

Susceptibility of pediatric HIV-1 isolates to recombinant CD4-IgG₂ (PRO 542) and humanized mAb to the chemokine receptor CCR5 (PRO 140)

To the Editor:

Although remarkable advances have been made in treating HIV-1 infection with antiretroviral agents, including reverse transcriptase inhibitors (RTIs) and protease inhibitors (PI),¹ the use of immune-based HIV-1 entry inhibitor proteins remains an attractive alternate approach, because the HIV-1 virion is prevented from attaching to target cells.² These molecules act via unexploited mechanisms, are active against multidrug-resistant viruses *in vitro*, and offer new, potentially long-acting modes of therapy.³ In an earlier analysis, we reported on the ability of PRO 542, a recombinant tetrameric CD4-IgG₂ fusion protein that binds to HIV-1 gp120, to reduce HIV-1 viral load in children being treated with combination antiretroviral agents including PI in the Pediatric AIDS Clinical Trials Group (PACTG) protocol 351.⁴ Subsequently, we have had the opportunity to compare the coreceptor usage and *in vitro* susceptibility of pretreatment viral isolates of protocol 351 study subjects to PRO 542 and PRO 140, a humanized mAb to the chemokine receptor CCR5.⁵ PRO 140 exhibits a broad spectrum of inhibition of primary R5 HIV-1 (binds to CCR5 receptor) isolates obtained from adult patients from the United States and Africa.⁶ PRO 140 also compares favorably with several other mAb and HIV-1 entry inhibitor molecules in its ability to inhibit acute and chronic HIV-1 viruses from European adult patients.⁷ Prolonged coating of CCR5⁺ lymphocytes (>60 d) by PRO 140 (5 mg/kg) was reported in healthy adult male human subjects in a dose-finding safety study.⁸ To provide a rationale for designing a clinical trial of PRO 140 in pediatric patients, we compared PRO 140 and PRO 542 for inhibition of pretreatment HIV-1 isolates of patients enrolled in PACTG protocol 351.

Study subjects were HIV-1 infected by perinatal transmission, 2 to 12 years of age, receiving stable antiretroviral medications for at least 3 months, and exhibiting an HIV-1 RNA viral load of $\geq 10,000$ copies/mL. Two separately recruited cohorts were studied: cohort I (1999; n = 6) and Cohort II (2000-2004; n = 13). Patient plasma and sera were preserved in liquid N₂ (−140°C). Five cohort I patients and 10 cohort II patients had adequate samples for study. Peripheral blood CD4⁺ T-cell counts (cells/ μ L) and percentages and plasma HIV-1 viral load (copies/mL) determination were performed as previously described.⁴ For the current study, only pretreatment HIV-1 viral isolates, HIV-1 RNA levels, and CD4⁺ T-cell values were used.

Tropism and neutralization data were obtained using a phenotypic assay (HIV PhenoSense Entry Assay; Monogram Biosciences, Inc, South San Francisco, Calif; formerly ViroLogic, Inc) on stored plasma samples collected before study treatment.³ In this assay, HIV-1

envelopes representing the circulating quasiespecies (typically 500-5000 quasiespecies) are amplified from plasma or sera, subcloned, and used to create single-round reporter viruses. The envelope-complemented reporter viruses are tested for infectivity on U87-CD4-CCR5 and U87-CD4-CXCR4 (chemokine receptor found on CD4⁺ T cells) cells in the presence and absence of inhibitors. IC₅₀ is the concentration of antibody (μ g/mL) required to inhibit the virus by 50%. The IC₅₀ values for a given patient isolate were divided by that observed for the dual-tropic control virus (92HT594) on the respective cell type, resulting in a relative IC₅₀ (rIC₅₀). This normalization process controls for minor day-to-day variations in assay performance.

Descriptive statistics, overall and classified by cohort, were computed for the following baseline characteristics: sex, race/ethnicity, age (years), weight (kg), CD4⁺ T-cell count/percentage, and HIV-RNA viral load. Summary statistics of IC₅₀ values were calculated for PRO 140 and PRO 542 (overall and by cohort). Estimates of the prevalence of R5X4 (HIV-1 that binds to both CCR5 and CXCR4 receptors) tropism were also calculated and bounded by 95% exact confidence intervals.

Previous antiretroviral use was quantified using the total number of drugs used in the following categories: PI, RTI (nucleoside and nonnucleoside), the fusion inhibitor enfuvirtide (T20), and total antiretroviral. Antiretroviral use was compared by cohort and by tropism using medians to summarize the results and the Kruskal-Wallis test to examine statistical significance. Associations between previous antiretroviral use and susceptibility to both PRO 140 and PRO 542 were tested using the Spearman rank correlation analysis.

Univariate correlations between viral tropism and various baseline factors such as viral load (HIV-RNA), CD4⁺ T-cell absolute count, and susceptibility to PRO 140 and PRO 542 using rIC₅₀ were assessed using Kruskal-Wallis test. Some dual-tropic patients had PRO 542 IC₅₀ and rIC₅₀ from both R5 and X4 (HIV-1 that binds to CXCR4 receptor) assays. Thus, in those cases, that is, PRO 542, the nonnormalized R5 and X4 IC₅₀ values were compared, and the higher IC₅₀ value and its corresponding rIC₅₀ value were used in the analyses, providing an overall measure of IC₅₀ and rIC₅₀, respectively. As expected, PRO 140 inhibited R5 virus entry but did not affect entry of viruses into U87-CD4-CXCR4 cells, and consequently R5 rIC₅₀ values were used exclusively for PRO 140.

The small sample size provides very limited statistical power; thus, many of the results are presented using descriptive statistics. Nonparametric analyses to test for significance were performed to avoid normality assumptions. The level of significance used was $\alpha = 0.05$.

Overall baseline characteristics of children (n = 15) who received multiple doses in the PACTG 351 study are as follows: 60% were male, 60% were Hispanic, 33%

TABLE I. Viral tropism by cohort: cohort I versus cohort II

Cohort/PATID	Tropism	rIC ₅₀		CD4 cells/ μ L (%)	HIV-1 RNA (copies/mL)
		PRO 542*	PRO 140†		
I/1	R5	4.10	3.10	882 (31)	10,806
I/2	R5	7.00	3.40	36 (4)	40,831
I/3	R5	59.32	2.50	1849 (46)	4710
I/4	R5	28.50	2.70	349 (26)	66,990
I/5	R5	4.30	5.20	400 (9)	59,433
Median (minimum-maximum)		7.00 (4.1-59.32)	3.10 (2.50-5.20)		
II/6	R5X4	R5: NA; X4: 3.30	NA	477 (18)	22,482
II/7	R5	0.30	3.30	724 (24)	39,140
II/8	R5	1.40	2.80	267 (17)	24,969
II/9	R5X4	R5: 1.47; X4: 1.70	0.52	113 (5)	72,142
II/10	R5	6.50	0.90	1005 (28)	7497
II/11	R5	3.22	2.47	813 (23)	3613
II/12	R5	11.00	1.59	1321 (33)	2590
II/13	R5X4	R5: 1.18; X4: NA	1.02	612 (28)	34,079
II/14	R5X4	R5: 19.00; X4: NA	2.30	121 (11)	48,562
II/15	R5X4	R5: 4.45; X4: 5.55	0.98	13 (1)	167,025
Median (minimum-maximum)		3.26 (0.3-19.00)‡	1.59 (0.52-3.30)§		

PATID, Patient identification number; NA, not available (insufficient level of viral replication to determine rIC₅₀).

*For R5X4 isolates, rIC₅₀ values in the R5 and X4 assays are indicated where available.

†rIC₅₀ values as measured in the R5 assay.

‡*P* = .0662; PRO 542 in cohort I vs PRO 542 in cohort II.

§*P* = .0196; PRO 140 in cohort I vs PRO 140 in cohort II.

were African American, and 7% were Caucasian. The median age was 8 years (range, 3-11). The median weight was 25.2 kg (range, 17.6-47.8). The median CD4⁺ T-cell count was 477 cells/ μ L (range, 13-1849; Table I). The median CD4⁺ T-cell percentage was 23 (range, 1-46). The median HIV-1 RNA was 34,079 copies/mL (range, 2590-167,025 copies/mL; Table I). Patients' baseline characteristics did not differ significantly across cohorts.

Patients who were enrolled to cohort II were relatively more susceptible to both PRO 140 and PRO 542 based on their lower median rIC₅₀ compared with patients enrolled to cohort I (Table I). The median PRO 140 rIC₅₀ of patients enrolled to cohort I was 3.10, whereas for cohort II, the median was 1.59, and this 2-fold difference was statistically significant (*P* = .0196), reflecting in part the modest variation in viral susceptibility to PRO 140 observed in this study. The difference in rIC₅₀ medians of PRO 542 across cohorts was marginally significant (*P* = .0662; cohort I median, 7.00; cohort II median, 3.26). The overall median nonnormalized IC₅₀ values were 0.25 μ g/mL (range, 0.043-0.48 μ g/mL) for PRO 140 and 5.5 μ g/mL (range, 0.39 to >76 μ g/mL) for PRO 542 (data not shown).

Patients of cohort I and cohort II had a median use of 1 PI and 2 RTI. Previous RTI use did not differ significantly between patients with R5 and R5X4 virus (*P* = .7920). However, previous PI use marginally differed (*P* = .0952) between patients with R5X4 (median, 2; *n* = 5) and R5 (median, 1; *n* = 10) virus. The Spearman rank correlations did not identify any significant correlations between the number of PI or RTI previously used and viral susceptibility to either PRO 140 or PRO

542, indicating no obvious cross-resistance between anti-retroviral and PRO 140 or PRO 542. Rather, there was a modest trend (*r* = -0.4073; *P* = .1483) toward increased susceptibility to PRO 140 with increased PI use.

Univariate analysis identified trends in lower CD4⁺ T-cell counts and higher HIV-1 RNA levels in R5X4 patients compared with R5 patients (Table II). R5 viruses had a median PRO 542 rIC₅₀ of 5.40 (*n* = 10), whereas R5X4 viruses were slightly more susceptible to PRO 542, showing a relatively lower median rIC₅₀ of 3.30 (*n* = 5). However, based on the *P* value of .4624, there was insufficient evidence to say that the difference was significant. R5 viruses had a median PRO 140 rIC₅₀ of 2.75 (*n* = 10). R5X4 viruses were slightly more susceptible to PRO 140 (rIC₅₀ = 1.00; *n* = 4) when tested on U87-CD4-CCR5 cells, and this difference was significant (*P* = .0237).

mAbs and immunoglobulin fusion proteins are assuming an important role in the treatment of numerous diseases in medicine, such as cancer, autoimmune disease, and infectious disease.⁹ The CCR5 mAb and CD4-Ig fusion protein studied here offer alternate molecular modes of therapy for HIV-1-infected patients. The results of this study suggest that PRO 140 effectively inhibits primary pediatric HIV-1 isolates, both R5 viruses and the CCR5-dependent component of R5X4 isolates. Also, the data suggest that R5 monotropism of pediatric HIV-1 strains is associated with higher CD4⁺ T-cell counts and lower HIV-1 RNA copy numbers, whereas R5X4 dual-tropism is associated with lower CD4⁺ T-cell counts and higher HIV-1 RNA copy numbers, results consistent with recent findings in adult patients.¹⁰

TABLE II. Univariate association between viral tropism and other baseline factors

Viral tropism	n	Median	Minimum	Maximum	P value*
Viral tropism and CD4 ⁺ T-cell count (cells/ μ L)					
R5	10	768.5	36	1849	.0662
R5-X4 (dual)	5	121.0	13	612	
Viral tropism and HIV-RNA viral load (copies/mL)					
R5	10	17,887.5	2590	66,990	.0864
R5-X4 (dual)	5	48,562.0	22,482	167,025	
Viral tropism and PRO 542 rIC ₅₀					
R5	10	5.4	0.30	59.32	.4624
R5-X4 (dual)	5	3.3	1.18	19.00	
Viral tropism and PRO 140 rIC ₅₀					
R5	10	2.75	0.90	5.2	.0237
R5-X4 (dual)	4	1.00	0.52	2.3	

*Kruskal-Wallis test. This test was chosen to avoid normality assumptions, thus avoiding effects of outliers. Note that the study has limited statistical power because of small sample size.

Some caveats need to be made in the interpretation of the results of this small pediatric study. It is apparent that the enrollment of patients into this study took several years, and the use of antiretroviral drugs changed considerably during this time, from 1999 to 2004. Thus, the later cohort used different background regimens as reflected in greater PI use. Therapy with PI and RTI can affect viral tropism.¹¹ Despite the preliminary nature of this *in vitro* study, it is reasonable to conclude that both PRO 140 and PRO 542 inhibit the growth of R5 and R5X4 viruses from children *in vitro*. In terms of PRO 140 and future phase I/II clinical trials in children, our findings provide support for a study to explore the potential of this CCR5 mAb as a potentially long-acting HIV-1 therapy.

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Duration of airborne Fel d 1 reduction after cat washing

To the Editor:

Allergic rhinitis and asthma triggered by pet exposure are a commonly encountered clinical problem. Allergic sensitization to cat or dog dander has been found in 22% to 67% of patients with asthma.¹

Allergic sensitization to Fel d 1, the major cat allergen, is present in more than 90% of patients with cat allergy.² Levels of Fel d 1 sufficient to promote sensitization and provoke symptoms are often encountered even in homes without cats.³

Because pet owners often develop emotional attachment to their pets, alternatives to animal removal from the have been studied. We were previously unable to demonstrate efficacy of several proposed allergen reduction methods.⁴ A subsequent report has suggested a reduction in recoverable Fel d 1 by a washing technique involving cat immersion.⁵ In this study, we analyze the duration of Fel d 1 reduction using an immersion technique.

Twelve adult female American domestic short-hair and medium-hair cats were washed by the immersion technique described by Avner et al.⁵ Briefly, cats were immersed in tap water to the level of their heads, and pelts were massaged for 3 minutes. This was followed by a 3-minute immersion in fresh water. Cats were then towel-dried and returned to their cages. The cats were treated and maintained in accordance with applicable animal care standards.

Fel d 1 collection was performed by placing cats in a 61 cm × 46 cm × 30 cm chamber with air samples collected for 30 minutes at a flow rate of 200 L/min onto a microporous filter mounted at one end of the chamber. A perforated Plexiglas shield (Altuglas International, Philadelphia, Pa) was placed between the cat and the filter to prevent direct contact. Chambers were thoroughly cleaned between collections.

All Fel d 1 assays were performed by a blinded investigator using a 2-site monoclonal immunoassay. Results were recorded as milliunits (1 mU = 4 ng) of Fel d 1 per 30 minute air sample. Prewash baseline Fel d 1 measurements were obtained daily for 1 week. Postwash measurements were then taken 3 hours after washing and daily for 1 week thereafter.

A natural log transformation was used for the analysis because of the variability of the Fel d 1 measurements. Mean values for all 12 cats are reported for each day of the study. In addition, the mean of 7 prewash days was determined to establish an average baseline value. This average value was compared to each of the postwash days using paired *t* tests.

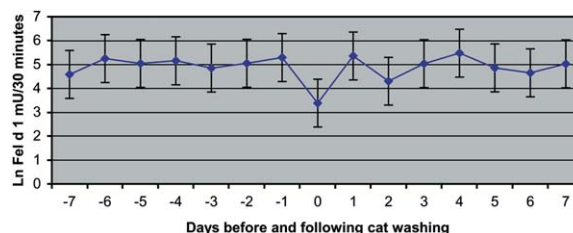


FIG 1. The averages of the Fel d 1 air sample collections for all 12 cats are depicted for each day of the study. The mean of the prewash days was compared with each day after washing the cat. Sampling for Fel d 1 three hours after washing (day 0) was lower than the mean prewash value ($P = .0062$). The remaining postwash values were not significantly different from the mean value.

Individual measurements varied significantly (<1.0-16,000 mU/30 min collection). Mean daily natural log Fel d 1 values are shown in Fig 1. Eight control measurements with empty sampling chambers were performed throughout the study to assess adequate cleaning methods between cat samples. The average of the prewash measurements (153.24 mU Fel d 1/30 min) was compared to each of the postwash measurements (3 hours and daily for 7 days). Only the 3-hour postwash measurement showed a significant reduction (29.54 mU Fel d 1; $P = .0062$). By 24 hours, Fel d 1 measurements were indistinguishable from baseline (212.09 mU Fel d 1; $P = .4542$).

Brief immersion of cats in water did result in a 4-fold to 5-fold decrease in Fel d 1 collection at 3 hours, with a return to baseline within 1 day after the washing.

Female cats were used in this study, because these are common household pets. However, Fel d 1 production in male animals is higher, and evaluation of immersion on uncastrated male cats may yield a different result.⁶

Our data confirm that significant reductions in Fel d 1 can be obtained by cat immersion. However, these reductions appear short-lived. Therefore, it is unlikely that the average cat owner will experience meaningful improvement in allergic symptoms with this environmental control method. Variability of Fel d 1 sampling between different cats on the same day, and within the same cat on different days, was observed in our study and reported by others.⁶ However, it appears that some cats were consistently high producers of Fel d 1. This may be a partial explanation for patients' claims of becoming symptomatic only when exposed to certain animals.

It is clear that many cat owners form strong emotional bonds with their pets and will continue to seek alternatives to pet removal, even if unproven. However, our study reinforces the lack of efficacy of washing cats as a means of reducing allergen exposure. In addition, keeping a cat out of a patient's bedroom appears to provide little benefit in reducing the Fel d 1 concentration throughout the home.⁷ In our study, only a single intervention was analyzed. Multifaceted environment manipulation may be effective for allergens such as cockroach.⁸ To our knowledge, studies involving multiple intervention approaches for cat allergy have not been published. However, removing upholstered furniture from the home after removal of