HIV DNA set point remains elevated in untreated vs. treated acutely infected Thais

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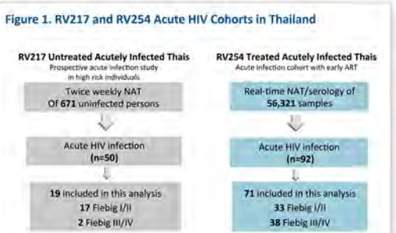
BACKGROUND

- The acute HIV infection (AHI) period usually spans the first month of infection when HIV serology is still non-reactive (1, 2). AHI individuals are rare due mainly to low HIV testing uptake and limitation of routine testing to diagnose AHI (3)
- The dynamic of HIV reservoir seeding in early AHI and the longitudinal comparison of reservoir markers in antiretroviral therapy (ART) untreated and treated AHI is poorly understood. Such knowledge could inform therapeutic strategies to mitigate the impact of HIV on the host (4)

METHODS

- This analysis included participants enrolled from 2 acute HIV infection cohorts in Thailand **(Figure 1)**

Figure 1. RV217 and RV254 Acute HIV Cohorts in Thailand



From June 2009 to June 2015, the RV217 cohort enrolled individuals at high risk for HIV at the ECHO clinic in Pattaya, Chonburi, who underwent twice-weekly HIV nucleic acid testing (NAT). NAT positivity was confirmed with HIV RNA and serology testing (5). Participants were followed frequently for phlebotomy and initiated ART according to standard of care at that time (CD4 < 350 cells/mm^3).

From April 2009 to November 2012, the RV254 cohort (NCT00796146) performed NAT and serology screening of individuals who sought HIV testing at the Thai Red Cross Anonymous Clinic in Bangkok (1). Individuals with non-reactive HIV IgG were enrolled, offered immediate ART (NCT00796263) and monitored (6).

- The Fiebig stages are categorized according to Fiebig and Busch (2) Fiebig I/II (positive HIV RNA, positive or negative p24 antigen and negative HIV IgM) Fiebig III/IV (positive HIV IgM, negative or indeterminate Western Blot)

The corresponding mean cumulative durations from onset of HIV viremia according to Fiebig et al. are 5 (Fiebig I), 10.3 (Fiebig II), 13.5 (Fiebig III) and 19.1 (Fiebig IV) days.

- Treatment in RV254 included standard doses of a 3-drug regimen (tenofovir, lamivudine or emtricitabine and efavirenz) with some participants receiving a 5-drug regimen with the addition of raltegravir and maraviroc during the first 24 weeks (7).
- The Thai Chulalongkorn University, the US Walter Reed Army Institute of Medical Research and all relevant US and Canadian institutional review boards approved these studies. All participants provided informed consent.

- Total and integrated HIV DNA were measured in the peripheral blood mononuclear cells (PBMCs) using a modified nested PCR assay for CRF01_AE and B (8).
- HIV DNA levels were log10 transformed prior to analysis. Differences between groups were assessed using two-tailed Student's *t* test. Generalized Estimating Equations (GEE) was used to assess factors associated with proviral burden. The model was constructed using exchangeable correlation matrix. Analyses were performed using StataCorp 2013 (StataCorp LP, College Station, TX). Figures were generated using Prism version 6.02 for Windows (GraphPad Software, La Jolla, California, USA).

RESULTS

Table1. Baseline characteristics 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)	-	
Duration since first detectable HIV RNA (days), Median (IQR)	1 (1 – 7)	-	
Duration since history of HIV exposure (days), Median (IQR)	-	10 (12 – 21)	
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			0.34
CRF01_AE	17 (89.4)	59 (83.1)	
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/mm3), Mean (SD) Median (IQR)	(n=13) 1027 (296) 993 (909 – 1193)	425 (198) 386 (293 – 532)	<0.0001
HIV RNA (log10copies/mL), Mean (SD) Median (IQR)A	4.5 (2.0) 4.1 (3.0 – 6.5)	5.7 (1.1) 5.7 (5.2 – 6.4)	0.0007
Total HIV DNA (log10copies/10Cells), Mean (SD) Median (IQR)	1.4 (1.5) 1.2 (0 – 2.3)	1.8 (1.1) 2.0 (0.8 – 2.9)	0.21
Integrated HIV DNA (log10Copies/10Cells), Mean (SD) Median (IQR)	0.6 (1.1) 0 (0 – 0.8)	(n=69) 0.7 (1.0) 0 (0 – 1.7)	0.81

- Individuals in the 2 cohorts were enrolled early following onset of infection
  RV217 untreated cohort: median of 1 day from the first detectable HIV RNA.
  RV254 treated cohort: median of 16 days from the history of HIV exposure. Median (IQR) time from enrollment to ART initiation was 2 (1 to 3) days. The proportions of individuals with HIV RNA < 50 copies/ml were 90% at week 24, 99% at week 48 and 97% at week 144
- The total and integrated HIV DNA values were similar between groups at baseline. The majority of men in both cohorts were Men who have Sex with Men. The most common HIV clade was CRF01_AE.
- However, the RV217 untreated participants were younger, more were transgender women and more were in Fiebig I/II; the latter resulting in higher CD4+ T cell count and lower HIV RNA compared to the RV254 treated cohort.

HIV DNA set-point is established early in untreated acutely infected individuals and proviral burden is significantly restricted by early ART

Figure 2: Total HIV DNA in PBMCs in RV217 Untreated vs. RV254 Treated Acutely HIV-Infected Thais

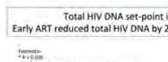


Figure 2A: Total HIF DNA in All participants

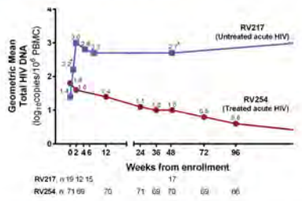
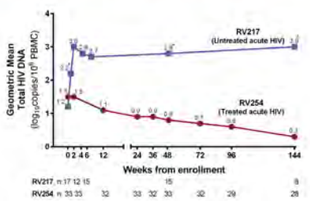


Figure 2B: Total HIV DNA in Fiebig I/II participants only



Frequencies of PBMCs harboring total HIV DNA values are shown in **Figure 2A**, which illustrates 3 important points

1) In the untreated RV217 cohort, the total HIV DNA values rose rapidly in the first 2 weeks reaching a set-point without significant changes thereafter.
2) In the RV254 treated cohorzt, the total HIV DNA values decreased over time reaching very low levels at week 144.
3) There is a marked divergent of HIV DNA values between the untreated and treated cohorts that occurred early **(Table 2)**. By week 2, there was a 1.3 log or 20-fold difference (p=0.0001) and by week 144, the difference was 2.5 log or 316-fold (p < 0.0001).

- Similar findings were observed in **Figure 2B** that included only individuals who were in the Fiebig I/II stages at enrollment.

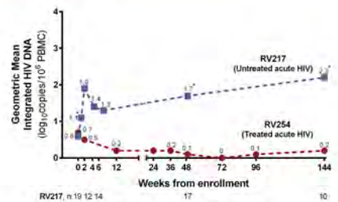
Table 2: Stark differences in frequencies of PBMCs harboring total and integrated HIV DNA between the RV217 untreated and RV254 treated acute HIV infection cohorts

	RV217 (Untreated Acute HIV)		RV254 Treated		Mean Log Difference 95%CI (RV254 - RV217)	p	Fold Difference
	n	Mean (SD)	n	Mean (SD)			
Total HIV DNA (log^{10}copies/ 10^6cells)							
Week 0	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/ 10^6cells)							
Week 0	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

footnote:
PBMCs: Peripheral Blood Mononuclear Cells

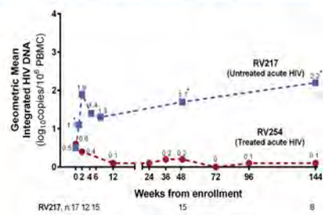
Figure 3: Integrated HIV DNA in PBMCs in RV217 Untreated vs. RV254 Treated Acutely HIV-Infected Thaists

Figure 3A: Integrated HIV DNA in all participants



- Frequencies of PBMCs harboring integrated HIV DNA are shown in all participants **(Figure 3A)** and participants in Fiebig I/II at enrollment **(Figure 3B)**. 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)**.

Figure 3B: Integrated HIV DNA in Fiegib I/II participants only



Factors affecting total HIV DNA levels

The multivariate analysis **(Table 3)** shows that proviral burden was significantly affected by treatment, and baseline CD4+ T cell count, HIV RNA level and total HIV DNA in PBMCs.

Table 3: Factors associated with frequencies of PBMCs harboring total HIV DNA (GEE model)

Factors at baseline	Univariate analysis		Multivariate analysis	
	Coefficient (95%CI)	p-value	Coefficient (95%CI)	p-value
Age	0.04 (-0.01 to -0.06)	0.004		
Gender				
Female	Ref			
Male	0.45 (-0.17 to 1.07)	0.15		
Transgender	1.44 (0.61 to 2.27)	0.001		
Fiebig stage	1 (1 – 7)			
I-II	Ref			
III-IV	-0.02 (-0.37 to 0.33)	0.90		
CD4 T cells (cells/uL) per 100 cells of cd4 increase	-0.29 (-0.23 to -0.34)	<0.001	-0.21 (-0.15 to – 0.27)	<0.001
HIV RNA (log10copies/mL)	0.43 (0.33 to 0.53)	<0.001	0.27 (0.15 to 0.40)	<0.001
Total HIV DNA in PBMC (log10copies/10Cells)	0.50 (0.44 to 0.57)	<0.001	0.33 (0.20 to 0.46)	<0.001
Treated				
No (RV217)	Ref.		Ref.	
Yes (RV254)	-1.14 (-0.71 to -1.55)	<0.001	-1.93 (-1.49 to -2.37)	<0.001

DISCUSSION

- This is among the first studies that compare proviral DNA in early AHI between untreated vs. treated individuals, with similar cohorts and DNA quantification methods (8).
- The HIV DNA levels at 2 to 4 weeks following the first HIV RNA detection in the RV217 untreated cohort determined the reservoir size in the chronic stage. This "HIV DNA set-point" appears to be established extremely early.
- Stark differences in HIV DNA levels were observed between cohorts, with a 20-fold lower total HIV DNA after 2 weeks of ART. As HIV DNA continues to decay in the treated group, the untreated cohort remained with high HIV DNA, resulting a difference of total HIV DNA of 300-fold after 3 years.
- The higher HIV DNA in untreated AHI may have long-term consequences as HIV DNA in PBMCs before ART is correlated with post-treatment HIV DNA levels, residual viremia and immune activation (6,7,9).
- In the SPARTAC trial, pre-treatment total PBMC HIV DNA predicted time to viral rebound when ART was removed (10). Therefore, lowering the frequencies of cells harboring HIV DNA with early ART may be critical in the efforts towards HIV remission.

CONCLUSION

- The **HIV DNA** set-point appears to be established early in AHI and determines the reservoir size in chronic infection.
- Over three years without ART, persons with AHI have total HIV DNA in PBMCs that is **300-fold** and integrated HIV DNA that is **100-fold** higher than those on ART.
- As there are currently no strategies that could markedly reduce proviral DNA burden, the opportunity to significantly alter HIV DNA levels is with very early ART.

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