CONCISE COMMUNICATION

Single-Dose Safety, Pharmacology, and Antiviral Activity of the Human Immunodeficiency Virus (HIV) Type 1 Entry Inhibitor PRO 542 in HIV-Infected Adults

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PRO 542 (CD4-IgG2) is a recombinant antibody-like fusion protein wherein the Fv portions of both the heavy and light chains of human IgG2 have been replaced with the D1D2 domains of human CD4. Unlike monovalent and divalent CD4–based proteins, tetravalent PRO 542 potently neutralizes diverse primary human immunodeficiency virus (HIV) type 1 isolates. In this phase 1 study, the first evaluation of this compound in humans, HIV-infected adults were treated with a single intravenous infusion of PRO 542 at doses of 0.2–10 mg/kg. PRO 542 was well tolerated, and no dose-limiting toxicities were identified. Area under the concentration-time curve, and peak serum concentrations increased linearly with dose, and a terminal serum half-life of 3–4 days was observed. No patient developed antibodies to PRO 542. Preliminary evidence of antiviral activity was observed as reductions in both plasma HIV RNA and plasma viremia. Sustained antiviral effects may be achieved with repeat dosing with PRO 542.

There is an urgent need for new human immunodeficiency virus (HIV) therapies that target additional stages of the viral replicative cycle. PRO 542 (tetravalent CD4-IgG2 fusion protein) [1] is a novel HIV-1 entry inhibitor that incorporates 4 copies of the virus-binding domains of CD4, the primary receptor for HIV-1. PRO 542 binds the HIV-1 envelope (env) glycoprotein gp120 with nanomolar affinity and neutralizes primary HIV-1 regardless of genotype or phenotype [2, 3]. The concentration required to achieve a 90% reduction in viral infectivity in vitro (IC_{90}) is ~20 μg/mL and is readily achieved in vivo. In ex vivo assays, PRO 542 is similarly effective at neutralizing the infectivity of plasma obtained from HIV-1–infected persons, which indicates that this agent is active against the diverse viral quasi species that are encountered clinically [4]. PRO 542 also protects against infection by primary isolates in the human peripheral blood lymphocyte (hu-PBL)–SCID mouse model of HIV-1 infection [5].

Compared with monovalent or divalent CD4–based proteins, PRO 542 has consistently demonstrated as much as 100-fold greater activity against primary HIV-1 isolates [1, 2, 4, 6]. PRO 542's antiviral activity compares favorably with that of the rare human monoclonal antibodies (MAbs) that broadly and potently neutralize primary viruses [1–3, 6]. In addition, PRO 542 therapy is, in principle, less susceptible to the development of drug-resistant viruses than are therapies that employ anti-env MAbs or portions of the highly mutable HIV-1 env glycoproteins. Thus, PRO 542 may have clinical utility as a therapeutic or prophylactic agent that neutralizes cell-free virus before it can establish new rounds of infection.

This report describes the results of the first clinical investigation of PRO 542. The phase 1 trial was conducted to evaluate the tolerability, pharmacokinetics, and immunogenicity of this compound in HIV-infected adults. Additional analyses examined the antiviral effects of PRO 542 when administered as a single intravenous infusion.

Methods

PRO 542. PRO 542 (Progenics Pharmaceuticals, Tarrytown, NY) was expressed in recombinant Chinese hamster ovary cells, was purified via column chromatography, and was supplied at 5 mg/mL in PBS [1].

Study design. This was an open-label, dose-escalation study

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All study participants provided written informed consent. The study protocol was approved by the Institutional Review Board, Mount Sinai Medical Center.

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of PRO 542 in HIV-infected adults. Cohorts of 3–6 patients were treated successively with a single 15–30-min intravenous infusion of PRO 542 at doses of 0.2, 1.0, 5.0, and 10 mg/kg. Inclusion criteria included stable or no anti-HIV therapy for ≥4 weeks prior to the study, >3000 copies of viral RNA/mL, >50 CD4 cells/μL, and normal hematologic and serum chemistry values.

Patient evaluation. Vital signs were recorded at frequent intervals on the day of treatment and on follow-up visits. Blood samples were collected ~2 weeks before treatment, immediately before dosing, and at specified time points ≤4 weeks after infusion. Complete blood counts, serum chemistries, and urine analyses were done 1 week after treatment. Samples were processed in the clinical laboratories of Mount Sinai Medical Center for routine hematology and chemistry. Plasma and serum fractions were stored at −70°C prior to virologic and immunologic evaluations.

Pharmacokinetic analysis. Cyropreserved serum samples were analyzed for PRO 542 by ELISA. In brief, patient serum containing PRO 542 was used to inhibit binding of the anti-CD4 antibody Leu-3a (Becton Dickinson, Franklin Lakes, NJ) to microtiter plates coated with recombinant soluble CD4 (Bartels, Issaquah, WA). Bound Leu-3a was detected by enzyme-conjugated goat antibody to mouse IgG. The lower limit of detection is 50 ng/mL. The terminal serum half-life was calculated by regression analysis of the terminal portion of the concentration-time curve. Area under the concentration-time curve, from time 0 to infinity ($AUC_{0\to\infty}$), was calculated by using the linear trapezoidal rule [7].

Immunogenicity analysis. Predose and 4-week serum samples were analyzed for the presence of antibodies to PRO 542. In the ELISA, microtiter plates were coated with PRO 542, contacted with patient serum or anti–PRO 542 antibody standards, and incubated with biotinylated PRO 542, which was detected by using streptavidin-horseradish peroxidase. With Leu-3a as a standard anti-CD4 antibody, assay sensitivity was 15 ng/mL.

Virologic analyses. Plasma HIV RNA levels were measured by reverse transcription–polymerase chain reaction (RT-PCR) by the Amplicor Monitor assay (Roche Diagnostic Systems, Branchburg, NJ). The changes in log-transformed HIV RNA levels were evaluated for statistical significance by the 2-tailed Wilcoxon signed-rank tests. Additional assays measured plasma levels of infectious HIV. In brief, CD4 lymphocytes were purified from activated peripheral blood mononuclear cells and cultured as described elsewhere [8]. We combined 2×10^6 CD4 lymphocytes with serially diluted patient plasma for 16–20 h at 37°C. Cultures then were washed and combined with an additional 5×10^5 cells. At weekly intervals thereafter, culture supernatant was collected for analysis and replaced with an equal volume of fresh medium containing 2.5×10^5 cells. The extent of HIV replication was determined by p24 ELISA as described elsewhere [2]. Samples were analyzed in 2 or 3 separate assays and were scored as positive if replication-competent HIV was detected in any analysis.

Results

Demographics. The median virus loads for the 0.2-, 1.0-, 5.0-, and 10-mg/kg cohorts were 5310, 207,000, 11,800, and 35,500 copies/mL, respectively, whereas the corresponding median numbers of CD4 lymphocytes were 310, 100, 390, and 314/μL, respectively. Seven patients were on stable combination antiretroviral therapy, and 8 were treatment naive. The latter group included subject 125, who received zidovudine and lamivudine therapy for ~2 months ~1 year before enrollment but who was otherwise treatment naive. All patients received a full infusion of PRO 542 and were available for follow-up.

Safety evaluation. PRO 542 was well tolerated, and no dose-limiting toxicities were identified. One patient in the lowest dose cohort presented 1 week after treatment with a grade 2 neutropenia that worsened to grade 3 at week 4 and then resolved without intervention by week 6. No other patient experienced neutropenia. One patient in the 1-mg/kg dose cohort experienced mild and transient headache. While these events could not be attributed directly to the study drug, a possible relationship could not be excluded. All other adverse events were mild and/or unlikely to be related to the study drug. There was no significant change in the number of CD4 lymphocytes (data not shown).

Pharmacokinetics and immunogenicity. Mean serum concentrations of PRO 542 observed for the different dose cohorts are plotted in figure 1. Serum concentrations of PRO 542 increased linearly with dose as did the mean $AUC_{0\to\infty}$ values ($r^2 = .99$), which were 13,500 ± 3500 and 23,800 ± 7800 hr × μg/mL for the 2 highest dose cohorts. A mean peak serum concentration of 564 ± 110 μg/mL was observed for the 10-mg/kg cohort. Mean terminal serum half-lives of 4.2 ± 0.9 and 3.3 ± 0.7 days were observed in the 5.0- and 10-mg/kg dose cohorts, respectively. About 2-fold shorter half-lives were observed for the lower dose cohorts, for which the 28-day time point was not evaluable. No patient developed measurable levels of antibodies to PRO 542.

Plasma HIV RNA. As indicated in figure 2*A,* a significant decline in plasma HIV RNA was observed after a single 10-mg/kg dose of PRO 542. The most consistent decreases were observed 4 h after treatment, when the mean reduction was

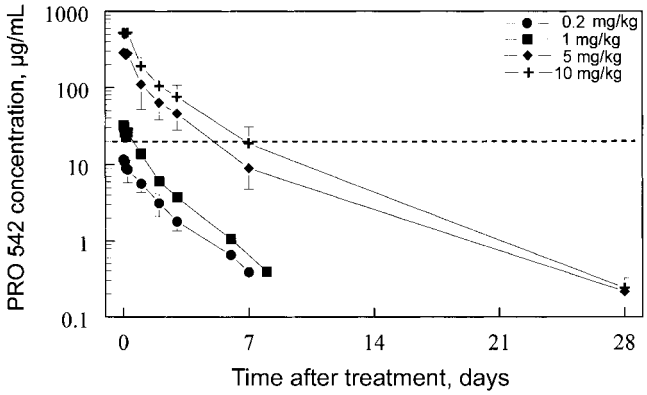

Figure 1. Pharmacokinetics of PRO 542. Mean serum concentrations (± SD) are plotted for each dose cohort. *Dashed line,* approximate in vitro IC_{90} value (20 μg/mL) of PRO 542 for primary human immunodeficiency virus–1 isolates [3].

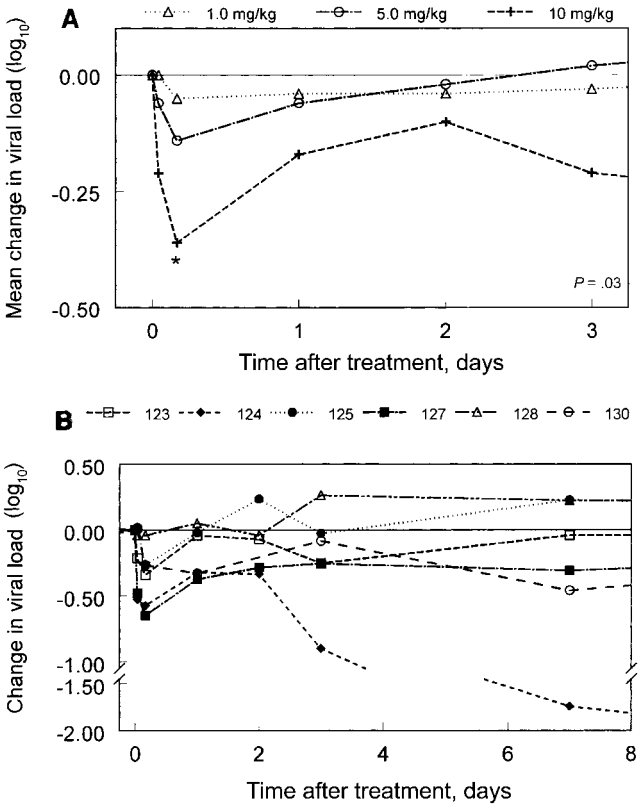

Figure 2. Virologic evaluations. *A,* Mean change in plasma human immunodeficiency virus RNA for patients in 3 highest dose cohorts. Virus load changes for lowest dose cohort were −0.09 to +0.07 log_{10} and have been omitted for clarity. *B,* Individual antiviral responses of subjects in 10-mg/kg cohort. Their predose virus loads were 8210, 498,000, 21,300, 3220, 23,000, and 413,000 copies/mL. Treatment-naive and -experienced patients are indicated by open and filled symbols, respectively.

0.36 log_{10} copies/mL ($P = .03$, 2-tailed Wilcoxon signed-rank test). Smaller and statistically nonsignificant reductions were observed at 1 and 24 h after injection. A similar trend was observed in the 5-mg/kg cohort. Spiking studies showed that assay performance was not affected significantly by the PRO 542 concentrations achieved in this study (data not shown).

Antiviral responses of individual patients in the 10-mg/kg cohort are plotted in figure 2*B.* Virus load reductions were observed in most patients at all posttreatment time points. Patient 124 experienced a 2-log reduction in virus load 7–14 days after treatment. The 10-mg/kg cohort comprised 3 treatment-naive individuals (subjects 123, 128, and 130) and 3 subjects who were receiving stable antiretroviral therapy at entry (subjects 124, 125, and 127). The 3 subjects on therapy experienced greater reductions in mean virus load at all postinfusion time points, including a 0.50 log_{10} mean reduction at 4 h, but the differences between the 2 groups did not reach statistical significance.

Plasma viremia. Plasma from patients 124 and 130 also was analyzed for levels of infectious HIV. These patients were in the 10-mg/kg cohort and had the highest baseline virus loads (498,000 and 413,000 copies/mL, respectively). Culturable virus was isolated from both patients at screening (1–2 weeks before treatment) and immediately before dosing. For subject 130, infectious virus could not be detected in plasma drawn at 1, 4, 24, 48, and 72 h and at 1 and 2 weeks after infusion but was detected at 4 weeks. Subject 124's plasma was culture negative at 1, 4, and 24 h and then was positive intermittently thereafter (i.e., positive at 72 h and 2 weeks but negative at 1 and 4 weeks). Of the subjects treated with 10-mg/kg PRO 542, subject 124 experienced the greatest sustained reduction in virus load, whereas subject 130 experienced a fairly typical pattern of response (figure 2*B*).

Discussion

PRO 542 was extremely well tolerated at all doses tested. This favorable safety profile is consistent with the compound's design to have minimal reactivities with molecules other than the HIV-1 surface glycoprotein. Serum concentrations of >500 μg/mL were attained after administration of a single 10-mg/kg dose, and serum concentrations remained above the in vitro IC_{90} for ~1 week. The terminal serum half-life of PRO 542 was 3–4 days in the high-dose cohorts. No subject developed measurable amounts of antibodies to PRO 542. Thus, any potential neoepitopes formed at the juncture of the CD4 and IgG domains were nonimmunogenic in this study. The pharmacologic and safety data support higher and/or repeat dosing of PRO 542.

Preliminary evidence of antiviral activity was observed as reductions in both plasma HIV RNA and plasma viremia. A statistically significant acute reduction in plasma virus load was observed after administration of a single 10-mg/kg dose of PRO 542, and 1 subject experienced a >2 log reduction in HIV RNA at 1–2 weeks after injection. Smaller virus load reductions were observed at lower doses. Since the in vivo half-life of virus-producing cells is ~2 days [9, 10], the rapid reduction in plasma HIV RNA is striking. One possible mechanism is active clearance of PRO 542–coated virus by the reticuloendothelial system. Although PRO 542 incorporates an IgG2 heavy chain constant region so as to minimize effector functions and does not measurably bind human Fc receptors in vitro [1], IgG2 molecules retain residual effector functions that may mediate clearance [11]. In addition, PRO 542 has the potential to cross-link virions and thereby form large complexes with heightened clearance rates. Conceivably, PRO 542 may also affect the budding of new viruses.

Numerous preclinical studies have established PRO 542's ability to neutralize primary HIV-1 isolates regardless of organ-based clearance mechanisms. Since RT-PCR measurements of

viral RNA cannot distinguish between infectious and neutralized virus, we examined the effects of PRO 542 on plasma viremia. In 2 of 2 subjects examined, infectious HIV was recovered from plasma samples taken immediately prior to treatment but not again for ⩾72 h after treatment. This result is similar to the ⩽8 h reductions in plasma viremia observed in subsets of patients treated with high-dose recombinant soluble CD4 [12, 13]. Taken together, the virus load and HIV culture analyses indicate that PRO 542 possesses antiviral activity in humans. Multiple-dose phase 2 trials of PRO 542 in this patient population will be required in order to determine the dosages and serum concentrations required for sustained antiviral activity.

In targeting cell-free virus, PRO 542 is unique among antiretroviral agents that are either approved or in late-stage clinical development, including other entry inhibitors [14, 15]. This agent may thus provide a useful complement to existing combination therapies. This mechanism of action offers a potential means to rapidly reduce plasma viremia and is consistent with the antiviral data obtained in this study. If borne out in multiple-dose studies, these properties might extend the potential applications of PRO 542 to prophylactic settings, such as perinatal or occupational transmission.

In summary, PRO 542 demonstrated favorable safety and pharmacologic profiles in this initial phase 1 study. In addition, measurable reductions in both plasma HIV RNA and plasma viremia were observed after treatment with a single dose of PRO 542, which indicates that the compound possesses antiviral activity in humans. These encouraging results support the design of multiple-dose phase 2 studies of PRO 542 as a novel inhibitor of HIV entry.

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