EARLY ANTIRETROVIRAL THERAPY REDUCES BUT DOES NOT ELIMINATE HIV DNA IN BLOOD

00387

Trevor A. Crowell[1,2], Justin Ritz[3], Lu Zheng[3], Asma Naqvi[4], Joshua Cyktor[4], Scott F. Sieg[5], Javier R. Lama[6], James F. Rooney[7], Lawrence Fox[8], Jintanat Ananworanich[9], Joseph J. Eron[10], Gert U. van Zyl[11], John W. Mellors[4], and Eric S. Daar[12]
for the AIDS Clinical Trials Group (ACTG) A5354 Study Team

[1] U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, USA; [2] Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, USA; [3] Harvard T.H. Chan School of Public Health, Boston, MA, USA; [4] University of Pittsburgh, Pittsburgh, PA, USA; [5] Case Western Reserve University, Cleveland, OH, USA; [6] Asociación Civil Impacta Salud y Educación, Lima, Peru; [7] Gilead Sciences, Foster City, CA USA; [8] Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA; [9] University of Amsterdam, Amsterdam, The Netherlands; [10] University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [11] Stellenbosch University, Cape Town, South Africa; [12] Lundquist Institute at Harbor–UCLA Medical Center, Torrance, CA, USA

BACKGROUND

- ART initiation during acute or early HIV infection (AEHI) may limit HIV reservoir establishment, enhance reservoir decay, and limit viral genetic diversification, thereby facilitating HIV remission
- Clinical trials that included analytic treatment interruption in early-treated people living with HIV (PLWH) have mostly found that viral rebound occurred within weeks or months, regardless of any specific intervention tested
- Hypothesis: ART prior to HIV antibody detection (Fiebig I/II) would be associated with limited or no detectable proviral DNA after 48 weeks of ART

METHODS

- Adults with anticipated AEHI were enrolled into a single-arm, open-label clinical trial at 30 sites in the Americas, Africa, and Southeast Asia (**Table 1**)
- Participants initiated ART during AEHI, either with study-provided EVG/COBI/FTC/TAF or any other regimen
- Fiebig stage at ART initiation was retroactively assigned by centralized testing and categorized per protocol as Group 1 (Fiebig I/II), Group 2 (Fiebig III/IV) or Group 3 (Fiebig V)
- Participants were followed longitudinally with visits at weeks 1, 4, 12, 24, 36, and 48 to monitor plasma HIV RNA in real-time
- HIV DNA was measured at ART initiation (in 1 million purified CD4+ T cells) and at week 48 (in 5 million purified CD4+ T cells) by sensitive qPCR assays targeting HIV *gag* and *pol*
  - Two HIV DNA targets were used to increase the likelihood of detection around potential polymorphisms
  - Primary study endpoint was undetectable HIV DNA at week 48 of ART
  - HIV DNA was also evaluated on the continuous scale
- Peripheral blood mononuclear cells were stimulated with potential T cell epitope peptide pools and stained for expression of CD3, CD4 and CD8 and intracellular interferon-gamma

Table 1. Acute or Early HIV Diagnostic Criteria

	Criterion to Diagnose HIV Infection	Days from entry		Criterion to Establish Recency	Days from entry		Estimated Fiebig Stage
A	Detectable HIV RNA	<28	+	Non-reactive HIV antibody	<7	=	I/II
D	ARCHITECT or GSCOMBO S/CO ≥10	<7	+	Non-reactive HIV antibody	<7	=	
E	ARCHITECT or GSCOMBO S/CO ≥1 AND S/CO <0.5	<7 <90	+	Non-reactive HIV antibody	<7	=	
F	Detectable HIV RNA	<7	+	Non-reactive HIV antibody AND ARCHITECT or GSCOMBO S/CO 0.5-9.9	<7	=	
B	Detectable HIV RNA OR Reactive HIV antibody	<28	+	Negative/Indeterminate WB OR NEG./INDET. Geenius HIV Assay	<7	=	III/IV
C	Reactive HIV antibody + Positive WB, Negative for P31 band OR POS Geenius HIV Assay, NEG P31 band	<7	+	Non-reactive HIV antibody OR Negative HIV RNA	<90	=	V

Study eligibility was assessed using available test results to satisfy one of six study-specific criteria, each mapped to an estimated Fiebig Stage group

Sensitive and specific assays performed on a large number of CD4+ T cells detected HIV DNA in all participants after 48 weeks of ART regardless of Fiebig stage at ART initiation.

These findings may explain why rapid viral rebound has been observed after ART cessation in early-treated individuals with undetectable HIV DNA by less sensitive methods.

RESULTS

- From January 2017 to December 2019, 188 participants initiated ART during AEHI (**Table 2**)
- Compared to all other Fiebig stages at enrollment, Fiebig I participants had:
  - Lower pre-ART HIV *gag* DNA (all p<0.05) and marginally lower pre-ART HIV *pol* DNA (all p<0.10; **Table 3**)
- Among 154 participants with HIV RNA <50 copies/mL without protocol-defined ART interruption, 100% had detectable HIV *gag* or *pol* DNA at 48 weeks of ART with negative intra-assay controls (**Table 4**).
  - HIV *gag* and *pol* DNA measurements after 48 weeks of ART were correlated (rho=0.34, p<0.001)
- Participants who started ART during the earliest Groups and Fiebig stages had significantly lower HIV *gag* and *pol* DNA levels at 48 weeks (all trend tests p<0.001, **Figure 1**).
- Week 48 HIV DNA did not correlate with CD4+ or CD8+ T cell interferon-gamma responses to *env*, *gag*, *nef*, or *pol* peptide stimulation (rho range -0.11 to +0.15, all p>0.05).

Table 2. Study Population Characteristics, by Fiebig Stage

	Fiebig I (n=6)	Fiebig II (n=43)	Fiebig III (n=56)	Fiebig IV (n=23)	Fiebig V (n=60)
Age, median years (IQR)	24 (19-32)	26 (23-35)	27 (23-39)	33 (26-43)	26 (23-38)
Male	83%	93%	91%	70%	82%
Cisgender	100%	95%	91%	96%	100%
Race					
Black/African American	17%	42%	39%	57%	65%
White	0%	30%	52%	30%	27%
Asian	67%	19%	2%	0%	2%
Initial ART Regimen					
EVG/COBI/FTC/TAF	17%	70%	84%	78%	83%
DTG/3TC/TDF	83%	30%	11%	17%	13%
CD4, median cells/mm^3 (IQR)	491 (426-853)	322 (207-466)	383 (277-548)	396 (254-495)	490 (366-652)
HIV RNA, median log$_{10}$ cp/mL (IQR)	4.6 (4.2-4.7)	6.6 (5.8-7.0)	6.5 (6.1-7.0)	6.6 (5.8-7.0)	5.4 (5.0-6.4)

Table 3. Pre-ART HIV DNA (copies per million CD4+ T-cells)

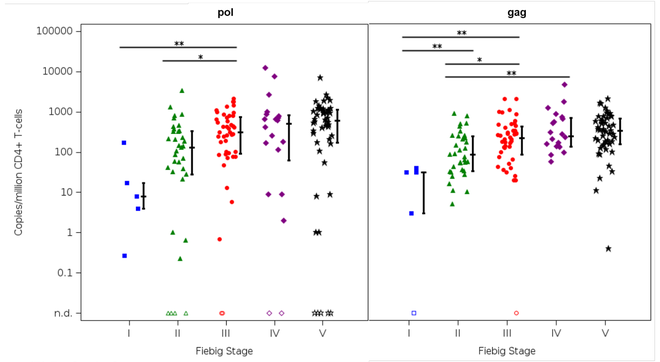
	Fiebig I (n=6)	Fiebig II (n=42)	Fiebig III (n=56)	Fiebig IV (n=22)	Fiebig V (n=60)
Pol, median (IQR)	100 (2-1375)	1719 (345-6005)	3203 (1003-6720)	2536 (26-8355)	2021 (267-5943)
Gag, median (IQR)	114 (28-233)	1235 (466-2637)	1207 (439-2853)	1994 (881-6261)	1078 (454-2588)

Table 4. Proportion (95% CI) with Undetectable Post-ART HIV DNA

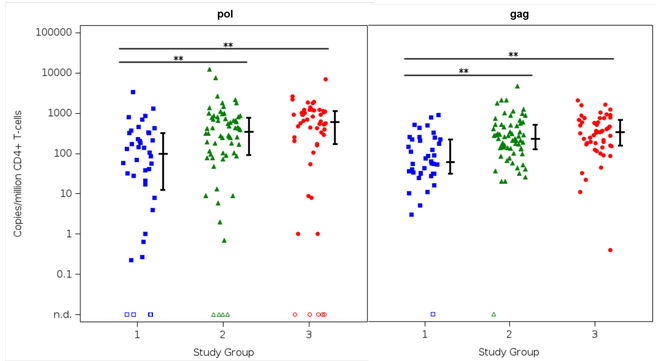
	Fiebig I (n=5)	Fiebig II (n=35)	Fiebig III (n=44)	Fiebig IV (n=20)	Fiebig V (n=50)
Overall	0 (0-0.52)	0 (0-0.10)	0 (0-0.08)	0 (0-0.17)	0 (0-0.07)
Pol	0 (0-0.52)	0.11 [n=4] (0.03-0.27)	0.05 [n=2] (0.01-0.15)	0.10 [n=2] (0.01-0.32)	0.10 [n=5] (0.03-0.22)
Gag	0.20 [n=1] (0.01-0.72)	0 (0-0.10)	0.02 [n=1] (0-0.12)	0 (0-0.17)	0 (0-0.07)

Figure 1. HIV DNA at 48 Weeks after Antiretroviral Therapy Initiation.

(A) By Fiebig Stage



(B) By Study Group



HIV DNA at 48 weeks was measured in 5 million purified CD4+ T cells. Undetectable HIV DNA measurements are indicated with open symbols. Two participants with undetectable HIV DNA by qPCR for *gag* at 48 weeks (1 in Fiebig I, 1 in Fiebig III; open symbols) were detectable by qPCR for *pol* (data not shown). Bars represent medians and interquartile ranges. Pairwise comparisons were done by Wilcoxon test (* < 0.05; ** < 0.01).

CONCLUSIONS

- ART initiation in earlier stages of AEHI reduced but did not eliminate the persistence of HIV-infected cells in blood
- In contrast to prior studies, sensitive and specific qPCR assays performed on a large number of CD4+ T cells detected HIV DNA in all participants after 48 weeks of ART regardless of Fiebig stage at ART initiation

ADDITIONAL INFORMATION

Funding: This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (grant numbers UM1 AI068634, UM1 AI068636, UM1 AI068618, UM1 AI106701 and P30 AI027757). Antiretroviral therapy for the study was donated by Gilead Sciences.
Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, the U.S. Army, the Department of Defense, or the Department of Health and Human Services. The investigators have adhered to the policies for protection of human subjects as prescribed in AR-70.
Acknowledgments: We would like to thank the study participants who made this research possible. The ACTG A5354 study was conducted at the following ACTG Clinical Research Sites: Alabama, Barranco, Brigham and Women's Hospital Therapeutics, Chapel Hill, Columbia Physicians and Surgeons, Cincinnati, Greensboro, Harbor-UCLA, Hospital Nossa Senhora da Conceição, Houston AIDS Research Team, Instituto de Pesquisa Clinica Evandro Chagas, Joint Clinical Research Centre/Kampala, Kenya Medical Research Institute/Walter Reed Project, Malawi, Massachusetts General Hospital, Milton Park, Miriam Hospital, New Jersey Medical School Clinical Research Center, Northwestern University, Ohio State University, Penn Therapeutics, Ponce de Leon Center, Rush University, San Miguel, Thai Red Cross AIDS Research Centre, Trinity Health and Wellness Center, UCLA CARE Center, UCSD Antiviral Research Center, University of Pittsburgh, University of Washington, Washington University Therapeutics, Weill Cornell Chelsea, Whitman-Walker Health.

Contact: Trevor A. Crowell, MD, PhD
6720-A Rockledge Drive, Suite 400
Bethesda, MD 20817
Office: +1 301-500-3990
E-mail: tcrowell@hivresearch.org