CONCISE COMMUNICATION

Recombinant CD4-IgG2 in Human Immunodeficiency Virus Type 1–Infected Children: Phase 1/2 Study

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The use of recombinant CD4-IgG2 in pediatric human immunodeficiency virus type 1 (HIV-1) infection was evaluated by single and multidose intravenous infusions in 18 children in a phase 1/2 study. The study drug was well tolerated, and dose proportionality was observed in terms of area under time-concentration curve and peak serum concentration. Acute decreases of >0.7 $\log_{10}$ copies/mL in serum HIV-1 RNA concentration were seen in 4 of the 6 children treated with 4 weekly 10 mg/kg doses. At 14 days after treatment, 3 children had sustained reductions in serum HIV-1 RNA; the other children had rebounded to baseline levels or above. By 28 days after therapy, the peak HIV-1 cellular infectious units was reduced in all 6 children, including the 2 who had experienced an earlier transient increase in values. Thus, recombinant CD4-IgG2 treatment of HIV-1–infected children appears to be well tolerated and capable of reducing HIV-1 burden.

Despite recent advances in the treatment of human immunodeficiency virus type 1 (HIV-1) with highly active antiretroviral therapy (HAART), long-lived reservoirs of virus, the increasing prevalence of drug-resistant viruses, and significant toxicities mandate the search for new therapies. Virtually all persons with HIV-1 infection have disease progression and eventually die of complications of HIV-1 disease. Since the early discovery that the CD4 molecule on cellular targets was actually a receptor for the HIV-1 surface glycoprotein 120 (gp120) [1], the concept of using CD4 as a soluble competitor molecule to treat HIV-1 infection and prevent perinatal HIV-1 transmission has been raised [2–7]. The recent discoveries of a second cognate receptor, such as CCR5 and CXCR4 (reviewed in [8]), have increased the enthusiasm for using receptor-based therapies.

In this pediatric phase 1/2 clinical trial, we used the CD4-

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Informed consent was obtained from parents or caretakers, and assent was obtained from children ≥7 years old. Human experimentation guidelines of the US Department of Health and Human Services and of the authors' institutions were followed in the conduct of this research.

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based molecule recombinant (r) CD4-IgG2 (PRO 542; Progenics Pharmaceuticals, Tarrytown, NY) [9]. This protein is an antibody-like molecule consisting of 4 copies of the D1D2 immunoglobulin super family domains of human CD4 that have been genetically fused to the highly conserved heavy and light chain constant regions of human IgG2. rCD4-IgG2 has several distinct advantages over earlier CD4-based molecules, including an extended peripheral blood half-life, multiple (tetrameric) binding to HIV-1 gp120, and potent neutralization of primary isolates in a variety of preclinical models of HIV-1 infection [9–13]. Phase 1/2 studies of this fusion protein in HIV-1–infected adults have shown excellent safety, tolerability, and pharmacokinetic properties, plus promising evidence for an antiviral effect on HIV-1 [14].

Methods

Study design and patient evaluation. Thirteen children were enrolled in single-dose studies of 0.2 ($n = 4$), 1.0 ($n = 3$), 5.0 ($n = 3$), and 10 ($n = 3$) mg/kg; 6 children were enrolled in multidose studies in which 10 mg/kg of rCD4-IgG2 was given as 4 weekly doses. Children in the multidose studies were required to have a stable HIV-1 RNA copy number ⩾10,000/mL. No children who were taking immunomodulating drugs, vaccines, or IgG were included.

The study drug, stored at −70°C in 5-, 20-, and 100-mg vials in PBS, was thawed, filtered through 0.20-μm filters, and infused intravenously for 15–30 min. For 2 h after infusion, vital signs were measured, and local and systemic reactions were monitored at 15- and 30-min intervals. The children were observed for side effects in the clinic at least hourly for ⩽12 h.

At intervals before and after infusion of rCD4-IgG2 into study subjects in the single-dose (⩽28 days later) and multidose study (⩽19 weeks later), blood specimens were obtained for complete blood counts and routine serum chemistry and lymphocyte subset determination. Standard Pediatric AIDS Clinical Trials Group (PACTG) toxicity tables were used to grade adverse events on a scale of 1–4 (grade 1, mild; grade 4, life-threatening adverse events). Adverse events were scored as not related, possibly related, and probably related to study drug.

Pharmacokinetic studies. Cryopreserved serum specimens were obtained at fixed time points before and after rCD4-IgG2 infusion for pharmacokinetic analysis, as described elsewhere [14]. Area under the concentration-time curve (AUC) from time 0 to the last measured concentration (C_{last}) was calculated according to the linear trapezoidal rule [15]. AUC from time 0 to infinity ($AUC_{0-\infty}$) was calculated as AUC from time 0 to $C_{last} + C_{last}/ke$, where ke is the elimination rate constant determined by regression analysis of the terminal portion of the concentration-time curve. The half-life was calculated as the natural logarithm of 2/ke. The steady-state AUC was calculated from the concentration-time data of subjects who participated in the multiple dose study as that within the 7-day interval following infusion 4.

Quantitative virology. Blood samples were obtained at fixed time points before and after rCD4-IgG2 infusion for HIV-1 RNA plasma levels, HIV-1 quantitative plasma cultures, and HIV-1 quantitative peripheral blood mononuclear cell (PBMC) cultures. Plasma HIV-1 RNA was measured by a quantitative polymerase chain reaction assay (Amplicor HIV Monitor assay; Roche Diagnostic Systems, Basel, Switzerland), as recommended in [16] and by the manufacturer. This assay has a lower limit of detection of 400 copies/mL. Because of the availability of frequently collected serum specimens from the pharmacokinetic studies, remaining serum specimens were also used to quantify HIV-1 RNA by the Amplicor assay. rCD4-IgG2 does not significantly affect this assay at the concentrations reached in this study [14].

Quantitative microcultures of PBMC and plasma were done by standard methods [16]. The titer of HIV-1 was expressed as the number of infectious units per million cells (IUPM) or per milliliter of plasma. The P value for the goodness-of-fit (PGOF) of the observed distribution of positive wells, given the estimated titer, was calculated for each assay. Assay results with PGOF < .05 were considered unreliable and were excluded.

Statistical analysis. Because of the small sample sizes for each treatment group, the study lacks the statistical power for tests of significance. Thus, the findings are presented as descriptive statistics and graphical displays.

Results

Patient cohort. Thirteen and 6 children were enrolled in the single- and multidose studies, respectively (1 child was in both). Of the 18 children, 56% were girls. The race/ethnicity breakdown was 17% white, 44% black, 33% Hispanic, and 6% other ethnic groups; the median age was 7 years (range, 2–13 years); the median CD4 cell count was 433/μL (range, 36–1849 cells/μL); the median CD4 cell percentage was 20 (range, 4–58); and the median $\log_{10}$ HIV-1 RNA level was 4.03 copies/mL (range, 2.6–5.04 copies/mL).

Safety, toxicity, and tolerability. In the single-dose study, there were no adverse experiences above grade 2 and no study drug–related events of any severity, as assessed by standard PACTG toxicity tables. In the multidose study, 1 child with a history of flu-like reactions to intravenous immunoglobulin experienced similar flu-like symptoms after the second of 4 doses. The grade 3 symptoms were easily controlled with an antipyretic/analgesic compound and were considered to be possibly related to the study product.

Pharmacokinetic measurements. Figure 1 illustrates the concentration-time profiles for single and multidose rCD4-IgG2, respectively. The AUC increased linearly ($r^2 = 0.997$; $P = .0016$) with dose. The median AUC after the fourth 10 mg/kg infusion in the multidose studies was 11,361 $\mu g \times h/mL$, which was in excellent agreement with that (12,335 $\mu g \times h/mL$) observed for a single 10-mg/kg dose. The elimination half-life of rCD4-IgG2 was >2 days (median, 2.27 days; range, 2.06–2.41 days) for the single-dose cohorts and 2.13 days for the multidose cohort. Peak serum concentrations were achieved shortly after infusion, and median values of 6.67, 57.4, 215, and 360 μg/mL were observed for the 0.2, 1.0, 5.0, and 10 mg/kg single-dose cohorts, whereas the corresponding median serum concentrations 7 days after infusion were 0.23, 1.01, 5.06, and 4.74 μg/mL. Median peak and

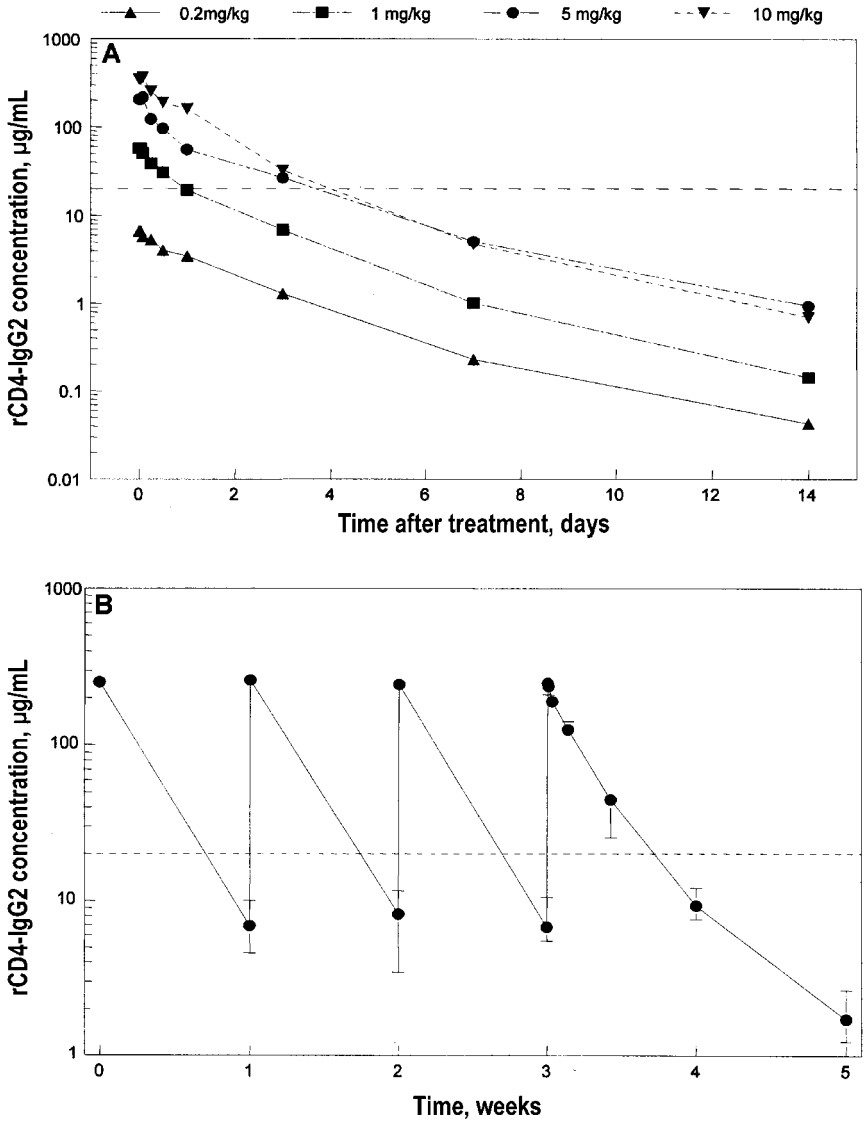

Figure 1. Pharmacokinetic data of recombinant CD4-IgG2 (rCD4-IgG2). *A,* Single-dose concentrations. *B,* Multiple-dose concentrations. Concentration-time profiles for 6 children who received 10 mg/kg of rCD4-IgG2 weekly for 4 doses. 95% Confidence intervals are shown where possible *(brackets)*.

7-day serum concentrations of 274 and 6.95 μg/mL were observed for the multidose cohort.

HIV-1 RNA levels. In the single-dose studies, there were modest acute reductions (~0.50 $\log_{10}$) in plasma HIV-1 RNA levels in some patients and acute increases ($\leq$1.50 $\log_{10}$) in others. In the multidose studies, 4 subjects (nos. 1–4) experienced decreases in serum HIV-1 RNA concentration >0.70 $\log_{10}$ (figure 2). One subject (no. 2) was noncompliant with her HAART program at the time of the second rCD4-IgG2 infusion, and a full adherence program was initiated by medical personnel at a camp (days 7–18); the patient's HIV-1 RNA level rose after she returned home (days 18–39), underscoring the importance of strict adherence to antiretroviral therapy in clinical trials with agents such as rCD4-IgG2. Rebound of HIV-1 RNA serum concentrations occurred in some subjects (e.g., patient 3 had a 2.61 $\log_{10}$ increase between the first and second rCD4-IgG2 infusions; figure 2). Overall, the median $\log_{10}$ RNA value for the 6 children given multidoses was 4.26 on day 0, with a nadir of 3.96 $\log_{10}$ 1 h after treatment at day 14 and an increase to near baseline levels (4.22 $\log_{10}$) at day 35, 14 days after the last infusion (data not shown).

Quantitative HIV-1 PBMC and plasma cultures. In the single-dose study arm in the 3 subjects with undetectable HIV-1 RNA at baseline, quantitative IUPM results remained below

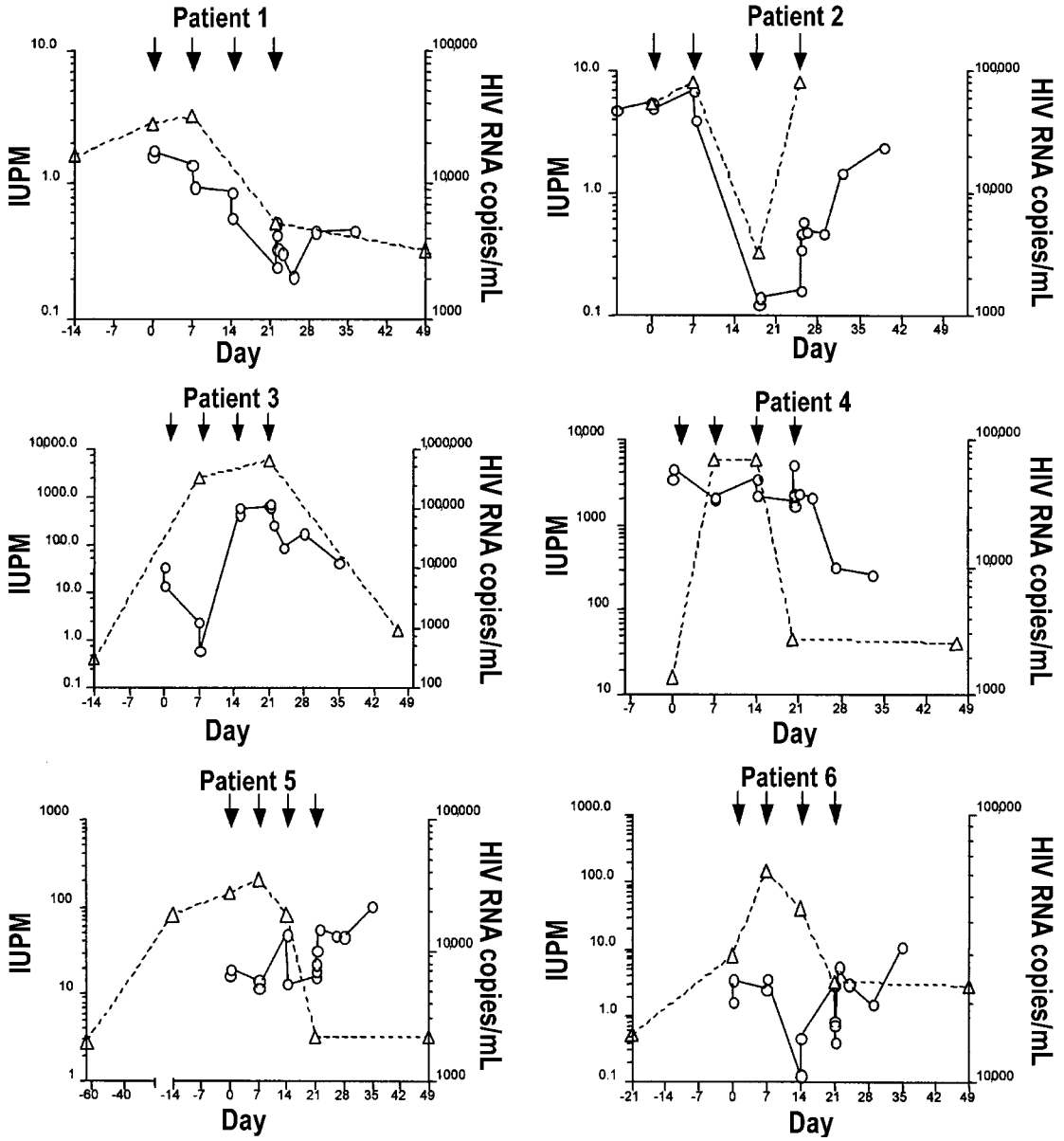

Figure 2. Comparison of serum human immunodeficiency virus (HIV)–1 RNA levels (copies/mL) and quantitative (peripheral blood mononuclear cell [PBMC]) microcultures (infectious units/million cells [IUPM]) in 6 children receiving 10 mg/kg of recombinant CD4-IgG2 (rCD4-IgG2) in multidose studies. Day 0 is beginning of study. Arrows indicate times of rCD4-IgG2 infusion. ○, HIV-1 RNA values (copies/mL). △, PBMC IUPM no. Screening test data (before day 0) are shown for most subjects.

the limit of detection. In subjects with detectable HIV-1 RNA levels at baseline, the IUPM generally reflected changes in plasma HIV-1 RNA levels (data not shown).

In the multidose study arm, there was a general lowering trend in IUPM values after treatment with rCD4-IgG2 (figure 2). The IUPM values decreased to near or below baseline in patients 4 and 6 after a transient increase from days 0 to 7. Patients 1, 5, and 6 experienced an increase in IUPM in the week(s) before drug infusion. Patients 1 and 5 experienced an ~10-fold decline in IUPM relative to that measured just before infusion (day 0), whereas patient 6 had an ~4-fold fall in IUPM from days 0 to 21. Patient 3 (baseline assessment not available) had a change from 2503 IUPM (day 7) to 5607 IUPM at day 21 (day 15 culture results were invalid) to 2 IUPM at day 49. Patient 4 went from 16 IUPM at day 0 to 5608 IUPM at days 7 and 14, a 350-fold increase, followed by a decline to ~45

IUPM thereafter. Patient 2 had an insignificant change from 5.4 IUPM at day 0 to 8.1 IUPM at day 7, followed by a decrease to 0.32 IUPM at day 18. Subsequently, the IUPM returned to 8.1 at day 25, which was concordant with an increase in HIV-1 RNA level after the child's removal from supervised antiretroviral drug therapy. Similarly, the IUPM values in patients 1, 3, and 5 fell to low levels after therapy. With the exception of patient 2 (likely to have been noncompliant with antiretroviral therapy), the IUPM levels after completion of the 4 doses of rCD4-IgG2 stayed depressed for at least 28 days. The IUPM decreases in all the children after infusion of rCD4-IgG2 appeared to be unrelated to CD4 cell count (data not shown). Most assessments of infectious titers in the plasma were at or below the level of assay detection at baseline and did not appear to change significantly with treatment.

Discussion

This new agent appeared to be well tolerated in HIV-1–infected children. Its pharmacokinetic properties also appeared to be excellent, and high concentrations of product were achieved shortly after infusion. At a dose of 10 mg/kg, rCD4-IgG2 produced a median peak level of 274 μg/mL and a trough level of 6.95 μg/mL 7 days later; the first of these values was well above that of 15–20 μg/mL, which was shown in laboratory and animal model systems to inhibit many wild-type HIV-1 strains [10, 11]. The AUC analysis suggests that this fusion protein at 10 mg/kg might be highly effective in binding HIV-1 virions for $\geq$5 days.

rCD4-IgG2 appeared to produce acute, sometimes sustained, decreases in serum HIV-1 RNA concentrations and cellular IUPM in individual pediatric patients. This is consistent with the findings of a phase 1 study of the molecule in HIV-infected adults, in which a statistically significant acute reduction in plasma HIV RNA was observed after administration of a single 10 mg/kg dose of rCD4-IgG2; reductions to 2 logs were observed 1–2 weeks after injection [14]. In 2 of 6 patients, an apparent early and substantial transient increase in circulating infected PBMC, but not in serum HIV-1 RNA level, was observed. Numerous possible explanations could account for the increases in circulating infected cells, including altered trafficking of HIV-1 in lymphoid tissue and formation of HIV-1 virion rCD4-IgG2 complexes by virtue of multimeric binding, but additional clinical trials will be necessary to better characterize their existence.

In summary, this first clinical trial of rCD4-IgG2 in children has established, in single and multidose studies, that the product is safe and well tolerated. This new product has excellent pharmacokinetic properties, and serum concentrations in excess of the in vitro inhibitory concentration were maintained for about 5 days after infusion. The trial results also suggest that rCD4-IgG2 may be effective in lowering the HIV-1 burden in children and clearly point the way toward additional pediatric clinical trials in which drug dosage and frequency of administration can be optimized.

Pediatric AIDS Clinical Trials Group Protocol 351 Study Team Study Coordinators

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