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Research Paper

HIV DNA Set Point is Rapidly Established in Acute HIV Infection and Dramatically Reduced by Early ART☆,☆☆

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ABSTRACT

HIV DNA is a marker of HIV persistence that predicts HIV progression and remission, but its kinetics in early acute HIV infection (AHI) is poorly understood. We longitudinally measured the frequency of peripheral blood mononuclear cells harboring total and integrated HIV DNA in 19 untreated and 71 treated AHI participants, for whom 50 were in the earliest Fiebig I/II (HIV IgM −) stage, that is ≤2 weeks from infection. Without antiretroviral therapy (ART), HIV DNA peaked at 2 weeks after enrollment, reaching a set-point 2 weeks later with little change thereafter. There was a marked divergence of HIV DNA values between the untreated and treated groups that occurred within the first 2 weeks of ART and increased with time. ART reduced total HIV DNA levels by 20-fold after 2 weeks and 316-fold after 3 years. Therefore, very early ART offers the opportunity to significantly reduce the frequency of cells harboring HIV DNA.

1. Introduction

HIV establishes reservoir within the human host early in infection, rapidly replicating and seeding peripheral blood mononuclear cell (PBMC) and tissue sanctuaries (Kulpa and Chomont, 2015). Frequencies of PBMCs that harbor HIV DNA are higher in later stages of acute HIV infection (AHI), suggesting that HIV reservoir seeding escalates with time (Ananworanich et al., 2013, Cheret et al., 2015). HIV reservoir size is pertinent to the goal of HIV remission (i.e. undetectable plasma viral load without treatment) because the size of the reservoir predicts time to viral load rebound after ART cessation (Williams et al., 2014, Li et al., 2016). It is hypothesized that people with a smaller reservoir size will have a greater chance of achieving HIV remission (Ananworanich and Fauci, 2015).

During early AHI, plasma viral RNA load rises exponentially and reaches a peak of over 6 logs in just two weeks from when it is first detected in the blood, This is followed by a sharp decline in viremia to a set-point level that is 2 logs lower 4 weeks later (Robb et al., 2016).

However, the kinetics of proviral DNA during early infection remains largely unknown. The earlier treatment is initiated in AHI, the lower the HIV reservoir size is after viral suppression (Ananworanich et al., 2012), but the extent of reductions compared to no treatment in AHI is not well documented.

Here we leverage our own well-characterized untreated and treated AHI cohorts to examine two questions: 1) what are the kinetics of peak and set-point of total and integrated HIV DNA and 2-LTR circles in PBMCs during untreated AHI? 2) what is the magnitude of difference in reservoir size with and without ART during AHI? As proviral DNA decays little in chronic HIV infection (Viard et al., 2004), identifying timing of DNA set-point will inform when to intervene. The magnitude of reservoir reduction may determine durability of HIV remission (Hill et al.,

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2014). Characterizing proviral reduction with early ART alone compared to no treatment will inform further interventions needed to achieve durable HIV remission (Martin and Siliciano, 2016).

2. Materials and Methods

The RV217 cohort enrolled individuals at high risk for HIV in Pattaya, Thailand who underwent twice-weekly HIV nucleic acid testing (NAT). Any NAT positive sample was confirmed with quantitative HIV RNA and serology (Robb et al., 2016). The 19 RV217 Thais were recruited from July 2009 to January 2012 and selected as they were enrolled in Fiebig stage I/II. Three additional Fiebig I/II participants were not included because they had insufficient PBMCs for analysis. The RV254 study (NCT00796146) enrolled individuals with non-reactive HIV IgG identified through routine NAT and serology in Bangkok (De Souza et al., 2015). Participants were offered immediate ART (NCT00796263). The median (IQR) time from enrollment to ART initiation in RV254 was 2 (1 to 3) days. This analysis includes the first 71 participants recruited from April 2009 to November 2012.

Fiebig stages were categorized according to Fiebig and Busch (2): Fiebig I/II: HIV RNA +, p24 antigen ±, HIV IgM − and Fiebig III/IV: HIV IgM +, Western Blot −/indeterminate. We estimated the duration from the advent of viremia for each individual in our two cohorts based on Fiebig et al. by assigning 10 days to those in Fiebig I/II and 14 days to those in Fiebig III/IV (Fiebig et al., 2003). Because RV254 had more Fiebig III/IV individuals than RV217, the estimated duration since the advent of viremia is 4 days longer in RV254 (Table 1). To account for this time gap between the two cohorts, 4 days was added to time from enrollment on the X axis in RV254 in Figs. 1 to 3, which resulted in an alignment of the peak viremia and proviral DNA between the two cohorts. Treatment in RV254 included 3-drug regimen (tenofovir, lamivudine or emtricitabine, and efavirenz) with 40/71 participants receiving additional raltegravir and maraviroc during the first 24 weeks (Ananworanich et al., 2015). All relevant ethics committees approved these studies and every participant provided informed consent. Samples and data were de-identified for analyses.

Total and integrated HIV DNA were measured in triplicates in PBMCs using a modified nested PCR assay for CRF01_AE and B (Vandergeeten et al., 2014), and the levels were $\log_{10}$ transformed prior to analysis. Differences between groups were assessed using two-tailed Student's *t*-test. Generalized Estimating Equations were used to assess factors associated with proviral burden. The model was constructed using an exchangeable correlation matrix. Analyses were performed using StataCorp 2013 (StataCorp LP, College Station, TX). Figures were generated with Prism version 6.02 for Windows (GraphPad Software, La Jolla, CA).

Table 1
Characteristics at enrollment of RV217 untreated and RV254 treated acute HIV infection participants.

Characteristics	RV217 (untreated acute HIV)	RV254 (treated acute HIV)	p
N	19	71	
Age, mean (SD)	24 (4.9)	29 (7.1)	0.001
Gender, n (%)			<0.001
Male	10 (53)	65 (92)	
Female	1 (5)	6 (8)	
Transgender	8 (42)	–	
Estimated duration from the advent of viremia			
Median (IQR)	10 (10 − 10)	14 (10–14)	0.001
Fiebig stage, n (%)			
I–II (RNA +, p24 Ag ±, HIV IgM −)	17 (89)	33 (46)	0.001
III–IV (HIV IgM +, Western Blot − or indeterminate)	2 (11)	38 (54)	
HIV-subtype			
CRF01_AE	17 (89.4)	59 (83.1)	0.34
B	1 (5.3)	3 (4.2)	
CRF01_AE/B recombinant	1 (5.3)	7 (9.9)	
Others	–	2 (2.8)	
CD4 T cells (cells/mm^3),	(n = 13)		
Mean (SD)	1027 (296)	425 (198)	<0.0001
Median (IQR)	993 (909–1193)	386 (293–532)	
HIV RNA ($\log_{10}$copies/ml),			
Mean (SD)	4.5 (2.0)	5.7 (1.1)	0.0007
Median (IQR)	4.1 (3.0–6.5)	5.7 (5.2–6.4)	
Total HIV DNA ($\log_{10}$copies/10^6 PBMCs),			
Mean (SD)	1.4 (1.5)	1.8 (1.3)	0.21
Median (IQR)	1.2 (0–2.3)	2.0 (0.8–2.9) (n = 69)	
Integrated HIV DNA ($\log_{10}$copies/10^{6}PBMCs),			
Mean (SD)	0.6 (1.1)	0.7 (1.0)	0.61
Median (IQR)	0 (0–0.8)	0 (0–1.7)	
2-LTR circles (log10copies/10^{6}PBMCs),			
Mean (SD)	0.4 (0.7)	1.2 (1.0)	0.005
Median (IQR)	0 (0–0.9)	1.2 (0–1.9)	

PBMCs: peripheral blood mononuclear cells.

3. Results

The RV217 untreated cohort started sampling at a median (IQR) of 1 (1–7) day from the first documented HIV RNA in the study (median of 13,183 copies/ml). The RV254 treated participants enrolled at a median (IQR) of 16 (12 − 21) days from the history of HIV exposure.

The majority were Men who have Sex with Men. The most common HIV clade was CRF01_AE. RV217 participants were younger, included transgender women and more were in Fiebig I/II; the latter resulted in shorter estimated duration from the advent of viremia, higher CD4 count and lower HIV RNA compared to the RV254 cohort. However, baseline total and integrated HIV DNA values were similar between groups.

The plasma HIV RNA kinetics in Fig. 1 illustrates the peak viremia at week 2 and set-point at week 4 from enrollment in the untreated group. In contrast, viremia declined rapidly after treatment initiation in RV254, and the proportions with HIV RNA < 50 copies/ml were 90% at week 24, 99% at week 48 and 97% at week 144.

The frequencies of PBMCs harboring total HIV DNA are shown in Fig. 2, which illustrates 3 important points 1) In the untreated RV217 cohort, the total HIV DNA values rose rapidly in the first 2 weeks after enrollment without significant changes thereafter. 2) In the RV254 treated cohort, the total HIV DNA values decreased over time reaching very low levels at week 144. 3) There was a marked divergence of total HIV DNA values between the untreated and treated cohorts that occurred early and this increased with time (Table 2).

The differences were 1.3 log or 20-fold at week 2 following enrollment, and 2.5 log or 316-fold by week 144. The integrated DNA values rose rapidly and peaked at week 2 followed by a significant decline at week 4 after which there was a gradual increase in integrated HIV DNA resulting in significantly higher integrated HIV DNA at week 144

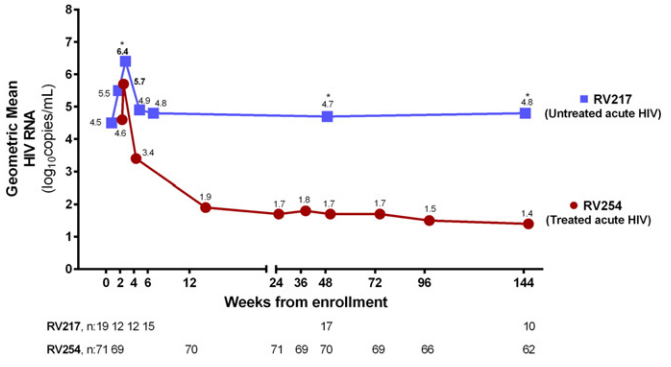

Fig. 1. Plasma HIV RNA of RV217 untreated and RV254 treated acute HIV infection participants. Footnote: The detection limit of HIV RNA was either 1.7 or 1.3 $\log_{10}$copies/ml.

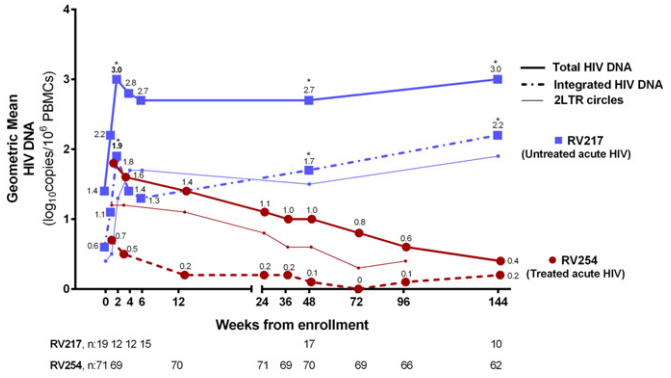

Fig. 2. Total and integrated HIV DNA and 2-LTR circles in peripheral blood mononuclear cells of Fiebig I to IV RV217 untreated and RV254 treated acute HIV infection participants. Footnote: PBMCs: peripheral blood mononuclear cells.

compared to week 4 ($p = 0.02$) (Fig. 2). By week 2, the treated cohort had 1.4 log or 25-fold lower integrated HIV DNA and by week 144, the difference was 2 log or 100-fold (Table 2). When only participants at the earliest AHI stages of Fiebig I/II were included (17 in RV217 and 33 in RV254), similar findings for total and integrated HIV DNA were observed as compared to the whole group (Fig. 3).

The frequency of cells harboring 2-LTR circles at baseline was higher in the RV254 group (Fig. 2 and Table 1) likely because of lower proportions of Fiebig I/II individuals in RV254 than in RV217. The baseline levels of 2-LTR circles did not differ between cohorts if only the Fiebig I/II participants were included: mean (SD) 0.7 (0.8) in RV254 vs. 0.4 (0.7) in RV217, $p = 0.19$ (Fig. 3). The levels subsequently declined significantly after 48 weeks of ART ($p < 0.0001$), whereas, in the untreated individuals, 2-LTR circles rose quickly between week 0 and week 2 ($p = 0.006$) and remained high throughout the follow up period (Fig. 2, Fig. 3 and Table 2).

As CD4 + T cell values differ significantly over time between the untreated vs. the treated groups, we also calculated the frequencies of CD4 + T cells that harbor total and integrated HIV DNA and 2-LTR circles using information of frequencies of CD4 + T cells from complete blood count (Supplemental Table 1). This resulted in greater differences between the treated and untreated cohorts with a 1585-fold difference in total HIV DNA and 501-fold difference in integrated HIV DNA observed at week 144.

Two baseline factors were strongly associated with total HIV DNA at week 144 in the multivariate analysis: Total HIV DNA (coeff 0.50, 95%CI 0.44 to 0.57, $p < 0.001$) and ART (coeff −1.39, 95%CI −1.14 to −1.64, $p < 0.001$). Similar relationships were observed for integrated HIV DNA at week 144: baseline integrated HIV DNA (coeff 0.57, 95%CI 0.47 to 0.68, $p < 0.001$) and ART (coeff −1.20, 95%CI −0.91 to −1.48, $p < 0.001$). HIV DNA outcomes did not differ for the 3- vs. 5-drug regimens. In the RV217 cohort, peak HIV RNA did not correlate with total HIV DNA at set-point (week 4) ($r = 0.17$, $p = 0.55$) or at week 48 ($r = 0.11$, $p = 0.68$).

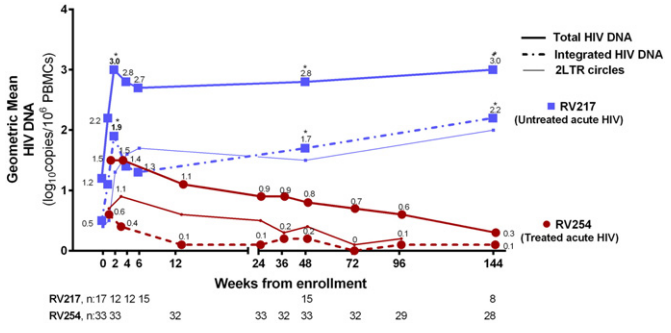

Fig. 3. Total and integrated HIV DNA and 2-LTR circles in peripheral blood mononuclear cells of Fiebig I/II RV217 untreated and RV254 treated acute HIV infection participants.

4. Discussion

This study compares proviral DNA in the earliest stages of AHI between untreated vs. treated individuals in two cohorts with similar gender, ethnicity, setting, HIV subtype and baseline total and integrated HIV DNA values, and with the same HIV DNA quantification methods (Vandergeeten et al., 2014). In the RV217 untreated cohort, the HIV DNA measurements performed within 4 to 6 weeks following the advent of viremia determined the reservoir size in the chronic stage. Therefore, the "HIV DNA set-point" is established very early and at the time of plasma HIV RNA set-point, at the conclusion of acute viremia (Robb et al., 2016). Stark differences in HIV DNA levels were observed between the treated and untreated cohorts, with a 20-fold lower total HIV DNA after 2 weeks of ART. As HIV DNA continues to decline in the treated group, it remains high in the untreated cohort, resulting in a 300-fold difference in total HIV DNA and a 100-fold difference in integrated HIV DNA after 3 years. Others have shown a marked decline in HIV DNA when ART was initiated by 15 days following infection but not later (Laanani et al., 2015). Continued HIV DNA reduction during long-term ART is observed when ART was initiated early (Buzon et al., 2014).

The rapidly-established pool of cells harboring HIV DNA in untreated AHI shows little decay over time (Cone et al., 1998) and may have long-term consequences. Indeed, HIV DNA levels before ART are correlated with post-treatment HIV DNA levels, residual viremia and immune activation (Groves et al., 2012, Jain et al., 2013). Higher HIV DNA also predicts HIV disease progression, independent of HIV RNA and CD4 count (Goujard et al., 2006, Rouzioux et al., 2005). Pre-treatment total HIV DNA predicted time to viral rebound when ART was interrupted (Williams et al., 2014), and post-treatment controllers maintained low HIV DNA after treatment interruption (Saez-Cirion et al., 2013). Generally reducing the frequencies of cells harboring HIV DNA with early ART may be critical in the efforts towards HIV remission. It must be noted, however, that failure to detect HIV DNA does not necessarily result in durable HIV remission. This was clearly exemplified in the Boston patients and the Mississippi child who had no detectable HIV DNA or other markers of active HIV reservoirs, they subsequently experienced resurgence of viremia when ART was removed (Henrich et al., 2014, Persaud and Luzuriaga, 2014). The best measures of HIV reservoirs that could predict HIV remission remain unknown (Siliciano and Siliciano, 2014, Eriksson et al., 2013). Additionally, the bulk of the HIV reservoirs may be in tissues particularly the lymph nodes (Lorenzo-Redondo et al., 2016, Rothenberger et al., 2015), which are more difficult to collect and often not measured in studies.

The lower plasma HIV RNA levels and frequencies of cells harboring 2-LTR circles at enrollment in RV217 were likely because of the higher proportion of people in the FIebig I/II stages (89% vs. 46% in RV254). Similar to plasma viremia, both total and integrated DNA peaked early at week 2 after enrollment in untreated AHI. The total HIV DNA showed a trend towards decay between weeks 2 and 4 from enrollment while this decline was significant for integrated HIV DNA. This could be from the preferential killing of productively infected cells during early AHI, as these cells harbor integrated HIV DNA (Laanani et al., 2015). Prolonged stable ART initiated in AHI resulted in a small frequency of cells harboring HIV DNA, mainly in its integrated form. In untreated infection, ongoing viral production continues, and both integrated HIV DNA and non-integrated HIV DNA accumulate, consistent with our data (Vandergeeten et al., 2014). Association with replication competent HIV appears stronger for integrated than total HIV DNA in the acutely treated (Eriksson et al., 2013) but the reverse is observed in chronically treated individuals (Kiselinova et al., 2016, Eriksson et al., 2013), which may be due to the high burden of mostly defective, non-integrated forms of DNA in the latter group (Vandergeeten et al., 2014).

Table 2
Differences in frequencies of peripheral blood mononuclear cells harboring total and integrated HIV DNA and 2-LTR circles between the RV217 untreated and RV254 treated acute HIV infection cohorts.

	RV217 untreated		RV254 treated		Mean log difference 95%CI (RV254-RV217)	p	Fold difference
	n	Mean (SD)	n	Mean (SD)			
Total HIV DNA (log_{10}copies/10^6PBMCs)							
Enrollment	19	1.4 (1.5)	71	1.8 (1.3)	0.4 (-0.2 to 1.1)	0.21	2.5
Week 2	12	3 (0.4)	69	1.6 (1.1)	−1.3 (−0.7 to −2.0)	0.0001	20
Week 48	17	2.7 (0.5)	70	1 (0.9)	−1.7 (−1.3 to −2.2)	<0.0001	50
Week 144	10	3 (0.6)	62	0.4 (0.7)	−2.5 (−2.1 to −3.0)	<0.0001	316
Integrated HIV DNA (log_{10}copies/10^6PBMCs)							
Enrollment	19	0.6 (1.1)	69	0.7 (1)	0.1 (−0.4 to 0.7)	0.61	1.3
Week 2	12	1.9 (0.5)	67	0.5 (0.8)	−1.4 (−1.0 to −1.9)	<0.0001	25
Week 48	17	1.7 (0.8)	69	0.1 (0.4)	−1.5 (−1.3 to −1.8)	<0.0001	32
Week 144	10	2.2 (0.6)	62	0.2 (0.5)	−2.0 (−1.7 to −2.3)	<0.0001	100
2-LTR circles (log_{10}copies/10^6PBMCs)							
Enrollment	17	0.4 (0.7)	70	1.2 (1.0)	0.8 (0.2 to 1.3)	0.005	6.3
Week 2	12	1.3 (0.8)	68	1.2 (1.1)	−0.1 (−0.7 to 0.6)	0.9	1.3
Week 48	16	1.5 (0.7)	66	0.6 (0.8)	−0.9 (-0.5 to -1.3)	0.0001	8
Week 144	9	1.5 (0.6)	ND	ND	ND	NA	NA

PBMCs: peripheral blood mononuclear cells, ND: not done, NA: not applicable.

Our study is limited by the small sample size and the lack of data on expressed reservoir markers (e.g. cell-associated HIV RNA), replication competent virus and tissue reservoirs that are relevant to HIV remission (Li et al., 2016). The longitudinal reservoir assessment in 50 Fiebig I/II is a major strength compared to past studies (Groves et al., 2012, Jain et al., 2013, Williams et al., 2014). Finally, the consequences of a small HIV reservoir size from early ART on clinical outcomes are not well understood.

In conclusion, the HIV DNA set-point is established early in AHI. Over three years without ART, persons with AHI carry a frequency of infected cells that is 300-fold higher than those on ART. As there are no strategies that could markedly reduce proviral burden, the opportunity to alter HIV DNA levels is with very early ART.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2016.07.024.

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The funders had no role in preparing this manuscript.

Conflict of Interest Statement

JA has received honoraria from ViiV Healthcare and Merck. Other authors declare no conflict of interest.

Author Contributions

JA and MLR designed and led the two clinical cohorts with input and contribution from EK, JLKF, ST, RC, NM and NP. NC, ST, MB, CV and MN designed and performed the reservoir testing. SP planned and conducted the statistical analysis. JA prepared the first draft of the manuscript and all authors contributed in the finalization of the work.

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