Higher CD4+ T Cell Counts Associated with Low Viral *pol* Replication Capacity among Treatment-Naive Adults in Early HIV-1 Infection

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Background. Infection with primary drug-resistant human immunodeficiency virus type 1 (HIV-1) has been associated with higher CD4+ T cell counts in drug-naive patients, suggesting that altered viral *pol* replication capacity (RC) associated with drug resistance diminishes immune injury in vivo, independent of exposure to drugs.

Methods. Virus replication over a single cycle was measured by use of a viral test vector containing patient-derived HIV-1 protease and reverse transcriptase gene segments.

Results. Among 191 recently infected patients, *pol* RC ranged widely, with only 6% of the variance explained by drug-resistance mutations. Patients infected with a virus with a low *pol* RC (≤43% of the reference virus) had significantly higher CD4+ T cell counts at study entry, independent of drug resistance and plasma HIV-1 RNA level, and over time, both before and during combination antiretroviral therapy.

Conclusions. Viral *pol* RC may influence HIV-1 disease progression by affecting the amount and tissue distribution of viral replication. The *pol* RC value of 43% may represent a threshold below which HIV-1 has lowered virulence and is less able to deplete CD4+ T cell counts.

Patients experiencing long-term virologic failure of a protease inhibitor (PI)–based regimen sustain CD4+ T cell counts above pretherapy levels for a median of 3 years, despite levels of viremia typically associated with rapid loss of CD4+ T cells in untreated patients [1, 2]. Furthermore, *pol* replication capacity (RC) decreases and remains very low (average, 40% of the reference virus) during long-term virologic failure, because of the accumulation of drug-resistance mutations [3]. On cessation of treatment, patients who had been receiving continuous treatment with a failing PI-based regimen experience a rapid outgrowth of a drug-sensitive virus with higher *pol* RC [4]. This outgrowth is accompanied by higher HIV-1 RNA levels and a resumption of rapid decrease in CD4+ T cell counts, suggesting that the restoration of *pol* RC increases the virulence of HIV-1.

Drug selection of viruses with decreased *pol* RC may account for the sustained partial suppression of plasma HIV-1 RNA levels among patients with detectable drug-resistant viremia during combination antiretroviral therapy (ART) [3–6]. A rapid and standardized assay has been developed for measuring single-cycle *pol* RC in re-

Received 11 September 2003; accepted 30 January 2004; electronically published 18 June 2004.

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The Journal of Infectious Diseases 2004;190:251–6
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0022-1899/2004/19002-0006$15.00

Presented in part: 11th International HIV Drug Resistance Workshop, Sitges, Spain, 2–5 July 2002; 11th Conference on Retroviruses and Opportunistic Infections, San Francisco, 8–11 February 2004 (abstract 388).

Financial support: Centers for Disease Control and Prevention (grant U64/CCU913941); University of California, San Francisco/Gladstone Center for AIDS Research (grant P30 MH59037); National Institutes of Health (grants AIEDRP AI 41531 and MH64384-01); University of California Universitywide AIDS Research Program; Gladstone Institute of Virology and Immunology. Kits for some genotypic testing were provided by Visible Genetics (Toronto, Canada). The replication capacity assays were performed at ViroLogic (South San Francisco, California).

Potential conflicts of interest: R.M.G. has received speaker's honoraria from the manufacturers of the genotyping assay and the replication capacity assay used in this study. J.O.K. owns stock options in and C.J.P. and T.W. are employees of ViroLogic, which provided the replication capacity assay results used in this article. N.S.H. was an employee of ViroLogic at the time that this study was performed. J.D.B. is a Burroughs Wellcome Fund Fellow in the Program for Quantitative Biology at the University of California, San Francisco.

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combinant viral vectors that contain protease and reverse transcriptase (RT) gene segments from patient-derived viruses [7]. Changes in *pol* RC correlate with changes in plasma HIV-1 RNA levels and $CD4^+$ T cell counts, during viral genetic shifts that occur when changing therapy [3] and stopping therapy [4]. However, each of these studies involves individuals who are initiating, stopping, or continuing ART, which has direct effects on viral replication and $CD4^+$ T cell counts. The effects of *pol* RC, independent of drug exposure, are difficult to discern during these treatment trials involving individuals with long and complicated treatment histories.

Higher $CD4^+$ T cell counts have been associated with primary drug resistance among treatment-naive patients in early HIV-1 infection [8]. It is not known whether *pol* RC varies significantly across recently infected individuals or is associated with altered virulence among treatment-naive, HIV-1–infected adults infected with a virus without evidence of drug resistance.

Treatment-naive patients in early HIV-1 infection provide a unique opportunity to (1) evaluate the relationship of low *pol* RC to $CD4^+$ T cell counts and plasma HIV-1 RNA levels, in the absence of drug-induced inhibition of viral replication, and (2) evaluate the relationship of low *pol* RC to $CD4^+$ T cell counts and plasma RNA responses to a first, potent ART regimen. To this end, we initiated a study of *pol* RC, drug resistance, HIV-1 RNA levels, and $CD4^+$ T cell counts in a cohort of treatment-naive, recently HIV-1–infected individuals seeking care at San Francisco General Hospital.

PATIENTS, MATERIALS, AND METHODS

Patients. All specimens were obtained from patients enrolled in the options study [9] of acute and early HIV-1 infection, which was conducted in a publicly funded urban HIV/AIDS care clinic. Patients for the university-based study were referred from a variety of sources, including physicians and HIV testing sites and by self-referral. All participants gave written, informed consent, in accordance with protocols approved by the Committee on Human Research, University of California, San Francisco. Human-experimentation guidelines defined by the US Department of Health and Human Services and those of the University of California, San Francisco, were followed. Patients were excluded from cross-sectional analysis if they had received ART before enrollment.

Criteria for recent HIV-1 infection. We identified individuals with acute and early HIV-1 infection if they met at least 1 of the following criteria: (1) 2 HIV-1 RNA test results of >3000 copies/mL, with a negative or indeterminate HIV-1 antibody test; (2) a positive HIV-1 antibody test, with a history of a negative HIV-1 antibody test within the previous 12 months; or (3) a history compatible with recent HIV-1 infection and a reactive standard HIV-1 antibody test, but a nonreactive less-sensitive EIA (LS-EIA), providing laboratory evidence of recent infection [10, 11].

Drug-resistance testing. The presence of mutations associated with drug resistance was evaluated using population-based sequencing of protease and RT reading frames, by use of the TRUGENE assay (Bayer Diagnostics) [12, 13]. In the analysis of predictors of *pol* RC and $CD4^+$ T cell counts, the following mutations were considered as markers of drug resistance: PR D30N, M46I/L, G48V, I50V, V82A/F/T, I84V, and L90M, for protease inhibitor (PI) resistance; RT M41L, E44D, A62V, K65R, D67N, T69D, K70R/E, L74V, V75M/S/A/T/I, F77L, W88G/S, Y115F, F116Y, V118I, Q151M, Q161L, V179N/E/D, M184V/I, L210W, H208Y, T215F/Y/C/D, and K219Q, for nucleoside RT inhibitor (NRTI) resistance; and A98G, L100I, K101E, K103N, V106A, V108I, Y181C/I, Y188C/H/I/L, and G190A/S, for nonnucleoside RT inhibitor (NNRTI) resistance.

pol *RC.* *pol* RC was assessed by use a modification of the Phenosense phenotypic drug susceptibility assay (ViroLogic) [4, 7]. The RC assay uses viral test vectors that involve extraction of HIV-1 RNA from the plasma of infected patients, followed by reverse-transcription polymerase chain reaction amplification of a gene segment containing the terminal 18 codons of *gag,* all of the *pro,* and a portion of the RT reading frame. Protease-RT *pol* RC values were expressed as percentage of the amount of replication of the NL4-3 reference vectors analyzed in parallel in every run. Values <100% have lowered *pol* RC, compared with those for NL4-3. The values for *pol* RC reported here are higher than values previously reported by our group [3] and by others, by a factor of 1.8, which reflects a change in the *pol* RC assay. In brief, the control virus is submitted to a similar extraction procedure as patient-derived viral RNA (C.J.P., unpublished data).

Cross-sectional analysis. The baseline date for this analysis was the first time point available during documented early HIV-1 infection. Patients were not receiving ART at this time point. All statistical analyses were performed with the SAS System for Windows (version 8.2; SAS Institute) or R for Solaris (version 1.6.2; available at: http://lib.stat.cmu.edu/R/CRAN/). Identification of putative break points in the relationship between *pol* RC and $CD4^+$ T cell counts was made by use of single-split regression trees, with subsequent evaluation by cross-validation and permutation testing; 95% confidence intervals (CIs) for the break-point value were derived by use of bootstrap analysis. For both the cross-sectional and the longitudinal analyses, HIV-1 RNA levels ⩽500 copies/mL were recoded to 200 copies/mL.

Longitudinal analysis. In the analysis of patients before initiation of ART, patients were right censored at the time of initiation of ART or when lost to clinical follow-up. In the analysis of patients receiving ART, patients were right censored at the time that they initiated treatment with interleukin-2, stopped ART, or were lost to clinical follow-up. Repeated-mea-

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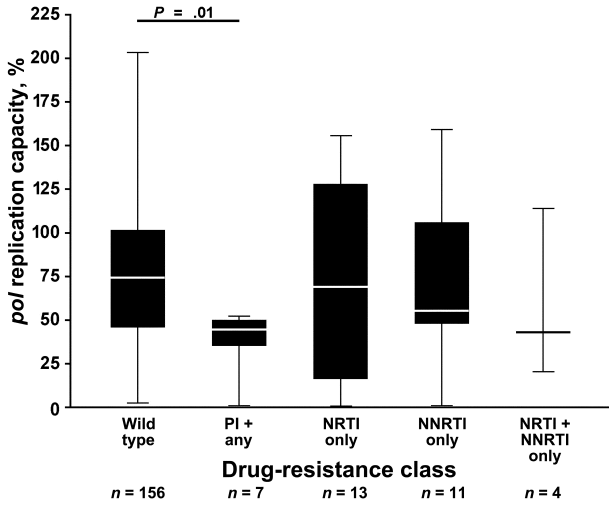

Figure 1. *pol* replication capacity (presented as percentage of the reference virus) at study entry, by resistance category. Individual patients were divided into mutually exclusive categories, according to the type of resistance that was detected. NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

sures, mixed-effects modeling [14, 15] was used to assess the relationship of study entry and longitudinal values to longitudinal outcome variables. A random effect for the individual was specified to account for variation at study entry (intercept).

RESULTS

Patient characteristics. *pol* RC data were available for 191 patients, composing the analysis cohort. Among this cohort, the median age of patients was 34 years (interquartile range [IQR], 30–39 years), and 177 patients (93%) were men, reflecting the epidemic of HIV-1 in San Francisco. For the cohort, the median $CD4^+$ T cell count was 519 cells/μL (IQR, 406–662 cells/μL; $n = 188$), the median plasma HIV-1 RNA level was 4.75 $\log_{10}$ copies/mL (IQR, 4.04–5.19 $\log_{10}$ copies/mL; $n = 188$), and the median LS-EIA standardized optical density [10, 11] was 0.19 (IQR, 0.02–0.48; $n = 184$). Drug resistance–associated mutations were identified in 35 viruses (18.6%) at study entry: 7 viruses (3.9%) had primary PI mutations, 22 (11.2%) had NRTI mutations, and 18 (10.3%) had NNRTI mutations.

Variation in* pol *RC and drug-resistance pattern at study entry. The median *pol* RC at study entry was 69% of the standard control (IQR, 43%–101%; $n = 191$). There was no difference in *pol* RC by approximate length of infection, as assessed by the 3 different study-entry eligibility categories, nor was there a relationship between *pol* RC and LS-EIA optical density in the range of LS-EIA optical density where there is a continuous relationship between optical density and duration of infection (LS-EIA > 0.5) [11].

pol RC was significantly lower for those patients infected with a virus with genotypic evidence of PI resistance than for those infected with a virus without genotypic evidence of drug resistance (figure 1; $P = .01$). Patients infected with a virus with genotypic evidence of resistance to other classes of drugs (NRTIs, NNRTIs, or both) tended to have lower *pol* RC, although they did not significantly differ from those infected with a virus without genotypic evidence of drug resistance (figure 1). Lower *pol* RC was associated with a greater number of primary PI-resistance mutations (table 1) but not with counts of NRTI- and NNRTI-resistance mutations. We assessed the proportion of variation in *pol* RC explained by resistance mutations, by use of partial correlation coefficients (type 1). These results indicated that 5.8% of the variation in *pol* RC was accounted for by the combination of terms. The relative contribution of each term was 3.6% for PI mutations, 0.5% for NRTI mutations, and 1.7% for NNRTI mutations. The remaining 94% variation in *pol* RC may be attributable to non-drug-resistance–associated sites or viral genetic interactions.

Relationship of* pol *RC with plasma HIV-1 RNA levels and $CD4^+$ T cell counts at study entry. At study entry, *pol* RC was not significantly associated with plasma HIV-1 RNA levels (Spearman's $\rho = 0.08$; $P = .25$). *pol* RC at study entry was significantly and negatively associated with $CD4^+$ T cell counts at study entry (Spearman's $\rho = -0.29$; $P < .0001$). An analysis of *pol* RC quartiles indicated that the association was primarily driven by higher $CD4^+$ T cell counts in the patients infected with a virus with a *pol* RC in the lowest quartile of the *pol* RC range (figure 2). Single-step regression-tree analysis was performed to determine the value in the continuous distribution of *pol* RC that best partitioned $CD4^+$ T cell count. The optimal break point occurred at a *pol* RC value of 42% (95% CI, 12%–93%), which coincides with the 25th percentile of *pol* RC (value of 43%) in this data set (figure 2). Those patients infected with a virus with a *pol* RC value ⩽42% had an average $CD4^+$ T cell count of 663 cells/μL, compared with a $CD4^+$ T cell count of 512 cells/μL for those infected with a virus with a *pol* RC value >42%. This result withstood cross-validation [12]. We performed a permutation test of the null hypothesis that there was no break point (i.e., the relationship between *pol* RC and $CD4^+$ T cell count is constant) and obtained a significant result ($P = .004$), indicating a

Table 1. Multivariate analysis of *pol* replication capacity and drug-resistance mutations at study entry.

Predictor variable	Estimate	SE	*P*
Primary PI mutation count	−29.1	10.3	.005
Primary NRTI mutation count	9.4	6.6	.15
Primary NNRTI mutation count	−14.2	8.3	.09
LS-EIA OD	−1.5	4.0	.70
HIV-1 RNA level at study entry, $\log_{10}$ copies/mL	4.9	3.6	.17

NOTE. LS-EIA OD, less-sensitive EIA optical density; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

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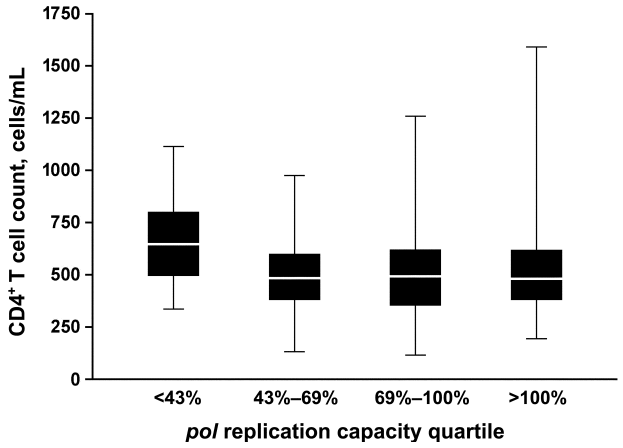

Figure 2. CD4+ T cell counts at study entry, by replication capacity

high likelihood for the existence and value of the identified break point at a *pol* RC value of 42%. All of these inferences and significance trends were maintained when patients infected with a virus with genotypic evidence of drug resistance were excluded from the analysis.

We adopted the 25th percentile value of *pol* RC as a threshold value when performing further assessments of the effect of *pol* RC on immunologic and virologic outcomes. In a multivariable analysis, a *pol* RC value ⩽43% was independently associated with higher CD4+ T cell counts, compared with the higher RC stratum (table 2).

Study-entry* pol *RC predictive of CD4+ T cell count over time in the absence of ART. Of the 191 patients studied at study entry, 65 patients were followed for at least 30 days before initiation of ART. These patients were followed for a median of 447 days (IQR, 112–764 days), or a total of 87.6 person-years. Over time, patients infected with a virus with a *pol* RC value >43% at study entry had lower CD4+ T cell counts over time (137.3 fewer CD4+ T cells/μL; $P = .03$). Adjusting for HIV-1 RNA levels did not alter the effect of *pol* RC on CD4+ T cell counts over time. These inferences and significance trends were not altered when patients infected with a virus with primary drug resistance were excluded from the analysis.

Study-entry* pol *RC predictive of CD4+ T cell count after initiation of therapy. Of the 191 patients, 122 (64%) elected to receive combination ART, after consultation with their clinicians. Of these, 114 could be followed over time and were observed for a median of 333 days (IQR, 114–569 days), or a total of 134.1 person-years. Among those with a *pol* RC value >43% at study entry, CD4+ T cell counts remained significantly lower than among those infected with a virus with a low *pol* RC (average, 99 fewer CD4+ T cells/μL; $P = .04$; figure 3). In this same model, poorer virologic responses to ART were independently associated with lower CD4+ T cell counts (average, 68.5 fewer CD4+ T cells/1 $\log_{10}$ increase in HIV-1 RNA copies/mL; $P < .0001$).

DISCUSSION

Among treatment-naive, recently HIV-1–infected adults infected with a virus with or without evidence of primary drug resistance, we observed a broad range of *pol* RC values. Only a small fraction (6%) of the broad range of variation in *pol* RC was attributable to drug-resistance mutations, suggesting that *pol* RC varies widely among wild-type *(wt)* viruses. Patients infected with a virus with a *pol* RC value ⩽43% of control had significantly higher CD4+ T cell counts at study entry than those infected with a virus with a high *pol* RC. This effect was independent of the influence of HIV-1 RNA levels, duration of infection, and presence of drug resistance. The higher CD4+ T cell counts among patients infected with a virus with a low *pol* RC at study entry persisted over time among patients who had not yet received treatment and among those who subsequently elected to receive treatment. In our limited follow-up, we did not detect a difference in rate of change of CD4+ T cell count by *pol* RC value. Each of these inferences remained when patients infected with a virus with primary drug resistance were excluded from the analysis. The *pol* RC of drug-sensitive, *wt* HIV-1 may vary significantly across adults in early HIV-1 infection, and low *pol* RC may be associated with diminished in vivo virulence.

pol RC varied widely in the viruses from recently infected patients, even after excluding viruses with genotypic evidence of drug resistance. Wide variation in *pol* RC has been observed in other settings as well. For example, analysis of a random sample of 500 specimens referred for clinical testing and having viruses with no genotypic evidence of drug resistance indicated a median *pol* RC of 99% (IQR, 62%–130%; N. T. Parkin, personal communication). The wide range of *pol* RC may partially reflect assay variation or incompatibilities between the patient-derived protease and RT and the Gag proteins derived from the test vector. Our finding that *pol* RC correlates with CD4+ T cell counts in vivo suggests that variation in *pol* RC has de-

Table 2. Multivariate analysis of CD4+ T cell counts and *pol* replication capacity (RC) at study entry.

Predictor variable	Estimate	SE	*P*
pol RC >43%	−121.5	36.4	.001
Primary PI mutation count	10.2	48.9	.8
Primary NRTI mutation count	26.9	31.3	.4
Primary NNRTI mutation count	23.1	39.6	.6
LS-EIA OD	−25.1	19.0	.2
HIV-1 RNA level at study entry, $\log_{10}$ copies/mL	−88.7	17.2	<.0001

NOTE. LS-EIA OD, less-sensitive EIA optical density; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

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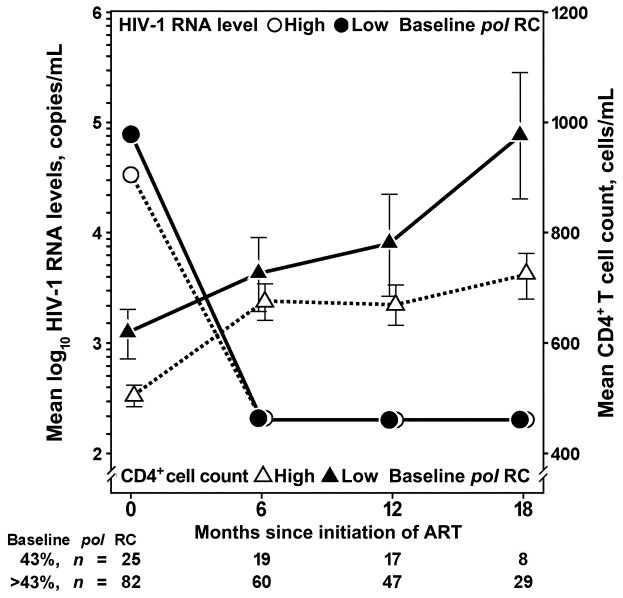

Figure 3. Greater CD4+ T cell counts during antiretroviral therapy (ART) among the group with low replication capacity (RC) at study entry.

tectable clinical relevance, despite assay variation. In addition, there are determinants of viral fitness that are not interrogated in the *pol* RC assay, including *env*-mediated fusion capacity, immunological escape, or small effective viral population sizes during transmission, each of which may allow fixation of deleterious alleles.

Among untreated patients with chronic viremia, the absolute HIV-1 RNA level predicts rates of loss of CD4+ T cells and long-term risk of clinical disease progression [16]. Among patients with chronic viremia despite receiving continuous, potent ART, the change in virus load from pretreatment baseline, rather than absolute level of virus load, is the better predictor of the rate of loss of CD4+ T cells [2]. Impairments in *pol* RC due to drug-resistance mutations have been associated with the degree of change in plasma HIV-1 RNA level that occurs when starting or stopping ART [3, 4]. Long-term sparing of CD4+ T cell counts has been observed among patients with highly drug-resistant viruses, with *pol* RC values durably lowered to <40% (22% on previous *pol* RC scale) despite substantial levels of plasma HIV-1 RNA [3].

Thus, in independent populations of patients, including both untreated and treated patients [3, 4], in both early and chronic infection, we have observed higher CD4+ T cell counts among patients infected with a virus with a *pol* RC value ⩽43%. The *pol* RC break-point value of 43% may represent a threshold value below which HIV-1 is not able to sustain virus populations in tissues involved in T cell production or a point at which the viral replication in tissue is less damaging to the host. PI-resistant HIV-1 isolates with lowered *pol* RC have been found to replicate poorly in human thymus tissue and to markedly spare thymocytes in explant cultures and SCID/hu thy/liv mice [17]. However, substantial levels of replication occurred in activated peripheral blood mononuclear cells that are highly permissive for HIV-1 [18, 19]. Hence, deficits in *pol* RC may be magnified in tissues that are less permissive for infection, thereby restricting the host tissue range to cells that contribute substantially to plasma HIV-1 RNA levels but are not required for regenerating the T cell pool. This would be reflected in higher circulating levels of CD4+ T cells among those patients infected with a virus with a low *pol* RC, despite substantial levels of plasma HIV-1 RNA, as we have observed in the present study of treatment-naive individuals and in prior studies of drug-experienced individuals [1, 3, 4, 20, 21].

The number of mutations associated with PI resistance was independently associated with diminished *pol* RC, whereas the numbers of mutations associated with NRTI and NNRTI resistance were not significantly associated with *pol* RC. There are multiple polymorphic sites in the patient-derived *gag/pol* gene segments that do not affect drug resistance but may determine *pol* RC in this assay. Several patient-derived viral *gag* cleavage sites, including the p6 cleavage site, are included in the *pol* RC construct. Mismatches between the patient-derived viral protease specificity and *gag* cleavage sites not included in the construct may influence *pol* RC assay values [22]. We are pursuing the genetic basis of the wide variation in *pol* RC among genotypically *wt* viruses in separate analyses and have presented preliminary results [23].

The finding that variation in *pol* RC among *wt* viruses is associated with CD4+ T cell counts before and during effective ART has broad implications for our understanding of the pathogenesis of HIV-1 and other viral diseases. The basic mechanisms that link low *pol* RC with preservation and expansion of circulating CD4+ T cell counts in HIV-1–infected individuals have not yet been fully elucidated. Further clinical research will be needed to determine how to use *pol* RC in decisions on when to stop or start ART.

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