Phase 2a Study of the CCR5 Monoclonal Antibody PRO 140 Administered Intravenously to HIV-Infected Adults

Jeffrey M. Jacobson,1* Jacob P. Lalezari, 2 Melanie A. Thompson, 3 Carl J. Fichtenbaum, 4 Michael S. Saag, 5 Barry S. Zingman, 6 Paul D’Ambrosio, 7 Nancy Stambler, 7 Yakov Rotshteyn, 7 Andre J. Marozsan, 7 Paul J. Maddon, 7 Stephen A. Morris, 7 and William C. Olson 7

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

Received 20 January 2010/Returned for modification 27 April 2010/Accepted 20 July 2010

The anti-CCR5 antibody PRO 140 has shown potent and prolonged antiretroviral activity in subjects infected with CCR5-tropic (R5) HIV-1. Prior studies have examined single intravenous doses ranging up to 5 mg/kg of body weight or up to three subcutaneous doses ranging up to 324 mg. Here we report the results of a randomized, double-blind, placebo-controlled trial that examined the antiviral activity, tolerability, and pharmacokinetics of single 5-mg/kg and 10-mg/kg intravenous infusions of PRO 140 in 31 treated subjects. Eligibility criteria included HIV-1 RNA levels of >5,000 copies/ml, CD4+ cell counts of >300/μl, no antiretroviral therapy for ≥12 weeks, and detection of only R5 HIV-1 in the original Trofile assay. Following poststudy testing with an enhanced-sensitivity Trofile assay, one subject treated with 10 mg/kg was reclassified as having dual/mixed-tropic virus at screening, and the data for that subject were censored from efficacy analyses. The mean maximum reduction of the HIV-1 RNA level from the baseline level was 1.8 log10 units for both the 5-mg/kg and 10-mg/kg doses (P < 0.0001 relative to placebo). Viral loads reached their nadir at day 12 posttreatment and remained significantly (P < 0.01) reduced through day 29 for both PRO 140 dose groups. Treatment was generally well tolerated, with no dose-limiting toxicity being observed. Peak serum concentrations and overall exposures increased proportionally with dose. In summary, single 5-mg/kg and 10-mg/kg doses of PRO 140 exhibited potent, long-lived antiviral activity and were generally well tolerated. The findings further delineate the safety and antiviral properties of this novel, long-acting antiretroviral agent.

Materials and Methods

Study design. A randomized, double-blind, placebo-controlled, parallel-group study was conducted with HIV-infected adults. Subjects (approximately 30 planned) were randomized 1:1:1 to receive a single intravenous infusion of placebo, 5 mg/kg PRO 140, or 10 mg/kg PRO 140. The protocol was approved by the institutional review board at each site. All subjects provided written informed consent. Eligibility criteria included age of ≥18 years, plasma HIV-1 RNA level of ≤5,000 copies/ml, CD4+ lymphocyte counts of ≥300/μl and no documented count being ≤250/μl, no antiretroviral therapy for ≥12 weeks, no history of an AIDS-defining illness, and only R5 HIV-1 detectable in the original Trofile assay.
Table 1. Demographic and baseline characteristics

| Characteristic         | Placebo (n = 11) | 5 mg/kg PRO 140 (n = 10) | 10 mg/kg PRO 140 (n = 10) | All subjects (n = 31) |
|------------------------|------------------|--------------------------|--------------------------|----------------------|
| Age (yr)               | 40.2 (22.3–56.6) | 44.7 (28.0–55.9)         | 45.3 (25.9–57.2)         | 42.7 (22.3–57.2)     |
| Sex (no. male/no. female) | 9/2              | 10/0                     | 10/0                     | 29/2                 |
| Race (no. black/no. white/no. other) | 4/7/0            | 2/8/0                    | 3/6/1                    | 9/21/1               |
| Wt (kg)                | 82.4 (65.7–101.5) | 79.1 (62.2–126.0)        | 82.3 (67.5–95.0)         | 81.4 (62.2–126.0)    |
| CD4⁺ cell count (no. of cells/μl) | 414.5 (316–738)  | 389 (321–519)            | 368 (264–595)            | 382 (264–738)        |
| HIV-1 RNA level (no. of log₁₀ copies/ml) | 4.52 (3.76–5.12) | 4.58 (3.88–4.75)         | 4.63 (3.79–5.53)         | 4.52 (3.76–5.53)     |

*Data are median (range) values, unless otherwise indicated. Data were collected during screening.*
maximum reductions for the 10-mg/kg group are 1.67 log_{10} and 1.82 log_{10} units, respectively, if the data for the censored subject are included (\(P < 0.0001\) relative to placebo). Individual viral nadirs were observed on day 10 (five subjects) or day 12 (five subjects) for subjects treated with 5 mg/kg PRO 140 and on day 12 (five subjects), day 15 (three subjects), or day 22 (one subject) for subjects in the 10-mg/kg group.

Similar mean log_{10} decreases in viral load were observed for the 5-mg/kg and 10-mg/kg dose groups through day 12, when the nadir reduction was observed in each group (Fig. 1). Thereafter, mean viral loads rebounded somewhat more slowly for the 10-mg/kg dose group; however, the differences between the 5-mg/kg and 10-mg/kg dose groups were not statistically significant at any time point (\(P > 0.1\)).

As noted above, all PRO 140-treated subjects with the exception of the individual who was reclassified as having dual/mixed virus prior to treatment experienced a \(\geq 1\)-log_{10} unit change in viral-1 RNA posttreatment. No subject in the placebo group experienced a \(\geq 1\)-log_{10} unit decline in viral load during the study (Table 2). Two subjects in the 5-mg/kg group and five subjects in the 10-mg/kg group (\(P < 0.01\) relative to placebo) experienced \(\geq 2\)-log_{10} unit decreases in viral loads.

Five subjects treated with 5 mg/kg PRO 140 (\(P = 0.012\) relative to placebo) and two treated with 10 mg/kg had viral loads reduced to \(<400\) copies/ml, but no subject in the placebo group did. One subject in the 10-mg/kg group had a viral load of 50 copies/ml on days 10 and 12.

Coreceptor tropism and viral susceptibility to PRO 140 in vitro. Tropism was assessed at screening and at the time of viral rebound in PRO 140-treated subjects. As noted above, one subject in the 10-mg/kg group was observed to have dual/mixed virus at day 15. This subject was later reclassified as having dual/mixed virus at screening, based on data generated poststudy using the enhanced-sensitivity Trofile assay. All other PRO 140-treated subjects maintained R5 coreceptor tropism following treatment.

Viral susceptibility to PRO 140 was measured in the PhenoSense Entry assay prior to treatment in all subjects and at the time of viral rebound in PRO 140-treated subjects. In the R5 Phenosense Entry assay, which examined CCR5-mediated viral entry into U87-CD4-CCR5 cells, PRO 140 inhibited all study viruses tested. Prior to treatment, the mean fold change ratio was 1.7 (range, 0.77 to 3.1). On the basis of the ratio of the fold change value at the time of viral rebound to the value prior to treatment, no appreciable change in R5 virus susceptibility was observed in PRO 140-treated subjects (median ratio, 0.83; range, 0.49 to 1.71). Threefold or lower differences in fold change are considered to be within the normal range of interassay variation (3, 8). The maximum inhibition was \(\geq 98\%\) in all cases prior to treatment and was \(\geq 99\%\) in all cases following treatment. CXCR4-mediated entry of dual/mixed viruses into U87-CD4-CXCR4 cells was not inhibited by PRO 140, as expected.

Safety. No serious adverse events (AEs) or dose-limiting toxicities were reported. All 11 subjects in the placebo group and 26 of 31 subjects overall reported at least one AE. AEs reported in more than two subjects were headache in one subject in the 5-mg/kg group and two subjects in the 10-mg/kg group, nasal congestion in two subjects in the placebo group and one subject in the 10-mg/kg group, and pruritus in three subjects in the placebo group. No obvious dose-related trend in the incidence of AEs was observed. There was no clinically relevant change in any electrocardiogram parameter, including QTc intervals, associated with administration of PRO 140 or placebo. There were no notable findings in clinical laboratory hematology or chemistry assessments or in vital sign measurements.

**TABLE 2. Change in HIV-1 RNA levels**

| Effect | Value (\(P\) value) for group* |
|--------|-------------------------------|
| Maximum log_{10} change in HIV-1 RNA load | -0.32 ± 0.24 | -1.83 ± 0.23 (<0.0001) | -1.83 ± 0.41 (<0.0001) |
| Day 12 log_{10} change in HIV-1 RNA load | 0.02 ± 0.18 | -1.69 ± 0.56 (<0.0001) | -1.75 ± 0.27 (<0.0001) |
| No. of subjects with a \(\geq 1\)-log_{10}-unit decrease in HIV-1 RNA level/total no. in group (%) | 0/11 (0) | 10/10 (100) (<0.0001) | 9/9 (100) (<0.0001) |
| No. of subjects with a \(\geq 2\)-log_{10} unit decrease in HIV-1 RNA level/total no. in group (%) | 0/11 (0) | 2/10 (20) | 5/9 (56) (<0.01) |

*Data are mean ± SD values, unless otherwise indicated. The analysis excludes data for one subject in the 10-mg/kg PRO 140 group who was reclassified as having dual/mixed virus at screening.
distribution (9.17 ± 5.52 and 10.8 ± 6.5 liters) were similar for the 5- and 10-mg/kg dose groups, respectively.

The relationship between viral load reductions and PRO 140 exposure was modeled using a hyperbolic $E_{\text{max}}$ equation. Combined data from the present study and a prior study of single-dose i.v. PRO 140 (6) were used in the analysis (Fig. 2B). The best-fit parameters for the combined data ($E_{\text{max}} = -2.06 ± 0.12 \log_{10}$ units, $\text{AUC}_{50} = 34.1 ± 9.7 \text{ mg} \cdot \text{day/liter}$) are similar to those reported previously for data from the prior study only ($E_{\text{max}} = -2.14 ± 0.22 \log_{10}$ units, $\text{AUC}_{50} = 43.6 ± 15.6 \text{ mg} \cdot \text{day/liter}$) (6).

Antibodies to PRO 140 were detected in two subjects in each of the PRO 140 dose groups. Antibodies were first detected on day 15 ($n = 1$), day 29 ($n = 2$), or day 59 ($n = 1$). In all cases, the anti-PRO 140 antibodies were of low titer (1:32 or less) and did not neutralize binding of PRO 140 to CCR5-positive (CCR5+) cells in vitro. The anti-PRO 140 antibodies did not have any apparent effect on the PKs or viral load reductions.

Lymphocyte and receptor occupancy analyses. The changes in CD4+ lymphocyte counts following treatment with PRO 140 were not statistically significant. For the combined PRO 140 dose groups, the median (range) changes in CD4+ lymphocyte counts were +1 (−225 to +407), +111 (−204 to +286), +57 (−198 to +386), and +18 (−362 to +370) cells/μl at days 8, 12, 15, and 22, respectively. The corresponding values for the placebo group were +24 (−250 to +279), +38.5 (−399 to +145), +45 (−117 to +339), and +82 (−173 to +291) cells/μl at these time points.

Receptor occupancy was assessed by flow cytometry using fluorescently labeled PRO 140. Occupancy of CCR5 by study drug results in a reduction in the number of lymphocytes with detectable levels of free CCR5. High levels of receptor occupancy (>85% reduction in the number of cells detected) were observed from day 3 through day 29 for both PRO 140 dose groups (Fig. 3). The results were statistically significant ($P < 0.01$ relative to placebo) throughout this time period. Significant receptor occupancy (81%, $P < 0.01$) was also observed at day 43 for the 10-mg/kg group. At day 59, receptor occupancy levels were not statistically significant for either PRO 140 dose group relative to that for the placebo group ($P > 0.05$). Lymphocytes were analyzed in parallel with a noncompeting fluorescently labeled CCR5 antibody, as described previously (6), and this analysis demonstrated that CCR5+ lymphocytes were not depleted from the circulation following treatment (data not shown).

**DISCUSSION**

In this study, PRO 140 demonstrated potent, rapid, and prolonged antiretroviral activity when it was administered as single 5-mg/kg or 10-mg/kg intravenous infusions to individuals infected with CCR5-tropic HIV-1. The mean maximum de-

**TABLE 3. Pharmacokinetic parameters**

| Dose (mg/kg) | $C_{\text{max}}$ (μg/ml) | $\text{AUC}_{0-\infty}$ (μg · day/ml) | $t_{1/2}$ (days) | CL (ml/day/kg) | MRT (days) | $V$ (liters) |
|-------------|------------------------|-------------------------------|----------------|----------------|-------------|--------------|
| 5           | 109 ± 31               | 224 ± 60                      | 3.13 ± 1.30    | 1.97 ± 0.61    | 2.76 ± 0.84 | 9.17 ± 5.52  |
| 10          | 211 ± 57               | 423 ± 150                     | 3.33 ± 0.70    | 2.36 ± 1.85    | 3.15 ± 0.39 | 10.8 ± 6.5   |

*Data represent arithmetic means ± standard deviations.
The HIV-1 RNA level was 1.8 log10 units for doses of 5 mg/kg PRO 140 (6). Remarkably, the mean maximum reduction in served in the present study and a prior study of intravenous safety profiles of this agent.

Understanding of the pharmacologic, pharmacokinetic, and concordance with the dynamics of inhibiting HIV-1 entry and cant reductions in viral load by at least 2 days. This result is critically significant levels of receptor occupancy preceded signifi- cant reductions in viral load by at least 2 days. This result is concordant with the dynamics of inhibiting HIV-1 entry and with the half-life of virus-producing T cells (12). Receptor occupancy values also appeared to rebound later than viral loads. This apparent discordance could reflect issues related to assay sensitivity and sampling. Given the modest numbers of CCR5+ lymphocytes at the baseline (~20 cells/μL, on average), the assay had a limited ability to determine mean receptor occupancy levels above 90%. Therefore, the times of maximum receptor occupancy and of initial rebound in receptor occupancy levels could not be determined precisely. In addition, receptor occupancy is measured on cells in the periphery, whereas HIV replication occurs primarily within tissues (20). PRO 140 concentrations and levels of receptor occupancy may differ at local sites of HIV-1 replication. As with viral load reductions, the duration of receptor occupancy was modestly greater with the 10-mg/kg dose relative to that with the 5-mg/kg dose, consistent with the higher serum concentrations of drug achieved at the higher dose.

To date, 84 HIV-infected individuals have been treated with i.v. or s.c. forms of PRO 140 in three short-term monotherapy studies (6, 7). In each study, 1.5- to 2.0-log10-unit mean reductions in HIV-1 RNA levels were observed with the higher dose levels. The viral load reductions were long-lived and highly statistically significant. No dose-limiting toxicity or pattern of toxicity was identified in these studies. In addition, no emergence of R5 viral
resistance was observed, even though >1-log10-unit reductions in viral loads were observed for up to 6 weeks in some subjects.

In the present study, the duration of antiviral activity increased somewhat as the i.v. dose was increased from 5 mg/kg to 10 mg/kg. However, neither i.v. dose would appear to support highly infrequent (e.g., monthly) administration, and E_{max} analysis indicated that further increases in i.v. dose would result in incremental increases in antiviral effects. In a study of s.c. PRO 140, significant antiviral effects were observed when the drug was administered weekly or every other week, and virologic suppression was maintained between successive doses (7). While both i.v. and s.c. dosage forms have demonstrated favorable antiviral and tolerability profiles, s.c. PRO 140 was selected for further development on the basis of its potential to be self-administered by patients. Self-administration may offer greater convenience for many patients. Nevertheless, the s.c. dosage form is undergoing clinical study, and i.v. administration may be preferred in certain treatment settings.

In summary, single intravenous infusions of 5 mg/kg and 10 mg/kg PRO 140 demonstrated potent, long-lived antiretroviral activity and a favorable tolerability profile in this study. The findings provide new insights into the safety and virological properties of this agent, which represents a novel and long-lived antiretroviral agent with a CCR5 entry inhibitor: AIDS Clinical Trial Group A5211. Clin. Infect. Dis. 45:1481–1487.

Kittinos, K. M., H. Amrine-Madsen, D. M. Irlebek, J. M. Word, and J. F. Demarest. 2009. Virologic failure in therapy-naive subjects on aplaviroc plus lopinavir-ritonavir: detection of aplaviroc resistance requires clonal analysis of envelope. Antimicrob. Agents Chemother. 53:4599–4607.

Kuhmann, S. E., P. Pugach, K. J. Kunstant, J. Taylor, R. L. Stanfield, A. Snyder, J. M. Strizki, J. Riley, M. B. Baronody, I. A. Wilson, B. T. Korber, S. M. Wolinsky, and J. P. Moore. 2004. Genetic and phenotypic analyses of human immunodeficiency virus type 1 escape from a small-molecule CCR5 inhibitor. J. Virol. 78:2790–2807.

Lalezari, J. M., M. Thompson, P. Kumar, P. Piliero, R. Davey, K. Patterson, A. Shachoy-Clark, K. Adkison, J. Demarest, Y. Lou, M. Berrey, and S. Pisitkul. 2005. Antiviral activity and safety of 873140, a novel CCR5 antagonist, during short-term monotherapy in HIV-infected adults. AIDS 19:1443–1448.

Lederman, M. M., A. Penn-Nicholson, M. Cho, and D. Mosier. 2006. Biology of CCR5 and its role in HIV infection and treatment. JAMA 296:515–526.

Markowitz, M., M. Louie, A. Hurley, E. Sun, M. Di Masico, A. S. Perelson, and D. Ho. 2003. A novel antiviral intervention results in month-long assessment of human immunodeficiency virus type 1 replication dynamics and T-cell decay in vivo. J. Virol. 77:5037–5038.

Marozsan, A. J., S. E. Kuhmann, T. Morgan, C. Herrera, E. Rivera-Troche, S. M. B. Baronody, J. F. Demarest, and J. P. Moore. 2005. Acne-related properties of a human immunodeficiency virus type 1 isolate resistant to the small molecule CCR5 inhibitor, SCH417690 (SCH-D). Virology 338:182–190.

Meyle, G. J., A. Wildfire, S. Mandalia, H. Mayer, J. Goodrich, J. Whitcomb, and B. G. Gazzard. 2005. Epidemiology and predictive factors for chemokine receptor use in HIV-1 infection. J. Infect. Dis. 191:866–872.

Murga, J., M. Franti, D. C. Peverar, P. J. Madden, and W. C. Olson. 2006. Potential antiviral synergy between monoclonal antibody and small-molecule CCR5 inhibitors of human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 50:3289–3296.

Pett, S. L., M. C. McCarthy, D. A. Cooper, K. MacRae, A. Tendolkar, R. Norris, J. M. Strizki, K. S. Williams, and S. Emery. 2005. A phase 1 study to explore the activity and safety of SCH32726, a small molecule chemokine receptor-5 antagonist in HIV type-1-infected patients. Antivir. Ther. 14:111–115.

Reeves, J. D., E. Coakley, C. J. Petropoulos, and J. M. Whitcomb. 2009. An enhanced-sensitivity TroploCMH HIV coreceptor tropism assay for selecting patients for therapy with entry inhibitors targeting CCR5: a review of analytical and statistical studies. J. Viral Entry 3:94–102.

Saag, M., J. Heera, J. Goodrich, E. DeJesus, N. Clumeck, D. Cooper, S. Murga, N. E. Ting, E. Coakley, J. Reeves, M. Westby, E. van der Ryst. 2008. Reanalysis of the MERIT Study with the Enhanced Trofile Assay (MERIT-ES), abstr. H-1232A. Abstr. 48th Annu. Intersci. Conf. Antimicrob. Agents Chemother. Am. (IDSA) and Ann. Meet. American Society for Microbiology and Infectious Diseases Society of America, Washington, DC.

Schuurmann, D., G. Fatkenheuer, J. Reyes, C. Michelet, F. Rabill, J. van Lier, M. Caceres, A. Keus, A. Sanju-Parsons, L. M. Dunkle, and C. Hoffmann. 2007. Antiviral activity, pharmacokinetics and safety of vicriviroc, an oral CCR5 antagonist, during 14-day monotherapy in HIV-infected adults. AIDS 21:1293–1299.

Stebbing, J. B., G. Gazzard, and D. C. Douek. 2004. Where does HIV live? N. Engl. J. Med. 350:1872–1880.

Su, Z., R. M. Gulick, A. Krambrink, E. Coakley, M. D. Hughes, H. Dong, C. Flexner, T. J. Wilkin, P. R. Skolnik, W. L. Greaves, D. Kuritzkes, and J. D. Reeves. 2009. Response to vicriviroc in treatment-experienced subjects, as determined by an enhanced-sensitivity coreceptor tropism assay. AIDS Clinical Trials Group A5211. J. Infect. Dis. 200:1724–1728.

Trokola, A., T. J. Ketas, K. A. Nagashima, L. Zhao, T. Cilliers, L. Morris, J. P. Moore, P. J. Madden, and W. C. Olson. 2001. Potent, broad-spectrum inhibition of human immunodeficiency virus type 1 by the CCR5 monoclonal antibody PRO 140. J. Virol. 75:579–588.

Whitcomb, J. M., W. Huang, S. Fransen, K. Limoli, J. Toma, T. Wrin, C. Chappely, L. D. Kiss, E. E. Paxinos, and C. J. Petropoulos. 2007. Development and characterization of a novel single-cycle recombinant-virus assay to determine human immunodeficiency virus type 1 coreceptor tropism. Antimicrob. Agents Chemother. 51:566–575.

Wilkin, T. J., Z. Su, D. R. Gulick, K. Kuritzkes, M. Hughes, C. Flexner, R. Gough, W. Graves, D. Cooper, P. J. Maddon, and R. M. Gulick. 2007. 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. 44:591–595.