

Anti-HIV-1 Activity of Weekly or Biweekly Treatment with Subcutaneous PRO 140, a CCR5 Monoclonal Antibody

Jeffrey M. Jacobson,¹ Melanie A. Thompson,² Jacob P. Lalezari,³ Michael S. Saag,⁴ Barry S. Zingman,⁵ Paul D'Ambrosio,⁶ Nancy Stambler,⁶ Yakov Rotshteyn,⁶ Andre J. Marozsan,⁶ Paul J. Maddon,⁵ Stephen A. Morris,⁶ and William C. Olson⁶

¹Drexel University College of Medicine, Philadelphia, Pennsylvania; ²AIDS Research Consortium of Atlanta, Atlanta, Georgia; ³Quest Clinical Research, San Francisco, California; ⁴University of Alabama, Birmingham; ⁵Montefiore Medical Center and Einstein/Montefiore Center for AIDS Research, Bronx, and ⁶Progenics Pharmaceuticals, Tarrytown, New York

Background. PRO 140 is a humanized CCR5 monoclonal antibody that has demonstrated potent antiviral activity when it is administered intravenously to adults infected with CCR5-tropic (R5) human immunodeficiency virus type 1 (HIV-1). This study is the first to evaluate subcutaneous administration.

Methods. A randomized, double-blind, placebo-controlled study was conducted among 44 subjects with HIV-1 RNA levels of >5000 copies/mL, CD4⁺ cell counts of >300 cells/ μ L, no receipt of antiretroviral therapy for \geq 12 weeks, and only R5 HIV-1 detectable. Subjects received placebo, 162 mg of PRO 140, or 324 mg of PRO 140 weekly for 3 weeks or 324 mg of PRO 140 every other week for 2 doses by means of subcutaneous infusion. Subjects were monitored for 58 days for safety, antiviral effects, and PRO 140 serum concentrations.

Results. Subcutaneous PRO 140 demonstrated potent and prolonged antiretroviral activity. Mean log₁₀ reductions in HIV-1 RNA level were 0.23, 0.99 ($P = .009$), 1.37 ($P < .001$), and 1.65 ($P < .001$) for the placebo, 162 mg weekly, 324 mg biweekly, and 324 mg weekly dose groups, respectively. Viral loads remained suppressed between successive doses. Treatment was generally well tolerated.

Conclusions. This trial demonstrates proof of concept for a monoclonal antibody administered subcutaneously in HIV-1 infected individuals. Subcutaneous PRO 140 offers the potential for significant dose-dependent HIV-1 RNA suppression and infrequent patient self-administration.

Trial registration. ClinicalTrials.gov identifier: NCT00642707.

Highly active antiretroviral therapy (HAART) has transformed the management of human immunodeficiency virus type 1 (HIV-1) infection and offers the potential for a normal life expectancy for many individuals who

have access to care [1, 2]. However, even when it is effective in reducing plasma viremia to levels that are undetectable in standard assays, HAART does not eradicate HIV-1, and long-term morbidities still occur [3–6]. In addition, treatment fails to achieve or maintain optimal viral suppression in many individuals. Further improvement in care may be realized with the development of new antiretroviral agents that inhibit viruses resistant to existing therapies, exhibit minimal drug or food interactions, reduce chronic toxicities, and permit dosing to be infrequent and flexible.

CCR5 coreceptor antagonists represent an emerging antiretroviral treatment class and are the first, to our knowledge, to target a host molecule. CCR5 is a chemokine receptor that mediates activation and migration of T cells and other leukocytes. CCR5 also binds the HIV-1 envelope glycoprotein gp120 and serves as a portal for HIV-1 entry into CD4⁺ cells [7]. CCR5-using

Received 21 August 2009; accepted 14 December 2009; electronically published 8 April 2010.

Potential conflicts of interest: P.D., N.S., Y.R., A.J.M., P.J.M., S.A.M., and W.C.O. are current or past employees of Progenics Pharmaceuticals and may hold stock or stock options in the company. Progenics has a proprietary commercial interest in PRO 140.

Presented in part: 16th Conference on Retroviruses and Opportunistic Infections, Montreal, Canada, 8–11 February 2009 (abstract 571a).

Financial support: National Institutes of Health (Public Health Service grant AI066329).

Reprints or correspondence: Dr Jeffrey M. Jacobson, Division of Infectious Diseases and HIV Medicine, Drexel University College of Medicine, 245 N 15th St, MS 461, Philadelphia, PA 19104 (jeffrey.jacobson@drexelmed.edu).

The Journal of Infectious Diseases 2010;201(10):1481–1487

© 2010 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/2010/20110-0007\$15.00

DOI: 10.1093/infdis/jin190

(R5) viruses typically mediate transmission and then predominate through the progression to symptomatic disease. Viruses can use an alternative chemokine receptor, CXCR4, either exclusively or in addition to CCR5. CXCR4-using viruses may be present early on but tend to become apparent in an increasing percentage of infected individuals in later phases of disease [8–11]. One small-molecule CCR5 antagonist (maraviroc; Pfizer) has been approved for use in antiretroviral treatment-experienced patients [12], and phase 3 clinical studies of a second small-molecule compound (vicriviroc; Merck) are ongoing [13, 14].

PRO 140 is a humanized CCR5 monoclonal antibody that potently inhibits R5 viruses and synergizes with small-molecule CCR5 antagonists, as shown in laboratory studies [15, 16]. PRO 140 does not inhibit CXCR4-using viruses. Previously, an intravenous form of PRO 140 was tested as monotherapy in HIV-1 subjects in whom only R5 virus was detectable [17]. Single doses, ranging up to 5 mg/kg, were generally well tolerated relative to placebo and demonstrated potent and prolonged antiviral activity, with a 1.83 log₁₀ mean reduction in HIV-1 RNA level observed at 5 mg/kg. These findings supported the development of subcutaneous formulations with the potential for patient self-administration. In the present study, we examined the antiviral effects, safety, and pharmacokinetics of subcutaneous PRO 140 administered weekly or every other week to HIV-infected individuals.

SUBJECTS AND METHODS

Study design. A phase 2a study was conducted to evaluate the antiviral activity, tolerability, and pharmacokinetics of weekly or biweekly subcutaneous doses of PRO 140 in adults with asymptomatic HIV-1 infection. The protocol was approved by the institutional review board at each site. All subjects provided written informed consent. Entry criteria included age of ≥ 18 years, plasma HIV-1 RNA level of ≥ 5000 copies/mL, CD4⁺ lymphocyte count of ≥ 300 cells/ μ L with no documented count of ≤ 250 cells/ μ L, no antiretroviral therapy for ≥ 12 weeks, no history of AIDS-defining illness, and only R5 HIV-1 detectable. PRO 140 was provided as a sterile 135-mg/mL solution in phosphate buffer (pH, 6.8). The placebo was a matched, sterile, buffer solution without PRO 140. Subjects were dosed on an outpatient basis with (1) placebo on days 1, 8, and 15; (2) 162 mg of PRO 140 on days 1, 8, and 15 (162 mg weekly); (3) 324 mg of PRO 140 on days 1 and 15 and placebo on day 8 (324 mg biweekly); or (4) 324 mg of PRO 140 on days 1, 8, and 15 (324 mg weekly). The study drug was administered by site personnel via subcutaneous infusion into the arm over ~ 5 min, using a syringe pump and Cleo 90 infusion set (Smiths Medical).

Virological evaluations. Plasma HIV-1 RNA level was determined with the Cobas Amplicor HIV-1 Monitor test (version

1.5; Roche Diagnostics) at screening, baseline (day 1), and days 3, 5, 8 (before treatment), 10, 12, 15 (before treatment), 22, 29, 43, and 59. Samples with HIV-RNA levels of < 400 copies/mL were reanalyzed with the Ultrasensitive method. Coreceptor tropism was determined at screening for all subjects and after viral rebound for PRO 140-treated subjects by means of the first-generation Trofile assay (Monogram Biosciences). The Enhanced Sensitivity (ES) Trofile assay, which was introduced after the study was initiated, was used in post hoc analyses. Viral susceptibility to PRO 140 was determined at baseline (day 1) for all subjects and after viral rebound for PRO 140-treated subjects by means of the PhenoSense Entry assay (Monogram Biosciences) as described elsewhere [17].

Safety evaluations. Vital signs, concomitant medications, and adverse events were recorded during screening and on days 1, 3, 5, 8, 10, 12, 15, 22, 29, 43, and 59. Physical examinations and clinical laboratory tests for serum chemistry, hematology, and urinalysis were performed during screening and on days 1, 8, 15, 29, and 59. Twelve-lead electrocardiograms were obtained during screening and on days 1, 3, 8, 10, and 59.

Bioanalytical methods. Serum concentrations of PRO 140 and of antibodies to PRO 140 were determined by enzyme-linked immunosorbent assay as described elsewhere [17]. Serum for pharmacokinetic analysis was collected before treatment and at 0.5, 1, 3, 6, 24, 32, 48, 56, and 96 h after treatment during the first week and then on days 8, 12, 15, 22, 29, and 59. Pharmacokinetic metrics were estimated after noncompartmental analysis by using WinNonlin (version 5.2; Pharsight). Serum samples for anti-PRO 140 antibodies were collected on days 1, 8, 15, 29, and 59. To assess the presence of PRO 140-neutralizing antibodies, human CCR5-expressing CEM-NKR cells were incubated with phycoerythrin-labeled PRO 140 in the presence of test serum. The resulting fluorescent signal was measured by flow cytometry and compared with the signal obtained from control serum. CD4⁺ and CCR5⁺ lymphocyte counts were determined essentially as described elsewhere [17]. Lymphocyte counts were determined at screening (CD4⁺ cells only) and on days 1, 3, 8, 15, 22, 29, 43, and 59. CCR5 receptor occupancy was assessed by comparing the numbers of lymphocytes that stained positive for binding of phycoerythrin-labeled PRO 140 before and after treatment. On treatment days, bioanalytical samples were collected before the study drug was administered, unless otherwise indicated.

Statistical methods. All subjects who received at least 1 dose of the study drug were included in the safety evaluations. Efficacy analyses were performed on log₁₀-transformed HIV-1 RNA level data, and changes were calculated relative to the baseline values (day 1, before treatment). Treatment and placebo groups were compared using an analysis of variance model. If the result of the overall *F* test was found to be statistically significant, then each treatment group was compared

with the placebo group by means of the pairwise *t* test. The Fisher exact test was used to compare the treatment groups with the placebo group for the percentage of subjects with a ≥ 1 log₁₀ reduction in HIV-1 RNA level from the baseline value at any time after treatment and the percentage of subjects with <400 copies of HIV-1 RNA at any time after treatment. The Mann-Whitney *U* test was used to compare CCR5 receptor occupancy data between placebo and treatment groups. Two-sided tests were used for all analyses.

RESULTS

Subject characteristics and disposition. Of the 138 subjects screened, 46 were randomized and 44 received at least 1 dose of the study drug. The most frequent reasons for screening failure were nonreportable coreceptor tropism (23 subjects), CD4⁺ cell counts of <300 cells/ μ L (21 subjects), dual/mixed coreceptor tropism (13 subjects), and HIV-1 RNA level of <5000 copies/mL (12 subjects). Demographic and baseline characteristics for the treated subjects are summarized in Table 1. For all subjects, the median age, median CD4⁺ cell count, and median plasma HIV-1 RNA level at baseline were 42 years, 410 cells/ μ L, and 25,100 copies/mL, respectively. Baseline characteristics were similar among the subjects in the different treatment groups; however, the median weights in the 324 mg weekly and biweekly dose groups differed by 28%. Fifteen subjects reported prior antiretroviral therapy. Forty-one of 44 treated subjects completed the study. Two subjects who received placebo discontinued because of adverse events, and 1 subject treated with 162 mg of the study drug withdrew for reasons unrelated to safety.

Antiviral effects. Significant, dose-dependent antiviral effects were observed for each PRO 140 group. Mean log₁₀ reductions in HIV-1 RNA level at virologic nadir were 0.23, 0.99, 1.37, and 1.65 for the placebo, 162 mg weekly, 324 mg biweekly, and 324 mg weekly groups, respectively. The reduction for each PRO 140 group was highly statistically significant relative to placebo (Table 2). Individual viral nadirs typically were ob-

served on day 22 (range, day 15 to day 29) for subjects in the 324-mg groups, with a range of day 15 to day 29.

The mean log₁₀ change in HIV-1 RNA level over time is depicted in Figure 1. Significant antiviral effects were observed following the first dose of PRO 140 ($P = .007$ to $<.001$ for the different treatment groups on day 8), and viral loads decreased with continued dosing. On day 22, the mean log₁₀ decreases in viral load were 0.15, 0.75, 1.20, and 1.51 for the placebo and ascending PRO 140 dose groups, respectively (Figure 1 and Table 2). Viral loads remained suppressed between successive weekly and biweekly 324-mg doses. For the 324 mg weekly and biweekly groups, mean viral load reductions exceeded 1 log₁₀ ($P \leq .001$) through day 29.

Antiviral response was defined as a ≥ 1 log₁₀ decrease in HIV-1 RNA level at any time after treatment. With inclusion of 1 subject each with a 0.99 log₁₀ reduction, antiviral response rates were 89% (10 of 11 subjects; $P \leq .001$) and 75% (9 of 12 subjects; $P \leq .001$) for the 324 mg weekly and biweekly groups, respectively. Six of 11 subjects treated with 162 mg (55%; $P = .012$) had a ≥ 1 log₁₀ decrease in viral load; no subject in the placebo group had a ≥ 1 log₁₀ decrease in viral load (Table 2). The 324 mg weekly subject who did not experience a ≥ 1 log₁₀ decrease in HIV-1 RNA level had only R5 virus after treatment. Pretreatment and posttreatment viral susceptibility to PRO 140, PRO 140 serum levels, and receptor occupancy data for this subject were similar to those of other subjects treated with 324 mg weekly. Seventy-three percent of subjects in the 324 mg weekly group had a viral load of <400 copies/mL, whereas no subject in the placebo group had a viral load of <400 copies/mL ($P = .001$) (Table 2).

Safety. Subcutaneous administration of PRO 140 was generally well tolerated. Forty of 44 subjects overall and 10 of 10 subjects receiving placebo reported at least 1 adverse event. Of these adverse events, approximately half were considered to be unrelated to the study drug. There were no drug-related serious adverse events or dose-limiting toxicities. The most frequently reported systemic adverse events were diarrhea (6 [14%] of 44), headache (6 [14%] of 44), lymphadenopathy (5 [11%] of

Table 1. Baseline Characteristics of Treated Subjects

Variable	Treatment with placebo (n = 10)	Treatment with PRO 140			All subjects (n = 44)
		162 mg weekly (n = 11)	324 mg biweekly (n = 12)	324 mg weekly (n = 11)	
Age, years	44.9 (32.3–51.6)	40.0 (29.1–44.6)	45.9 (31.0–59.6)	41.1 (34.8–53.6)	42.3 (29.1–59.6)
Sex, no. male/no. female	9/1	10/1	11/1	10/1	40/4
Race, no. black/no. white	3/7	5/6	5/7	4/7	17/27
Weight, kg	82.3 (59.4–107)	77.0 (59.3–94.4)	88.3 (58.9–102)	69.0 (60.8–83.6)	79.1 (58.9–107)
CD4 ⁺ cell count, cells/ μ L	410 (312–878)	352 (307–611)	493 (357–911)	389 (341–638)	410 (307–911)
HIV-1 RNA level, log ₁₀ copies/mL	4.09 (3.94–5.13)	4.43 (3.92–4.97)	4.60 (4.03–6.68)	4.19 (3.61–4.77)	4.40 (3.61–6.68)

NOTE. Data are median (range) values, unless otherwise indicated. HIV-1, human immunodeficiency virus type 1.

Table 2. Changes in Human Immunodeficiency Virus Type 1 (HIV-1) RNA Level and CD4⁺ T Cell Count

Effect	Treatment with placebo (n = 10)	Treatment with PRO 140					
		162 mg weekly (n = 11)	P	324 mg biweekly (n = 12)	P	324 mg weekly (n = 11)	P
Maximum log ₁₀ change in HIV-1 RNA level	-0.23 (0.29)	-0.99 (0.56)	.009	-1.37 (0.89)	<.001	-1.65 (0.57)	<.001
Day 22 log ₁₀ change in HIV-1 RNA level	-0.15 (0.20)	-0.75 (0.41)	.007	-1.20 (1.00)	<.001	-1.51 (0.65)	<.001
No. (%) of subjects with HIV-1 RNA decrease ^a	0 (0)	6 (55)	.012	9 (75)	<.001	10 (91)	<.001
No. (%) of subjects with <400 copies/mL HIV-1 RNA ^b	0 (0)	1 (9)	...	2 (17)	...	8 (73)	.001
Change in CD4 ⁺ cell count, cells/ μ L							
Day 8, median (range)	4 (-70 to 71)	29 (-169 to 198)	...	104 (-97 to 317)	...	76 (-52 to 219)	...
Day 15, median (range)	12 (-101 to 104)	50 (-204 to 278)	...	44 (-46 to 178)	...	76 (-84 to 246)	...
Day 22, median (range)	14 (-184 to 179)	55 (-140 to 153)	...	99 (-21 to 243)	...	84 (-110 to 296)	...

NOTE. Data are mean (standard deviation) values, unless otherwise indicated.

^a Subjects with ≥ 1 log₁₀ decrease in HIV-1 RNA level at any time after treatment. Includes 1 subject each in the 324 mg biweekly and 324 mg weekly dose groups with a 0.99 log₁₀ reduction in HIV-1 RNA level.

^b Subjects with HIV-1 RNA levels of <400 copies/mL at any time after treatment.

44), and hypertension (4 [9%] of 44). No obvious dose-proportional trend in the frequency of adverse events was observed. There was no clinically relevant drug-related effect on corrected QT intervals or other electrocardiogram parameters. There were no notable findings in laboratory safety tests.

Administration-site reactions were infrequent, mild, transient (duration, 1–2 days), and self-resolving. Rates of reactions were similar for the placebo and PRO 140 groups. Adverse events reported in >5% of subjects were induration (9 [20%] of 44), pain (4 [9%] of 44), and irritation (3 [7%] of 44). No subcutaneous infusions were paused or discontinued for any reason.

Pharmacokinetics. Serum concentrations observed during the first week of treatment are depicted in Figure 2 and were used to calculate pharmacokinetic metrics. Data for the 324 mg weekly and biweekly groups were pooled for this analysis. Peak concentrations typically were observed 32–56 h after treatment, with a mean of 6.1 and 13.8 mg/L for subjects treated with 162 and 324 mg of PRO 140, respectively. The corresponding mean terminal half-lives were 3.4 and 3.7 days, respectively. During the first week after treatment, the mean area under the PRO 140 concentration-time curve (AUC) values were 24.4 and 58.8 mg · day/L for 162-mg and 324-mg doses, respectively. Mean AUC values from time 0 to infinity were ~36% higher. Mean trough concentrations for the 162 mg weekly group increased from 1.86 μ g/mL on day 8 to 2.89 and 3.55 μ g/mL on days 15 and 22, respectively. The corresponding values for the 324 mg weekly group were 5.45, 8.50, and 8.75 μ g/mL, respectively.

Low titers of anti-PRO 140 antibodies ($\leq 1:32$) were detected on days 29 and/or 59 in 2 subjects treated with 162 mg weekly, 2 subjects treated with 324 mg biweekly, and 3 subjects treated with 324 mg weekly. All other subjects tested negative for anti-PRO 140 antibodies. Anti-PRO 140 antibodies had no obvious

effect on pharmacokinetics or antiviral responses. For example, subjects with positive anti-PRO 140 antibody test results experienced a mean 1.38 log₁₀ decrease in HIV-1 RNA level, whereas the mean decrease was 1.32 log₁₀ in PRO 140-treated subjects who tested negative for anti-PRO 140 antibodies. All serum samples that tested positive for anti-PRO 140 antibodies were nonneutralizing; ie, the serum samples did not block binding of PRO 140 to CCR5⁺ cells in vitro.

Coreceptor tropism and viral susceptibility to PRO 140. Subjects were eligible for the study if only R5 virus was detected with the first-generation Trofile assay at screening. Coreceptor tropism was determined using the same method after viral rebound in subjects treated with PRO 140. All subjects in the

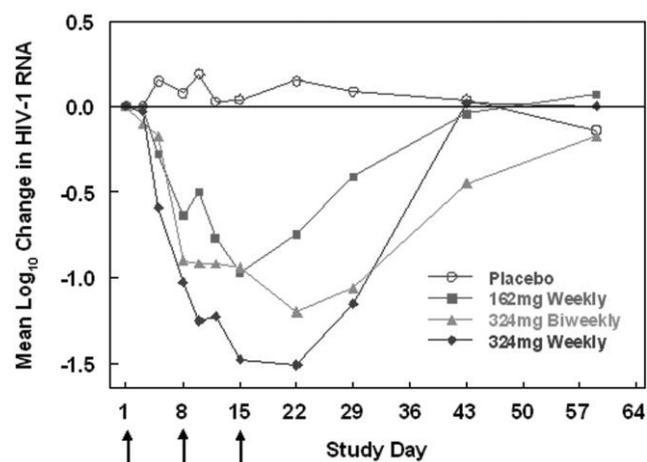


Figure 1. Mean log₁₀ change from the baseline value in human immunodeficiency virus type 1 (HIV-1) RNA level over time for the different treatment groups. The arrows indicate days that the study drug, PRO 140, was administered (days 1, 8, and 15).

162 mg weekly and 324 mg weekly groups maintained R5-only coreceptor tropism following treatment. However, dual/mixed virus was detected in 3 subjects in the 324 mg biweekly group during the study. One of these subjects experienced a 1.1 log₁₀ nadir in viral load on day 8. Minimal antiviral responses (nadir reduction, <0.5 log₁₀) were observed for the other 2 subjects. When the 3 subjects were censored, the mean maximum decrease in viral load for the 324 mg biweekly group was 1.60 log₁₀.

Screening samples from the 3 subjects with dual/mixed virus were analyzed after the study by means of the ES Trofile assay. One subject had detectable levels of dual/mixed virus before treatment and would have been excluded from the study if the ES Trofile assay had been available for the screening. Dual/mixed virus was first detected on day 15 and on day 29 in the other 2 subjects. Studies are ongoing to determine whether these subjects had preexisting dual/mixed virus at levels below the limit of detection of the ES Trofile assay. At the end of the study (day 59), only R5 virus was detected in samples from both subjects with the ES Trofile assay.

All viruses were susceptible to inhibition by PRO 140 in the R5 PhenoSense Entry assay. The mean fold change at baseline was 1.92 (range, 0.83–3.94). There was no significant change in fold change values after treatment ($P > .8$). All posttreatment values were ≤ 1.7 -fold greater than the pretreatment values. The maximum percent inhibition of R5 viruses was 98%–100% both before and after treatment for all subjects, indicating essentially complete inhibition by PRO 140.

Lymphocyte and receptor occupancy analyses. The subjects had CD4⁺ lymphocyte counts of 307–911 cells/ μ L at baseline (Table 1). An increase in CD4⁺ lymphocyte count was observed for subjects in each PRO 140 treatment group on days 8, 15, and 22 (Table 2); however, the changes were not statistically significant.

The median baseline CCR5⁺ lymphocyte count was 35 cells/ μ L (range, 3–266 cells/ μ L). CCR5⁺ lymphocytes were not depleted following treatment; however, high levels of receptor occupancy were observed. The median number of cells that stained positive with fluorescently labeled PRO 140 ex vivo was reduced to $\leq 7\%$ of the pretreatment values between days 3 and 43 for all PRO 140 treatment groups ($P \leq .004$ relative to placebo at each time point). On day 59, receptor occupancy levels were not significantly different from those measured in the placebo group for any PRO 140 treatment group ($P \geq .08$).

DISCUSSION

In this study to evaluate subcutaneous delivery of a monoclonal antibody for the treatment of HIV-1 infection, PRO 140 demonstrated potent and prolonged antiretroviral activity. The mean maximum reduction in HIV-1 RNA level observed for

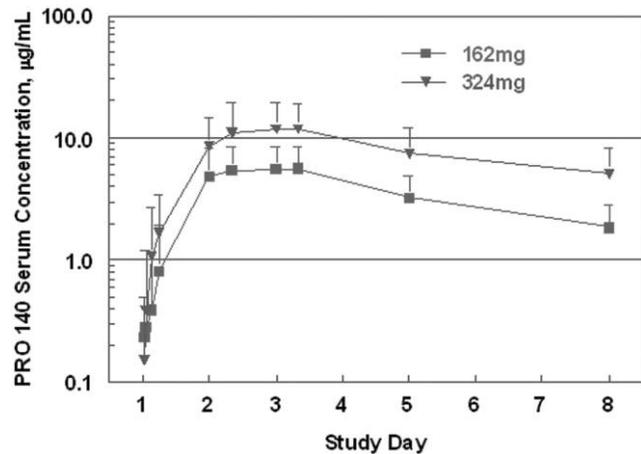


Figure 2. Mean serum concentrations of PRO 140 following a 162-mg or 324-mg subcutaneous dose. The error bars depict standard deviations. The curve for 324 mg includes data for the 324 mg weekly and biweekly treatment groups.

the 324 mg weekly dose was 1.65 log₁₀ and is similar in magnitude to that observed in short-term monotherapy studies, both of small-molecule CCR5 antagonists and of intravenous PRO 140 [17–20]. Treatment was generally well tolerated. These findings provide clinical proof of concept for subcutaneous PRO 140 as a potent and long-acting antiretroviral agent. The subcutaneous dosage form offers the potential for infrequent self-administration by patients, and this issue will be explored in future studies.

Viral load reductions and antiviral response rates increased as the total amount of PRO 140 administered over 3 weeks was increased from 486 mg (162 mg weekly) to 648 mg (324 mg biweekly) to 932 mg (324 mg weekly). Because trough concentrations increased after repeat subcutaneous dosing, a loading dose potentially could be used to increase the initial rate of virologic suppression. Faster initial viral decay rates have been found to be correlated with improved long-term virologic outcomes [21, 22]. After an initial loading dose, potent virologic suppression and steady-state trough concentrations of PRO 140 might be attainable with subcutaneous maintenance doses similar to those examined here.

Significant antiviral effects were observed for PRO 140 administered both weekly and every other week, and virologic suppression was maintained between successive doses. A weekly regimen may offer appreciable latitude of 1 or more days in the timing of subsequent dosing. Frequent dosing can provide a barrier to adherence [23–25], such that once-daily regimens are recommended over twice-daily regimens [26]. However, there currently are a limited number of once-daily antiretroviral regimens, especially for patients with drug-resistant virus. The availability of a long-acting antiretroviral agent would provide an additional option for constructing alternative treatment reg-

imens that are more accommodating. The mean reduction observed at virologic nadir following 3 weekly 324-mg subcutaneous doses ($1.65 \log_{10}$) (Table 2) was similar in magnitude to the reduction of $1.83 \log_{10}$ observed previously for a single 5 mg/kg intravenous dose [17]; however, pharmacodynamic differences were evident. Peak serum concentrations were ~10-fold lower after subcutaneous dosing ($13.8 \mu\text{g/mL}$) than they were after intravenous dosing ($173 \mu\text{g/mL}$ [17]), indicating that the high serum concentrations achieved with intravenous dosing are not required for potent virologic suppression. Although subcutaneous and intravenous PRO 140 exhibited similar serum half-lives, the apparent overall exposure, as determined by AUC analysis of serum concentrations, was ~3.5-fold lower after a single 324-mg subcutaneous dose, compared with a single 5 mg/kg intravenous dose (mean total dose, 367 mg).

Proteins and other macromolecules drain from subcutaneous sites into both blood capillaries and the lymphatic system. In animals, proteins with molecular weights of $>16,000$ Da have been observed to drain primarily into the lymphatic system following subcutaneous administration [27]. Such proteins transit through lymph fluid and typically are not absorbed significantly into the blood until they reach the thoracic duct. Because the molecular weight of PRO 140 is ~150,000 Da, a substantial amount of subcutaneous PRO 140 can be expected to drain into the lymphatic system and potentially encounter CCR5⁺ cells in lymphoid tissues prior to reaching the bloodstream. For these reasons, serum concentrations may not provide a full picture of the overall exposure following subcutaneous dosing of PRO 140.

In order to evade inhibition by CCR5 drugs, HIV-1 can adapt either to use CCR5 in the presence of drug or to use an alternative coreceptor [28]. In clinical trials of CCR5 inhibitors, adaptation to an alternative coreceptor typically has reflected outgrowth of preexisting CXCR4-using viruses rather than de novo mutation of R5 viruses [29–32]. Three PRO 140–treated subjects (9%) had dual/mixed virus detected in their serum samples during the study. Each of these subjects was in the 324 mg biweekly dose group. This imbalance may be due to chance because of the small number of subjects in each group. These 3 subjects had minimal or blunted antiviral responses, as expected. One subject had preexisting dual/mixed virus at levels that would have excluded the subject from the study if the ES Trofile assay had been available at screening. Further analysis of the pretreatment viruses of the other 2 subjects is ongoing and will be reported separately. A loading dose may decrease the potential for dual/mixed virus to emerge following initiation of treatment with PRO 140, as may the presence of additional antiretroviral agents in future combination regimens. Finally, because the potential for outgrowth of CXCR4-using viruses is related to the sensitivity of the tropism test used for screening, this resistance pathway may diminish in clinical im-

portance as tropism assays become increasingly sensitive in detecting CXCR4-using viruses.

There was no change in R5 viral susceptibility to PRO 140 following 3 weeks of monotherapy, indicating no adaptation of virus to use CCR5 in the presence of drug. Similarly, no development of R5 viral resistance was observed following treatment with single intravenous doses of PRO 140, which produced significant antiviral effects for 2 weeks [17]. In contrast, phenotypic and/or genotypic resistance has been reported within 2 weeks of monotherapy with some nonnucleoside reverse transcriptase inhibitors [33–36]. The PRO 140 results are especially notable in that monotherapy was followed by slow washout of the drug. Such conditions can foster development of antiretroviral drug resistance [37, 38]. Overall, our findings suggest that PRO 140 presents a high barrier to resistance.

This study is the first to examine subcutaneous administration of PRO 140 in humans. An infusion pump was used to control and potentially pause administration, if necessary. Subcutaneous administration was well tolerated. No infusions were interrupted for any reason. Subcutaneous infusion currently is used by individuals with primary immunodeficiency to self-administer at home considerably larger amounts (~11 g) and volumes (~70 mL total, up to 15 mL/site) of immunoglobulin weekly [39, 40]. Self-administration of 324 mg of subcutaneous PRO 140 would be much simpler in comparison. In addition, the favorable local tolerability that was observed in the present study supports further studies to examine self-administration of PRO 140 by subcutaneous injection.

In summary, the current study establishes proof of concept for subcutaneous infusion of a therapeutic monoclonal antibody for the treatment of HIV-1 infection. Subcutaneous PRO 140 offers the potential for significant suppression of HIV-1 replication and infrequent patient self-administration.

Acknowledgments

We thank the subjects for their participation in the study. We gratefully acknowledge the assistance of all site personnel and study investigators, including Nicholaos Bellos, Daniel Berger, Gary Blick, Stephen Brown, Cynthia Brinson, Edwin DeJesus, Paul DenOuden, Ralph Liporace, David Prelutsky, Robert Redfield, Peter Ruane, Peter Shalit, and Michael Wohlfeiler.

References

1. Bhaskaran K, Hamouda O, Sannes M, et al. Changes in the risk of death after HIV seroconversion compared with mortality in the general population. *JAMA* 2008; 300(1):51–59.
2. Antiretroviral Therapy Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet* 2008; 372(9635): 293–299.
3. Effros RB, Fletcher CV, Gebo K, et al. Aging and infectious diseases: workshop on HIV infection and aging: what is known and future research directions. *Clin Infect Dis* 2008; 47(4):542–553.

4. Weber R, Sabin CA, Friis-Moller N, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. *Arch Intern Med* **2006**;166(15):1632–1641.
5. Mondy K, Tebas P. Cardiovascular risks of antiretroviral therapies. *Annu Rev Med* **2007**;58:141–155.
6. Robertson KR, Smurzynski M, Parsons TD, et al. The prevalence and incidence of neurocognitive impairment in the HAART era. *AIDS* **2007**;21(14):1915–1921.
7. Lederman MM, Penn-Nicholson A, Cho M, Mosier D. Biology of CCR5 and its role in HIV infection and treatment. *JAMA* **2006**;296(7):815–826.
8. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CCR5 structural gene: Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* **1996**;273(5283):1856–1862.
9. Moyle GJ, Wildfire A, Mandalia S, et al. Epidemiology and predictive factors for chemokine receptor use in HIV-1 infection. *J Infect Dis* **2005**;191(6):866–872.
10. Wilkin TJ, Su Z, Kuritzkes DR, et al. HIV type 1 chemokine coreceptor use among antiretroviral-experienced patients screened for a clinical trial of a CCR5 inhibitor: AIDS Clinical Trial Group A5211. *Clin Infect Dis* **2007**;44(4):591–595.
11. Brumme ZL, Goodrich J, Mayer HB, et al. Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naïve individuals. *J Infect Dis* **2005**;192(3):466–474.
12. Gulick RM, Lalezari J, Goodrich J, et al. Maraviroc for previously treated patients with R5 HIV-1 infection. *N Engl J Med* **2008**;359(14):1429–1441.
13. Vicriviroc in HIV-treatment experienced subjects (study P04889AM7). ClinicalTrials.gov Web site. <http://www.clinicaltrials.gov/ct2/show/NCT00474370>. First received 15 May 2007. Last updated 4 March 2010. Accessed 6 August 2009.
14. Vicriviroc in HIV-treatment experienced subjects (study P04405AM4). ClinicalTrials.gov Web site. <http://www.clinicaltrials.gov/ct2/show/NCT00523211>. First received 30 August 2007. Last updated 4 March 2010. Accessed 6 August 2009.
15. Murga J, Franti M, Pevear DC, Maddon PJ, Olson WC. Potent antiviral synergy between monoclonal antibody and small-molecule CCR5 inhibitors of human immunodeficiency virus type 1. *Antimicrob Agents Chemother* **2006**;50(10):3289–3296.
16. Trkola A, Ketas TJ, Nagashima KA, et al. Potent, broad-spectrum inhibition of human immunodeficiency virus type 1 by the CCR5 monoclonal antibody PRO 140. *J Virol* **2001**;75(2):579–588.
17. Jacobson JM, Saag MS, Thompson MA, et al. Antiviral activity of single-dose PRO 140, a CCR5 monoclonal antibody, in HIV-infected adults. *J Infect Dis* **2008**;198(9):1345–1352.
18. Fatkenheuer G, Pozniak AL, Johnson MA, et al. Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nat Med* **2005**;11(11):1170–1172.
19. Schurmann D, Fatkenheuer G, Reynes J, et al. Antiviral activity, pharmacokinetics and safety of vicriviroc, an oral CCR5 antagonist, during 14-day monotherapy in HIV-infected adults. *AIDS* **2007**;21(10):1293–1299.
20. Lalezari J, Thompson M, Kumar P, et al. Antiviral activity and safety of 873140, a novel CCR5 antagonist, during short-term monotherapy in HIV-infected adults. *AIDS* **2005**;19(14):1443–1448.
21. Polis MA, Sidorov IA, Yoder C, et al. Correlation between reduction in plasma HIV-1 RNA concentration 1 week after start of antiretroviral treatment and longer-term efficacy. *Lancet* **2001**;358(9295):1760–1765.
22. Kuritzkes DR, Ribaudo HJ, Squires KE, et al. Plasma HIV-1 RNA dynamics in antiretroviral-naïve subjects receiving either triple-nucleoside or efavirenz-containing regimens: ACTG A5166s. *J Infect Dis* **2007**;195(8):1169–1176.
23. Maitland D, Jackson A, Osorio J, Mandalia S, Gazzard BG, Moyle GJ. Switching from twice-daily abacavir and lamivudine to the once-daily fixed-dose combination tablet of abacavir and lamivudine improves patient adherence and satisfaction with therapy. *HIV Med* **2008**;9(8):667–672.
24. Boyle BA, Jayaweera D, Witt MD, Grimm K, Maa JF, Seekins DW. Randomization to once-daily stavudine extended release/lamivudine/efavirenz versus a more frequent regimen improves adherence while maintaining viral suppression. *HIV Clin Trials* **2008**;9(3):164–176.
25. Molina JM, Podsadeci TJ, Johnson MA, et al. A lopinavir/ritonavir-based once-daily regimen results in better compliance and is non-inferior to a twice-daily regimen through 96 weeks. *AIDS Res Hum Retroviruses* **2007**;23(12):1505–1514.
26. Hammer SM, Eron JJ Jr, Reiss P, et al. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. *JAMA* **2008**;300(5):555–570.
27. Porter CJ, Charman WN. Transport and absorption of drugs via the lymphatic system. *Adv Drug Deliv Rev* **2001**;50(1–2):1–2.
28. Moore JP, Kuritzkes DR. A piece de resistance: how HIV-1 escapes small molecule CCR5 inhibitors. *Curr Opin HIV AIDS* **2009**;4(2):118–124.
29. Tsimbris AM, Korber B, Arnaout R, et al. Quantitative deep sequencing reveals dynamic HIV-1 escape and large population shifts during CCR5 antagonist therapy in vivo. *PLoS ONE* **2009**;4(5):e5683.
30. Westby M, Lewis M, Whitcomb J, et al. Emergence of CXCR4-using human immunodeficiency virus type 1 (HIV-1) variants in a minority of HIV-1-infected patients following treatment with the CCR5 antagonist maraviroc is from a pretreatment CXCR4-using virus reservoir. *J Virol* **2006**;80(10):4909–4920.
31. Lewis M, Simpson P, Fransen S, et al. CXCR4-using virus detected in patients receiving maraviroc in the phase III studies MOTIVATE 1 and 2 originates from a pre-existing minority of CXCR4-using virus. *Antivir Ther* **2007**;12:S65.
32. Marozsan AJ, Parsons T, Huang W, et al. Clonal analysis of HIV-1 co-receptor tropism change following treatment with PRO 140, a CCR5 monoclonal antibody. Paper presented at: 48th Annual ICAAC/IDSA 46th Annual Meeting (abstract H-1218); 25–28 October **2008**; Washington, DC.
33. Saag MS, Emini EA, Laskin OL, et al. A short-term clinical evaluation of L-697,661, a non-nucleoside inhibitor of HIV-1 reverse transcriptase. L-697,661 Working Group. *N Engl J Med* **1993**;329(15):1065–1072.
34. Richman DD, Havlir D, Corbeil J, et al. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. *J Virol* **1994**;68(3):1660–1666.
35. Havlir DV, Eastman S, Gamst A, Richman DD. Nevirapine-resistant human immunodeficiency virus: kinetics of replication and estimated prevalence in untreated patients. *J Virol* **1996**;70(11):7894–7899.
36. Vazquez-Rosales G, Garcia Lerma JG, Yamamoto S, et al. Rapid screening of phenotypic resistance to nevirapine by direct analysis of HIV type 1 reverse transcriptase activity in plasma. *AIDS Res Hum Retroviruses* **1999**;15(13):1191–1200.
37. Chi BH, Sinkala M, Mbewe F, et al. Single-dose tenofovir and emtricitabine for reduction of viral resistance to non-nucleoside reverse transcriptase inhibitor drugs in women given intrapartum nevirapine for perinatal HIV prevention: an open-label randomised trial. *Lancet* **2007**;370(9600):1698–1705.
38. Darwich L, Esteve A, Ruiz L, Bellido R, Clotet B, Martinez-Picado J. Variability in the plasma concentration of efavirenz and nevirapine is associated with genotypic resistance after treatment interruption. *Antivir Ther* **2008**;13(7):945–951.
39. Ochs HD, Gupta S, Kiessling P, Nicolay U, Berger M. Safety and efficacy of self-administered subcutaneous immunoglobulin in patients with primary immunodeficiency diseases. *J Clin Immunol* **2006**;26(3):265–273.
40. Gardulf A, Andersen V, Bjorkander J, et al. Subcutaneous immunoglobulin replacement in patients with primary antibody deficiencies: safety and costs. *Lancet* **1995**;345(8946):365–369.