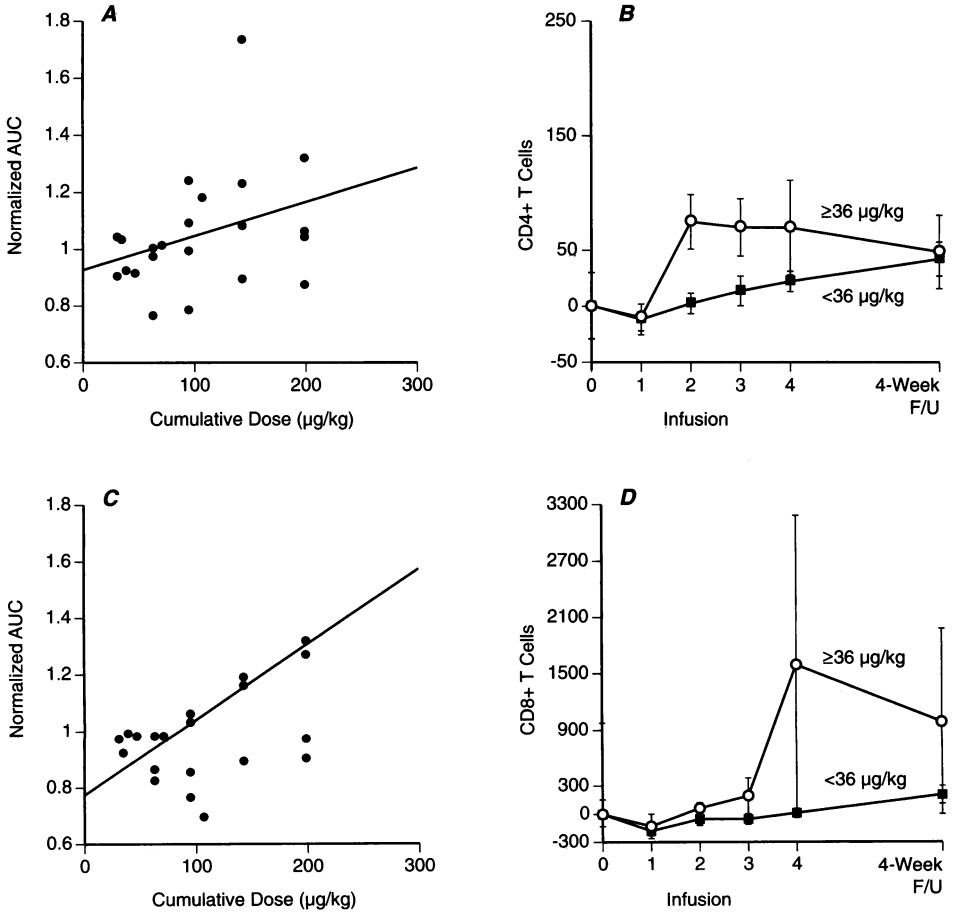

FIG. 2. Changes in $CD4^+$ and $CD8^+$ T cells over time. (A) Absolute change in mean $CD4^+$ T cell counts versus week of study. (B) Normalized AUC for $CD4^+$ T cells versus cumulative dose of GLQ223 administered. (C) Absolute change in $CD8^+$ T cells versus week of study. (D) Normalized AUC for $CD8^+$ T cells versus cumulative dose of GLQ223 administered. F/U, follow-up.

related increase in β_2-microglobulin levels observed in the present study thus seems paradoxical and potentially worrisome. However, our results are consistent with the proposed mechanism of action of GLQ223, which differs from those of zidovudine and other inhibitors of reverse transcriptase. Selective cytotoxic effects on infected cells might well trigger transient increases in β_2-microglobulin levels, with decreases occurring after treatment is discontinued. Alternatively, nonspecific activation of immune cells or the flu-like illness with fevers and myalgias resulting from GLQ223 administration may also result in the release of circulating β_2-microglobulin. The return of β_2-microglobulin levels toward normal when therapy is stopped and the absence of correlative clinical or laboratory findings suggestive of disease exacerbation argue against disease activation as the underlying basis of the observed increase in β_2-microglobulin levels. Treatment-associated changes in surrogate markers persisted in the presence of circulating antibodies, which is consistent with the effects of other immunogenic therapeutic proteins, such as OKT3, for which continued drug activity in the presence of a humoral immune response has been documented (11, 16). The greatest increases in β_2-microglobulin and $CD4^+$ T-cell levels were observed after the third and fourth doses, when anti-GLQ223 antibody levels were detectable in most patients. While circulating antibodies to GLQ223 may confirm prior exposure, they do not necessarily reduce the in vivo biological activity of the drug.

Evaluation of drug activity in our study was complicated by the two patients who discontinued the study drug, by the two patients who continued to receive zidovudine therapy, and by the lack of direct measures of viral load. Furthermore, since the study was designed to assess safety and pharmacokinetics, it did not have prospectively defined activity end points. However, the two patients who continued zidovudine treatment had received zidovudine for 307 and 718 days, respectively, prior to infusion with GLQ223, so it is unlikely that the simple continuation of zidovudine therapy would independently explain the observed increases in $CD4^+$ T lymphocytes. The increases in $CD4^+$ T lymphocyte counts during drug administration, with greater increases occurring in the group receiving one of the two highest doses of GLQ223, suggest that GLQ223 has activity in HIV-infected individuals with AIDS or ARC.

The in vitro activity profile of GLQ223, particularly its effects on chronically infected macrophages, suggests that the drug may be beneficial, either alone or in combination with other antiviral agents, for patients infected with HIV. Our study demonstrates that repeated dosing with GLQ223 is safe. A flu-like illness was associated with GLQ223 treatment. Specific common adverse events included allergic reactions, asthenia, pharyngitis, nausea, and anorexia. The majority of adverse events were classified as mild or moderate and were reversed with simple treatments. Long-term safety data will require a larger study that follows subjects for a longer period of time. Controlled, multicenter clinical trials, which are needed to evaluate the true potential of this drug in the treatment of patients with HIV infection and to assess the long-term tolerability of GLQ223, are under way.

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