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BRIEF REPORT

Continued Slow Decay of the Residual Plasma Viremia Level in HIV-1–Infected Adults Receiving Long-term Antiretroviral Therapy

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We measured plasma human immunodeficiency virus type 1 (HIV-1) RNA levels by means of single-copy assay in 334 participants receiving virologically suppressive antiretroviral therapy (ART). A residual viremia load of ≥1 copy/mL after 4 years of ART was predicted by a higher pre-ART HIV-1 RNA level, higher $CD8^+$ T-cell count during treatment, and a lower ratio of CD4+ T cells to CD8+ T cells during treatment but not by initial ART regimen. In a longitudinal subset of 64 individuals, continued decay of the plasma HIV-1 RNA level was observed, with an average annual decrease of 6% and an estimated half-life of 11.5 years. In contrast to prior reports, the persistent viremia level continues to slowly decline during years 4–12 of suppressive ART.

Clinical Trials Registration: NCT00001137.

Keywords. HIV/AIDS; residual viremia; viral decay; single-copy assay; CD4/CD8 ratio.

Despite long-term suppression of the human immunodeficiency virus type 1 (HIV-1) RNA level below the reportable limit for Food and Drug Administration (FDA)–approved assays (20–75 copies/mL), antiretroviral therapy (ART) does not eradicate HIV-1 infection, because of the persistence of infected long-lived cells. The decay half-life of latently infected memory CD4+ T-cells has been estimated to be >40 months, requiring >70 years of ART to eliminate them [1, 2].

Studies have shown that the majority of patients receiving ART with an HIV-1 RNA level of <50 copies/mL have residual viremia when tested with a quantitative polymerase chain reaction (qPCR) assay with single-copy HIV-1 RNA sensitivity [3–5]. The source of this virus is likely to be long-lived cells that continually or intermittently express virus that, in most patients, does not result in ongoing rounds of replication [6]. Prior modeling of the decay in viremia level from week 60 to week 384 of suppressive ART revealed a biphasic pattern consisting of an initial phase in which decay was observed (half-life, 39 weeks), followed by a plateau phase during which no significant decline in the viremia level was detected, suggesting a half-life of >7 years [4]. The level of persistent viremia has been correlated with the pre-ART HIV-1 RNA level [3, 4], but other correlates of residual viremia have yet to be identified. In the current study, we sought other predictors of persistent viremia and assessed whether the viremia level decayed in a large cohort of patients receiving virologically suppressive ART for up to 12 years.

MATERIALS AND METHODS

Ethics Statement

The AIDS Clinical Trials Group (ACTG) Longitudinal Linked Randomized Trials (ALLRT) study was a longitudinal cohort study that prospectively enrolled HIV-1–infected participants who were participating in randomized ART trials between 1998 and 2011 [7]. An institutional review board at each site approved the study, and all participants provided written informed consent.

Study Participants

ALLRT participants meeting the following criteria were identified for this study: initiation of first ART within an ACTG clinical trial (Supplementary Table 1), plasma HIV-1 RNA level of <50 copies/mL from week 32 of ART until the last available measurement, and an available cryopreserved plasma specimen (volume, at least 3 mL) at weeks 192 and 208 of ART. A subset of these individuals also had additional plasma samples available for testing at approximately 7, 10, and 12 years after ART initiation.

Single-Copy HIV-1 RNA Assay

Plasma samples were obtained from ethylenediaminetetraacetic acid–anticoagulated blood that was collected and processed at participating ACTG clinical sites between 1998 and 2011; all plasma samples were stored at −80°C before use in this study. Plasma samples were tested for HIV-1 RNA at a single laboratory between 2011 and 2013, using a single-copy qPCR assay (SCA) targeting HIV-1 gag. The method and performance characteristics of the SCA have been previously reported [3, 4]. For the current study, the limit of detection ranged from 0.4 to 1 copy/mL, depending on the plasma volume available. The amplification efficiency of HIV-1 RNA by SCA was

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determined by testing pretherapy plasma specimens obtained from participants who had an SCA result of < 1 copy/mL at both weeks 192 and 208. Participants with pretherapy samples showing inefficient HIV-1 RNA amplification (<10% amplification, compared with the Roche HIV Monitor assay v1.0 or 1.5) were excluded.

Statistical Analyses

Factors associated with a residual viremia level of ≥1 (vs <1) copy/mL at weeks 192 and 208 were assessed by odds ratios, using logistic regression for repeated measures (via generalized estimating equations). Each subject contributed an observation at week 192 and at week 208.

Longitudinal decay in the residual viremia level was evaluated with a random-effects regression model with first-order kinetics [2], using maximum likelihood methods and estimation specifically developed to accommodate longitudinal censored responses (ie, SCA findings of <1 copy/mL) [8]. The correlations between pre-ART HIV-1 RNA level and SCA-results at weeks 192 and 208 (all $\log_{10}$ transformed) were estimated by maximum likelihood, using similar censored-data methods [9].

RESULTS

Participant Characteristics

The study population was derived from 3172 ART-naive participants from 5 ACTG randomized clinical trials (Supplementary Table 1). Samples from 405 participants with sufficient available plasma specimens from weeks 192 and 208 were tested by SCA. Seventy-one were excluded because of inefficient amplification efficiency (including 11 with a missing pre-ART plasma specimen). The characteristics of the 334 final study participants at the time of ART initiation and after approximately 4 years of ART are provided in Supplementary Table 2. The majority of participants (82%) were male, with a median pre-ART age of 40 years, a median pre-ART $CD4^+$ T-cell count of 248 cells/mm^3, and an HIV-1 RNA level of 4.7 $\log_{10}$ copies/mL. Initial ART regimens were nonnucleoside reverse transcriptase inhibitors plus nucleoside reverse transcriptase inhibitors (NRTIs; 61%), protease inhibitors plus NRTIs (28%), and other regimens (11%). After 4 years of suppressive ART, the median $CD4^+$ T-cell count (average of weeks 192 and 208) increased to 588 cells/mm^3, and the ratio of $CD4^+$ T cells to $CD8^+$ T cells increased from 0.29 before ART initiation to 0.83 after ART initiation.

Predictors of Plasma Viremia Level After 4 Years of ART

The SCA results (668 samples from 334 participants) measured at weeks 192 and 208 of ART ranged from <0.4 to 41.4 copies/mL (median, <1 copy/mL; interquartile range, <1 to 2.5). The SCA result was ≥1 copy/mL at both week 192 and week 208 for 85 subjects (25%) and <1 copy/mL at both weeks for 123 subjects (37%); for the remaining 126 (38%), the SCA result was ≥1 copy/mL at either week 192 or week 208 and <1 copy/mL at the other week. SCA results at weeks 192 and 208 were significantly correlated ($r = 0.46$; $P < .001$). In univariate repeated-measures analysis, the likelihood of a residual viremia level of ≥1 copy/mL for the 668 samples tested was predicted by a higher pre-ART HIV-1 RNA level (odds ratio [OR], 1.79 per 1 $\log_{10}$ [95% confidence interval {CI}, 1.32–2.42]; $P < .001$), a higher $CD8^+$ T-cell count during treatment (median average of weeks 192 and 208, 724 cells/mm^3; OR, 1.07 per 100 cells/mm^3 [95% CI, 1.02, 1.12]; $P = .008$), and a lower ratio of $CD4^+$ T cells to $CD8^+$ T cells (median, 0.83; OR, 0.72 per 0.5 [95% CI, .58, .90]; $P = .004$). The initial ART regimen type, sex, race/ethnicity, injection drug use status, age, $CD4^+$ T-cell count before or after ART initiation, and pre-ART $CD8^+$ T-cell count or ratio of $CD4^+$ T cells to $CD8^+$ T cells were not associated with residual viremia (all $P > .05$; Table 1).

After adjustment for pre-ART HIV-1 RNA load, a higher average $CD8^+$ T-cell count and a lower ratio of $CD4^+$ T cells to $CD8^+$ T cells at weeks 192 and 208 remained significant predictors of a plasma HIV-1 RNA load of ≥1 copy/mL ($P = .014$ and $P = .031$, respectively). The pretherapy HIV-1 RNA load was significantly associated with week 192 and week 208 SCA results ($r = 0.18$ [$P = .004$] for week 192 and 0.20 [$P = .001$] for week 208).

Longitudinal Assessment of Residual Viremia

For 64 participants, SCA was performed on an additional 2–4 plasma specimens (median, 3 specimens) obtained at 2–3-year intervals after week 208 (Supplementary Table 3). In this longitudinal cohort, the median interval from the time of ART initiation to the time when plasma HIV-1 RNA was last measured by SCA was 11.2 years (minimum interval, 8.1 years). Participants with persistent viremia during year 4 (SCA result, ≥1 copy/mL at both week 192 and week 208) generally had a viremia level of ≥1 copy/mL at years 7 and 10 (65% and 56%, respectively); by contrast, participants with undetectable viremia during year 4 rarely had a viremia level of ≥1 copy/mL at years 7 and 10 (14% and 9%, respectively). The distribution of the plasma HIV-1 RNA load by year after ART initiation for all 64 participants is provided in Figure 1*A*. Using a random-effects repeated-measures regression model developed for censored assay data, there was evidence of a decay in SCA levels over time ($P = .023$), estimated to be 0.026 $\log_{10}$ per year. This decay corresponds to a 6% decline in the plasma HIV-1 RNA level per year and an estimated half-life of 11.5 years (95% CI, 6.2–83). The rate of decay was similar for the 27 subjects with a SCA result of ≥1 copy/mL at week 192 (Figure 1*B*).

DISCUSSION

In the large, cross-sectional component of this study, the presence of measurable residual viremia (≥1 copy/mL) after approximately 4 years of suppressive ART was predicted by a

Table 1. Predictors of a Residual Viremia Level of ≥1 copy/mL 192 and 208 Weeks After Antiretroviral Treatment (ART) Initiation

Predictor	Observations, No.[a]	Unadjusted		Adjusted[b]	
		OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Pre-ART HIV-1 RNA level (log_{10} copies/mL)	668	1.79 (1.32–2.42)	<.001		
Pre-ART $CD4^+$ T-cell count (per 100 cells/mm^3)	668	0.91 (.83–1.00)	.053	0.98 (.89–1.08)	.63
Pre-ART $CD8^+$ T-cell count (per 100 cells/mm^3)	668	1.01 (.98–1.05)	.45	1.02 (.99–1.06)	.21
Pre-ART ratio of $CD4^+$ to $CD8^+$ T cells (per 0.5 increase)	668	0.71 (.37–1.36)	.30	0.83 (.56–1.22)	.34
Age at parent study entry (per 10 y)	668	1.09 (.92–1.29)	.35	1.07 (.90–1.28)	.41
$CD4^+$ T-cell count during treatment[c] (per 100 cells/mm^3)	664	0.99 (.92–1.07)	.88	1.02 (.95–1.10)	.53
$CD8^+$ T-cell count during treatment[c] (per 100 cells/mm^3)	664	1.07 (1.02–1.12)	.008	1.06 (1.01–1.11)	.014
Ratio of $CD4^+$ to $CD8^+$ T cells during treatment[c] (per 0.5 increase)	664	0.72 (.58–.90)	.004	0.78 (.63–.98)	.031
Female sex (vs male sex)	668	0.70 (.44–1.11)	.13	0.69 (.44–1.10)	.12
Initial ART regimen	668				
PIs + NRTIs (vs NNRTIs + NRTIs)		1.16 (.79–1.71)	.45	1.30 (.88–1.92)	.19
Other (vs NNRTIs + NRTIs)		1.06 (.60–1.87)	.83	1.03 (.58–1.85)	.91
Race/ethnicity	668				
Hispanic (vs white non-Hispanic)		0.89 (.57–1.39)	.60	0.91 (.58–1.42)	.68
Black non-Hispanic (vs white)		0.79 (.52–1.20)	.26	0.83 (.54–1.28)	.41
Other (vs white)		1.17 (.31–4.39)	.82	1.21 (.31–4.84)	.78
Past history of injection drug use (vs no history)	668	1.08 (.56–2.07)	.82	1.13 (.59–2.18)	.71

Abbreviations: CI, confidence interval; HIV-1, human immunodeficiency virus type 1; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor.

[a] There were 2 values per participant, unless otherwise indicated.

[b] Analyses were adjusted for pre-ART HIV-1 RNA level.

[c] Data are for 332 participants (2 values per participant) and reflect the average of values at weeks 192 and 208.

higher pre-ART HIV-1 RNA level, a higher $CD8^+$ T-cell count during treatment, and a lower ratio of $CD4^+$ T cells to $CD8^+$ T cells during treatment. The pre-ART HIV-1 RNA level has previously been associated with the level of persistent viremia, but the $CD8^+$ T-cell count during treatment and a lower ratio of $CD4^+$ T cells to $CD8^+$ T cells during treatment have not, which provides new clues into the mechanisms underlying persistent viremia. Key factors that were not associated with persistent viremia in well-powered analyses were age, sex, race/ethnicity, ART regimen, pre-ART $CD4^+$ T-cell count, pre-ART $CD8^+$ T-cell count, and pre-ART ratio of $CD4^+$ T cells to $CD8^+$ T cells.

Importantly, in the longitudinal cohort followed for a median of 11.2 years, a significant decay in the plasma HIV-1 RNA level was observed. This result provides the first evidence of slow but ongoing decay of the plasma viremia level after 4 years of suppressive ART. The decay in the viremia level occurred with an estimated half-life of 11.5 years and a wide CI (95% CI, 6.2–83). The statistical approach to assess decay in our study was robust and addressed the issue of assay censoring by use of longitudinal statistical models developed to address this issue [8]. The larger size and longer follow-up in the current study, compared with prior ones, is likely to have increased the power to detect decay. A more precise estimate of the decay rate for the plasma viremia level would require a larger sample size and longer-term follow up.

The estimate for the rate of ongoing decay of plasma viremia level demonstrated by our data was very small (0.026 log_{10} per year), indicating that the plasma viremia level measured by SCA is a valid end point to assess changes in viremia level after short-term experimental interventions to enhance viral expression from latent proviruses or to deplete HIV-1 reservoirs. The estimated half-life of 11.5 years for the plasma viremia level during suppressive ART is longer than the reported estimate of 3.7 years (95% CI, 2.3–9.5) for the half-life of the latent, infectious viral reservoir in resting $CD4^+$ T cells, but both estimates have broad and overlapping CIs [2].

The cellular sources and mechanisms of persistent viremia during long-term suppressive ART are still undefined. Ongoing viral replication is unlikely to be a common source of residual viremia, based on the lack of impact of ART intensification [10] and the lack of evolution in the rebounding virus population after ART interruption, compared with the pre-ART virus population [6]. Persistent viremia from clonal expansion of HIV-1–infected cells in individuals receiving suppressive ART has recently been described, but the frequency of this phenomenon needs to be better defined [11, 12].

In prior studies, no association has been demonstrated between the level of residual viremia and the degree of immune activation [10, 13]. In the current study, we demonstrated that a lower ratio of $CD4^+$ T cells to $CD8^+$ T cells during ART was associated with greater likelihood of persistent viremia. Serrano-Villar et al have shown that a lower ratio of $CD4^+$ T cells to $CD8^+$ T cells during effective ART was associated with higher immune activation (ie, an increased percentage of $CD38^+DR^+$

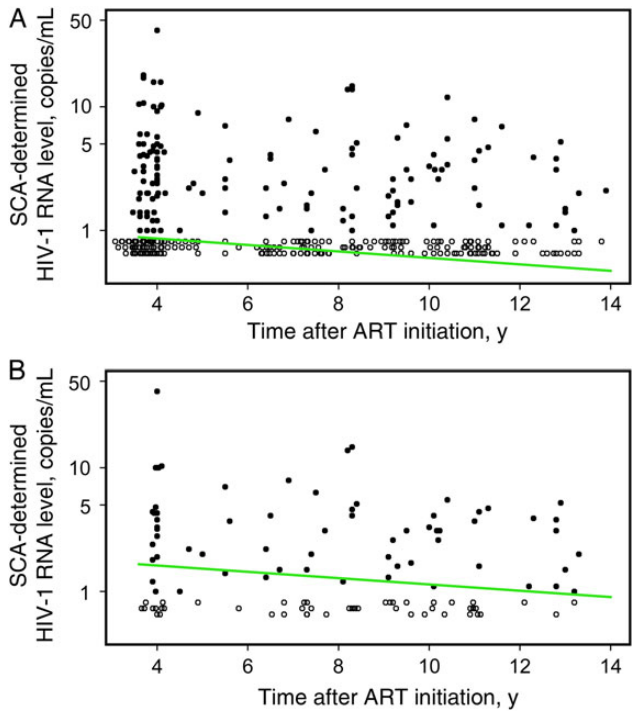

Figure 1. Distribution of human immunodeficiency virus type 1 (HIV-1) RNA levels determined by a single-copy assay (SCA) for the longitudinal cohort of 64 individuals, by time after initiation of suppressive antiretroviral therapy (ART). *A*, SCA findings for all participants. *B*, SCA findings for the subset of 27 participants with an HIV-1 RNA load of ≥1 copy/mL at the 4-year (192-week) time point after ART initiation.

$CD8^+$ T cells) and immune senescence (ie, an increased number of $CD57^+CD28^-$ T cells) [14]. Whether residual viremia is a cause or consequence of a lower ratio of $CD4^+$ T cells to $CD8^+$ T cells requires further investigation, including analysis of interventions that affect residual viremia, T-cell reconstitution, or persistent immune activation.

This study has some limitations. The ART regimens used by the participants did not include integrase inhibitors or other more recently approved drugs. Additionally, the gag SCA used has higher rates of inefficient HIV-1 RNA amplification due to primer mismatch, compared with the recently optimized SCA targeting integrase [15]. However, individuals with inefficient amplification by gag SCA were, appropriately, excluded. Finally, the study is limited by the lack of simultaneous data on immune activation and systemic inflammation, and data on cellular reservoirs including blood and tissues were not available.

Nevertheless, this study shows that the persistent viremia level decays gradually over time and suggests that cells capable of expressing HIV-1 do not persist indefinitely. Interventions to accelerate the death of HIV-1–producing cells should be vigorously pursued, including monoclonal antibodies, therapeutic vaccinations, latency reversal agents, and immune checkpoint inhibitors, and should include measurement of the persistent viremia level as an indicator of response.

STUDY PERSONNEL AND SITES

The participating personnel (and sites) are as follows: Susan Koletar, MD, and Mark Hite, RN (Ohio State University); Linda Meixner, RN, and Edward Seefried, RN (University of California–San Diego); Jorge L Santana Bagur, MD, and Sigrid Perez, MD (Puerto Rico AIDS Clinical Trials Unit CRS); Connie Funk, RN, MPH, and Bartolo Santos, RN (University of Southern California); Vicki Bailey and Brenda Jackson (Vanderbilt University); Judith Feinberg, MD, and Michelle Saemann, RN, BSN (University of Cincinnati); Elizabeth Lindsey, RN, and Tamara James (University of Alabama–Birmingham); Catherine Kronk, BA, and Jonathan Oakes, BA (University of North Carolina-Chapel Hill); Mary Adams, RN, and Christine Hurley, RN (University of Rochester); Shelia Dunaway, MD, and Sheryl S. Storey, PA-C (University of Washington); Cornelius Van Dam, MD, and Kim Epperson, RN, BSN (Greensboro CRS); Pablo Tebas, MD, and Aleshia Thomas, BS, RN (University of Pennsylvania); Lisa Klevens, RN, BSN, and Sara Mattiucci, BS, RN, CCRP (University of Pittsburgh); Trisha Walton, RN, and Jane Baum, RN (Case Western Reserve University); Teresa Spitz, RN, and Debra DeMarco, RN, BSN (Washington University); Beverly E. Sha, MD, and Tondria Green, RN, BSN, ACRN (Rush University Medical Center); Cathi Basler and Christine Griesmer (University of Colorado); Judith A. Aberg, MD, and Michelle S Cespedes, MD, MS (New York University/Bellevue ACTU); Sandra Valle, PA-C, and Debbie Slamowitz, RN (Stanford University); Judith Currier, MD, and Vanessa Cajahuaringa (UCLA Care Center); Babafemi Taiwo, MBBS, and Donna McGregor, NP (Northwestern University); Princy Kumar, MD, and Joseph Timpone, MD (Georgetown University); Mary Albrecht, MD, and Andrea Kershaw, ANP (Beth Israel Deaconess); Paul Sax, MD, and Cheryl Keenan, RN, BC (Brigham and Women's Hospital); Eric Daar, MD, and Ruben Lopez, MD (Harbor-UCLA); Annie Luetkemeyer, MD, and Jay Dwyer, RN (University of California–San Francisco); Jerrold Ellner, MD, and Benjamin Linas, MD (Boston Medical Center); Valery Hughes, NP, and Luis Lopez-Detres, BA (Weill Cornell-Chelsea CRS); Traci Davis and Kim Whitely (MetroHealth Medical Center); and Melody Palmore, MD, and Clifford Gunthel, MD (Ponce de Leon Center).

Members of the A5276s study team, in addition to the authors, are as follows: Amy Mirand (data manager), Michael Klebert (field representative), Carrie Fry (laboratory data manager), and Joan Dragavon (laboratory technologist).

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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