Earlier Initiation of ART Reduces Intact Proviruses but not Residual Viremia after 48 Weeks

514

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Background & Methods

The timing of antiretroviral therapy (ART) initiation during acute or early HIV-1 infection may affect the size of the latent and expressed HIV-1 reservoir but its specific influence on intact proviral DNA (IPD) or low-level viremia has not been fully described.

ACTG study **A5354** enrolled 192 adults who started ART during AEHI at 30 sites in the Americas, Africa, and Asia. Participants were retrospectively centrally categorized as:

Group 1 (Fiebig I/II) | **Group 2** (Fiebig III/IV) | **Group 3** (Fiebig V)

Measures of HIV-1 persistence were evaluated on **a subset of 106** US study participants or those with confirmed subtype B.

All participants had HIV-1 RNA <50 copies/mL at week 48 with **no ART interruption for** ≥7 consecutive days.

The **Intact Proviral DNA Assay (IPDA®)** was performed on CD4⁺ T cells isolated from peripheral blood at weeks 24, 48, and 72 after ART initiation.

Plasma HIV-1 RNA was measured in 5mL of EDTA plasma by automated **Single Copy Assay (SCA)** at week 48.

Wilcoxon Rank-Sum and Fisher's Exact Tests were used for pairwise comparisons between Groups and Jonckheere-Terpstra tests for trends across the Groups.

Table 1 Participant Characteristics

		Group 1 (Fiebig I-II) (N=20)	Group 2 (Fiebig III-IV) (N=51)	Group 3 (Fiebig V) (N=35)	Total (N=106)
Age (years)	Median (IQR)	26 (24, 34)	31 (24, 43)	28 (24, 39)	29 (24, 39)
	18-29	14 (70%)	22 (43%)	20 (57%)	56 (53%)
	30-39	3 (15%)	15 (29%)	8 (23%)	26 (25%)
	40-49	1 (5%)	6 (12%)	4 (11%)	11 (10%)
	50+	2 (10%)	8 (16%)	3 (9%)	13 (12%)
Sex	Female	1 (5%)	8 (16%)	2 (6%)	11 (10%)
	Male	19 (95%)	43 (84%)	33 (94%)	95 (90%)
Gender Identity	Cisgender	19 (95%)	48 (94%)	35 (100%)	102 (96%)
	Transgender Spectrum	0 (0%)	3 (6%)	0 (0%)	3 (3%)
	Not reported	1 (5%)	0 (0%)	0 (0%)	1 (1%)
Race	Asian	0 (0%)	0 (0%)	1 (3%)	1 (1%)
	Black or African American	11 (55%)	26 (54%)	21 (62%)	58 (57%)
	White	9 (45%)	22 (46%)	12 (35%)	43 (42%)
Ethnicity	Hispanic or Latino	7 (35%)	13 (25%)	8 (23%)	28 (26%)
	Not Hispanic or Latino	13 (65%)	38 (75%)	27 (77%)	78 (74%)
Initial ART Regimen	EVG/COBI/FTC/TAF	20 (100%)	48 (94%)	35 (100%)	103 (97%)
BMI (kg/m²)	Median (IQR)	23.1 (20.5, 25.3)	26.3 (21.6, 29.5)	23.6 (21.3, 30.6)	24.8 (21.4, 28.7)
	Min, Max	18.0, 32.2	17.1, 56.8	16.1, 48.0	16.1, 56.8
	Underweight (<18)	2 (10%)	5 (10%)	1 (3%)	8 (8%)
	Normal (18 - <25)	12 (60%)	16 (31%)	19 (54%)	47 (44%)
	Overweight (25 - <30)	3 (15%)	18 (35%)	6 (17%)	27 (25%)
	Obese (30+)	3 (15%)	12 (24%)	9 (26%)	24 (23%)

Figure 1 IPDA® Across Study Groups After 24, 48, or 72 Weeks on ART

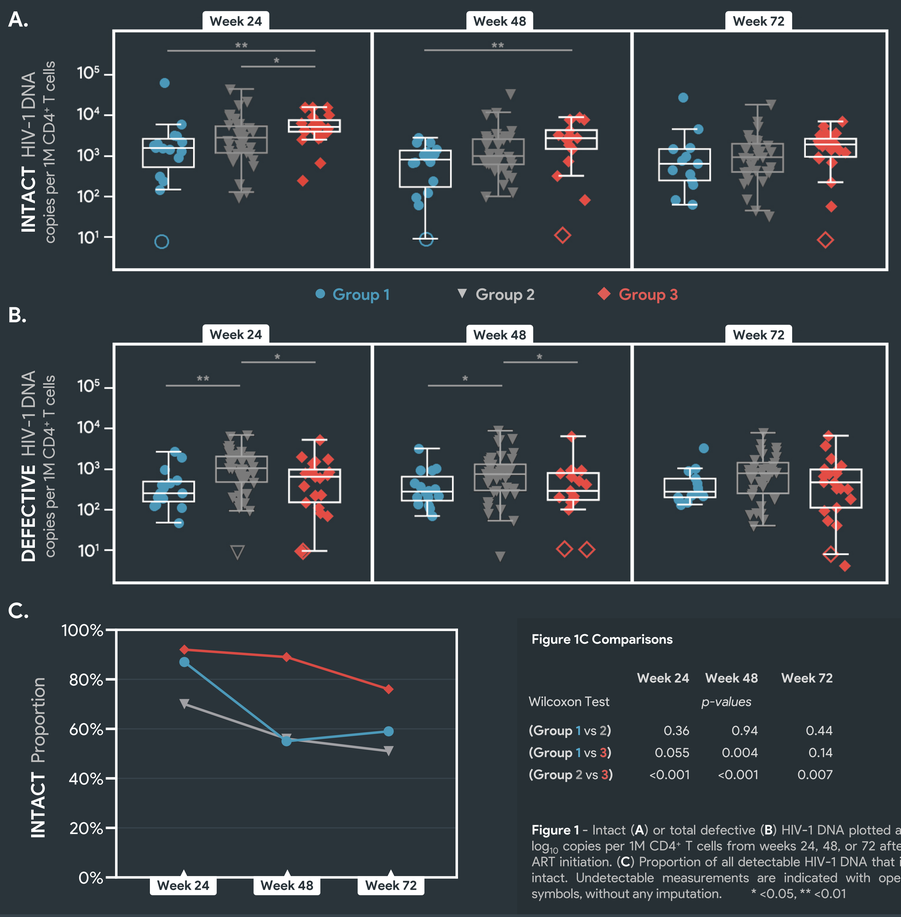

Figure 1C Comparisons

	Week 24	Week 48	Week 72
Wilcoxon Test	*p-values*		
(Group 1 vs 2)	0.36	0.94	0.44
(Group 1 vs 3)	0.055	0.004	0.14
(Group 2 vs 3)	<0.001	<0.001	0.007

Figure 1 - Intact (**A**) or total defective (**B**) HIV-1 DNA plotted as $\log_{10}$ copies per 1M CD4⁺ T cells from weeks 24, 48, or 72 after ART initiation. (**C**) Proportion of all detectable HIV-1 DNA that is intact. Undetectable measurements are indicated with open symbols, without any imputation. * <0.05, ** <0.01

Figure 2 Plasma HIV-1 RNA by **SCA** Across Study Groups at **Week 48**

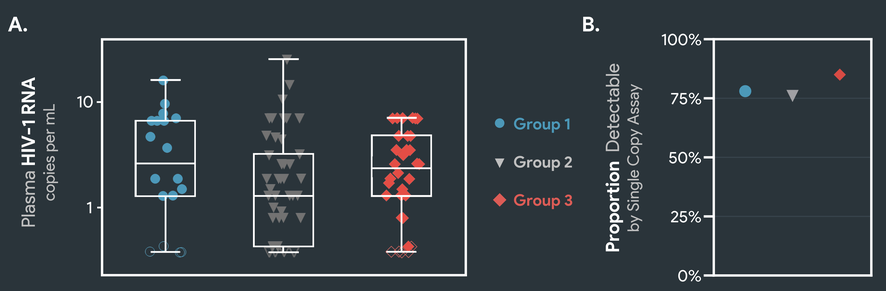

Figure 2 – Plasma HIV-1 RNA measured by **Automated SCA** at week 48 after ART initiation displayed as linear copy number (**A**) or categorical proportion detectable (**B**). Measurements reported as 'undetectable' are indicated with open symbols, without any imputation.

Results Summary

Participant Characteristics are described in **Table 1.**

Total HIV-1 DNA

- Participants in Group 1 (Fiebig I-II) had **lower levels** of total proviral DNA (IPD + defective DNA) than Groups 2 and 3 at weeks 24 and 48 ($p \leq 0.038$).
- This confirms earlier A5354 data showing total HIV-1 DNA by sensitive qPCR on 5M CD4⁺ T cells was lower with earlier ART initiation (**Crowell *et al*, in press, AIDS**).

Intact and Defective HIV-1 DNA

- Participants who started ART during the earliest study groups had significantly lower IPD levels at all three timepoints (trend tests $p \leq 0.033$) but with substantial variation and overlap in IPD copies per million CD4⁺ T cells (**Figure 1A, 1B**).
- No significant longitudinal trends were observed in the **decline of IPD** across Groups.
- The **proportion** of total proviruses that were intact varied across Groups but generally decreased over time while remaining highest in Group 3 (**Figure 1C**).

Plasma HIV-1 RNA SCA

- Persistent plasma HIV-1 RNA was detected by SCA in >75% of participants at week 48 with no significant differences in the proportions with detectable HIV-1 RNA (76-85%; $p \geq 0.41$) or the levels of HIV-1 RNA between pairwise groups ($p \geq 0.22$) (**Figure 2**).
- The magnitude of **IPD and plasma HIV-1 RNA** at week 48 were significantly but modestly correlated ($r=0.42$, $p<0.001$).

Conclusions & Acknowledgements

1. **Earlier ART lowers the levels of total and intact HIV-1 DNA** in Groups 1 and 2 (Fiebig I-IV) compared with Group 3 (Fiebig V), but **substantial variation** and overlap was observed between Groups.
2. Residual **plasma HIV-1 RNA** was detected in >75% of participants at week 48 with **no significant differences** in proportions or levels between study Groups.
3. These results provide new evidence that **earlier ART does not affect the active HIV-1 reservoir** measured by plasma HIV-1 RNA.

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