

Short-term ART Interruption has Little Effect on Levels of Integrated Proviral DNA

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Background

- Analytic treatment interruption (ATI) trials are necessary to assess strategies for sustained ART-free remission.
- The impact of ATI on reservoir size after resumption of ART is unclear.
- Levels of integrated HIV DNA can be used to evaluate the effect of interventions on the size of the proviral reservoir.

Objectives

- To validate a novel integrated HIV DNA assay, termed the HIV Integrated DNA size-exclusion (HIDE) assay
- To assess the impact of short-term ATI on the size of the total proviral reservoir

Methods

Assay Validation

- Genomic DNA size-selected for >20kb fragments by the automated BluePippin pulsed-field gel electrophoresis system to eliminate unintegrated DNA species¹.
- Proviral DNA levels were measured by qPCR and normalized to cellular input by CCR5 qPCR.
- HIV-negative DNA was spiked with either linear near-full-length HIV amplicons or a 12kb HIV-encoding plasmid to confirm elimination of unintegrated DNA.
- HIV DNA levels were assayed before and after size-selection in three replication-incompetent HIV infected cell lines: J-Lat, 8E5 and an integrated DNA standard for the Alu-gag assay.
- HIV-infected cell lines and participant samples were measured by the standard Alu-gag assay for comparison.

Study Samples

- Cryopreserved PBMCs were obtained from 12 participants from 4 previously completed ACTG ATI trials.
- Participants samples were obtained prior to ATI, during ATI and a median of 27 weeks after ART reinitiation and assayed for levels of integrated HIV DNA as described above.

Table 1: Median Participant Characteristics

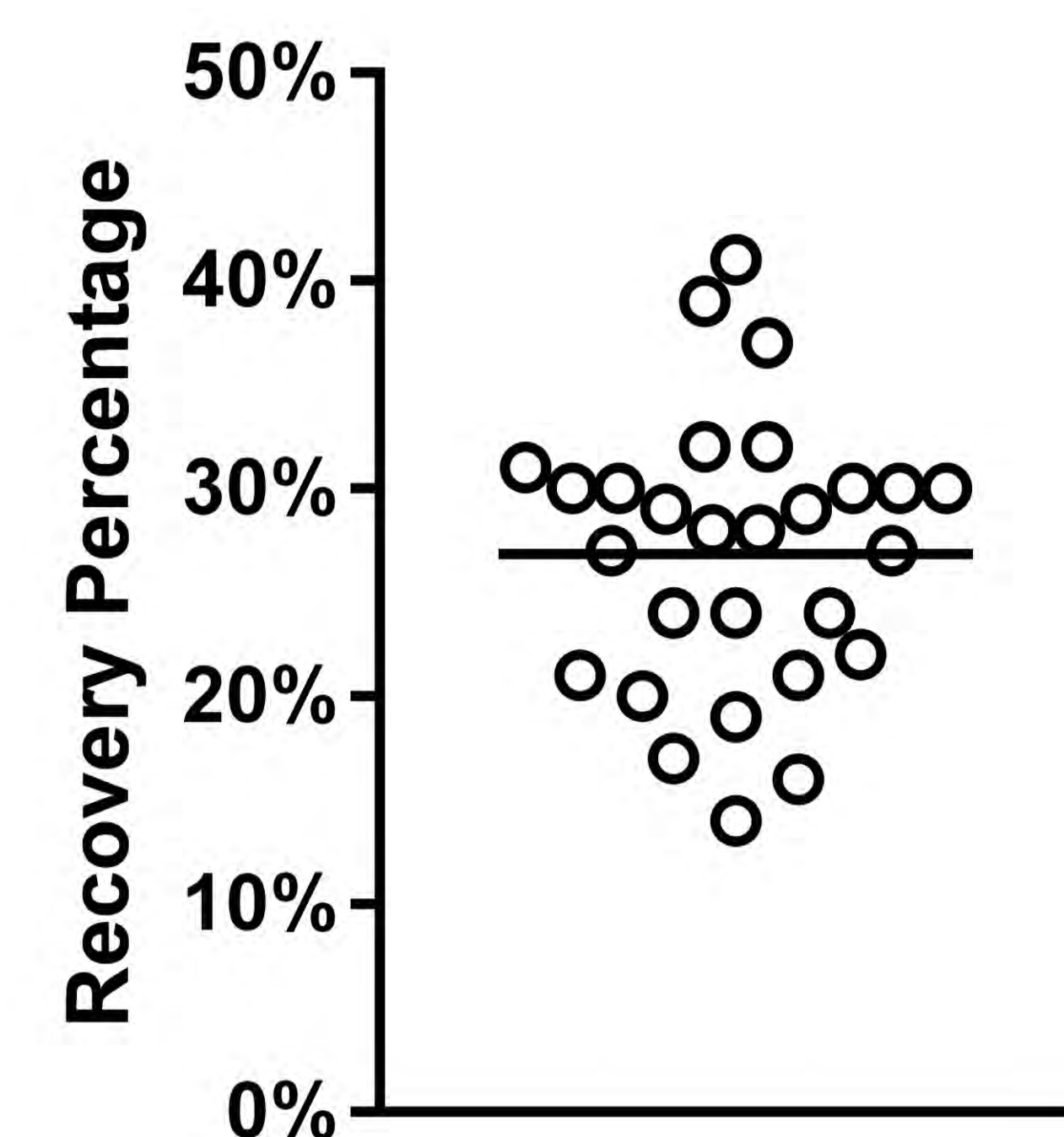
ART duration prior to ATI (years)	ATI Duration (weeks)	ART duration post-ATI (weeks)	Plasma viral load prior to ATI (cp/mL)	CD4+ count prior to ATI (per mm ³)
3.9	12	27	<50	852

Statistical Analysis

- Wilcoxon matched-pairs signed rank test was used in analysis of pre- and post-ATI integrated DNA levels.

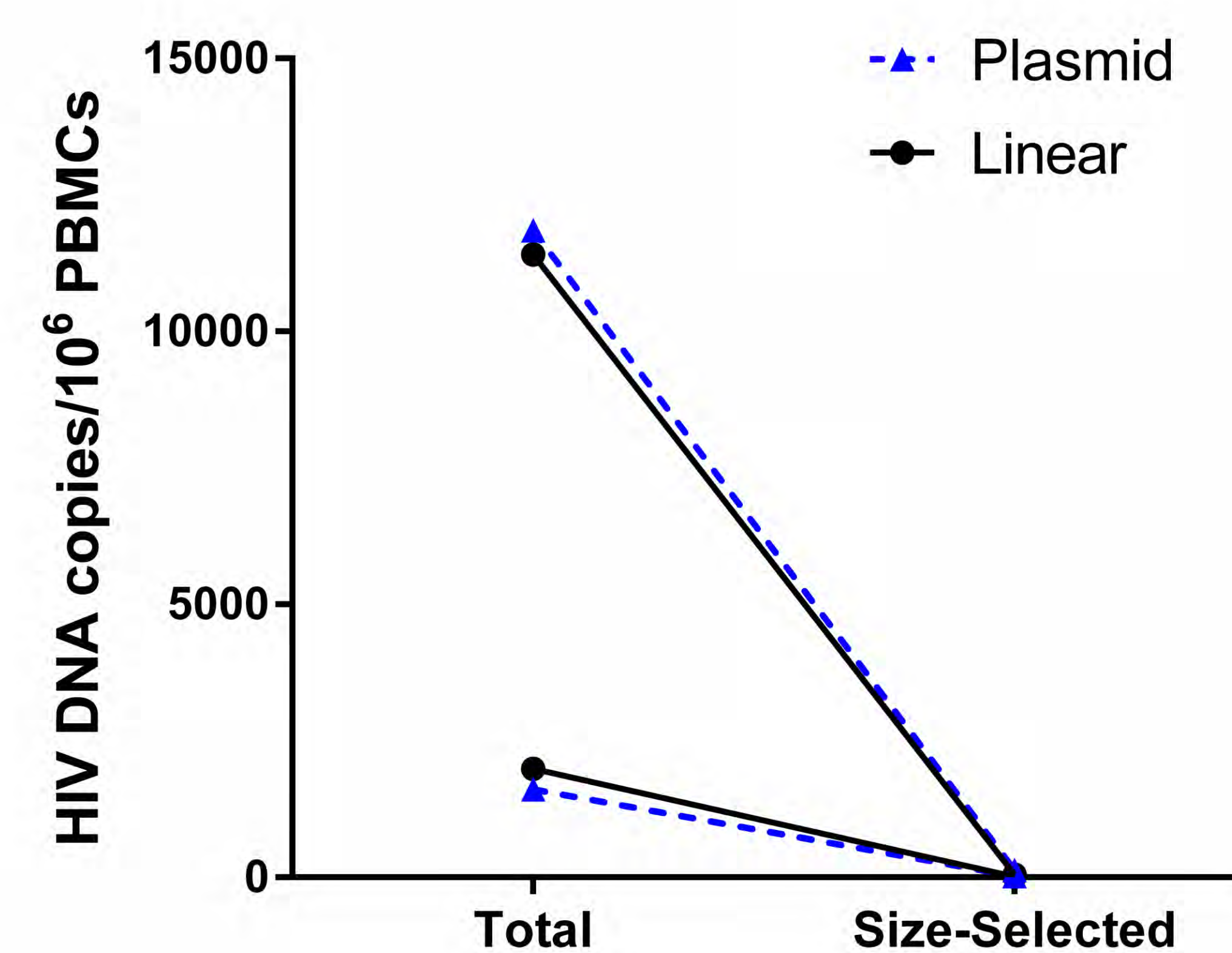
Results

BluePippin Recovery Rate



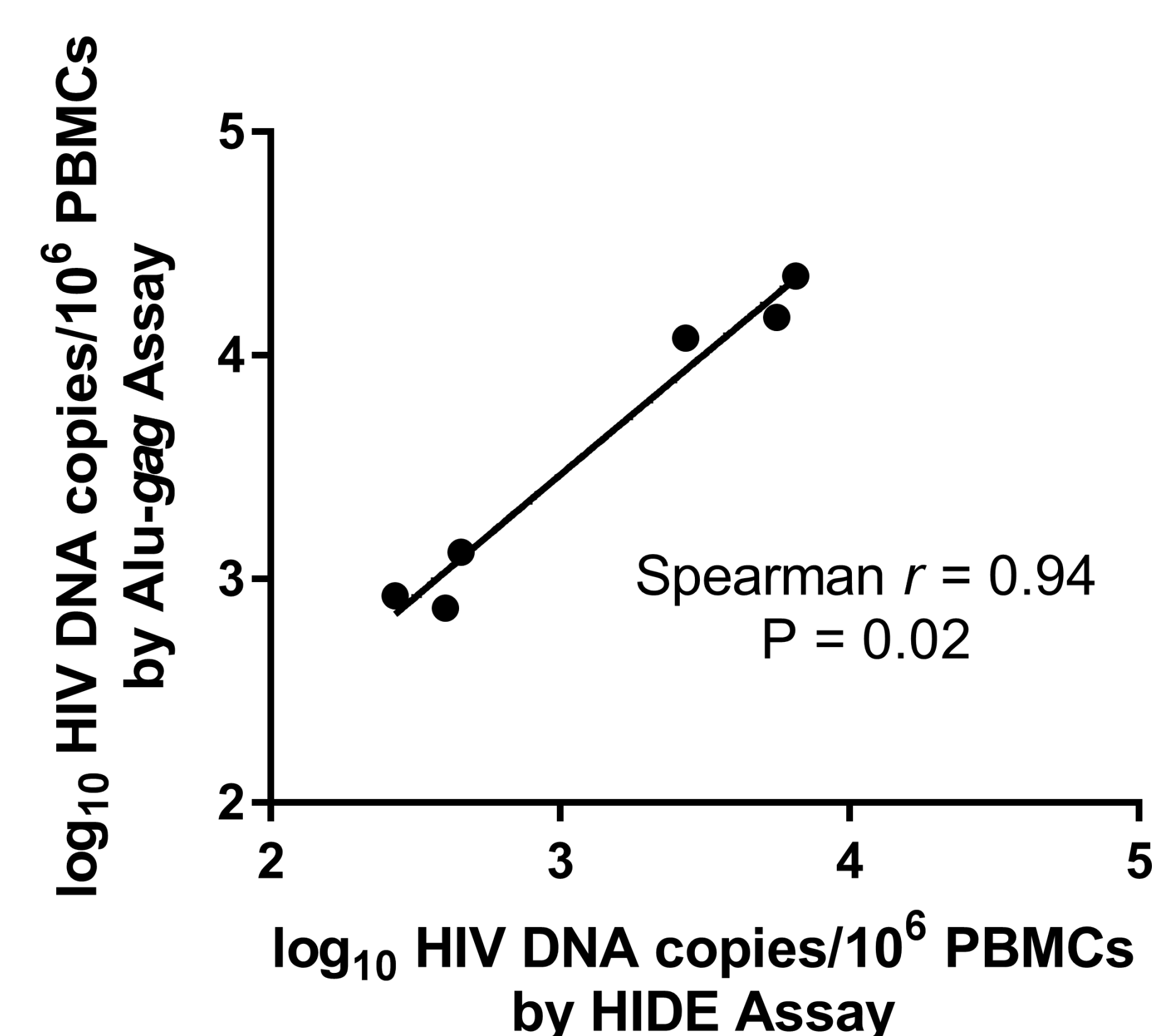
- A median 28% of total input DNA was recovered after size-selection for >20kb.

Elimination of Unintegrated HIV DNA Species



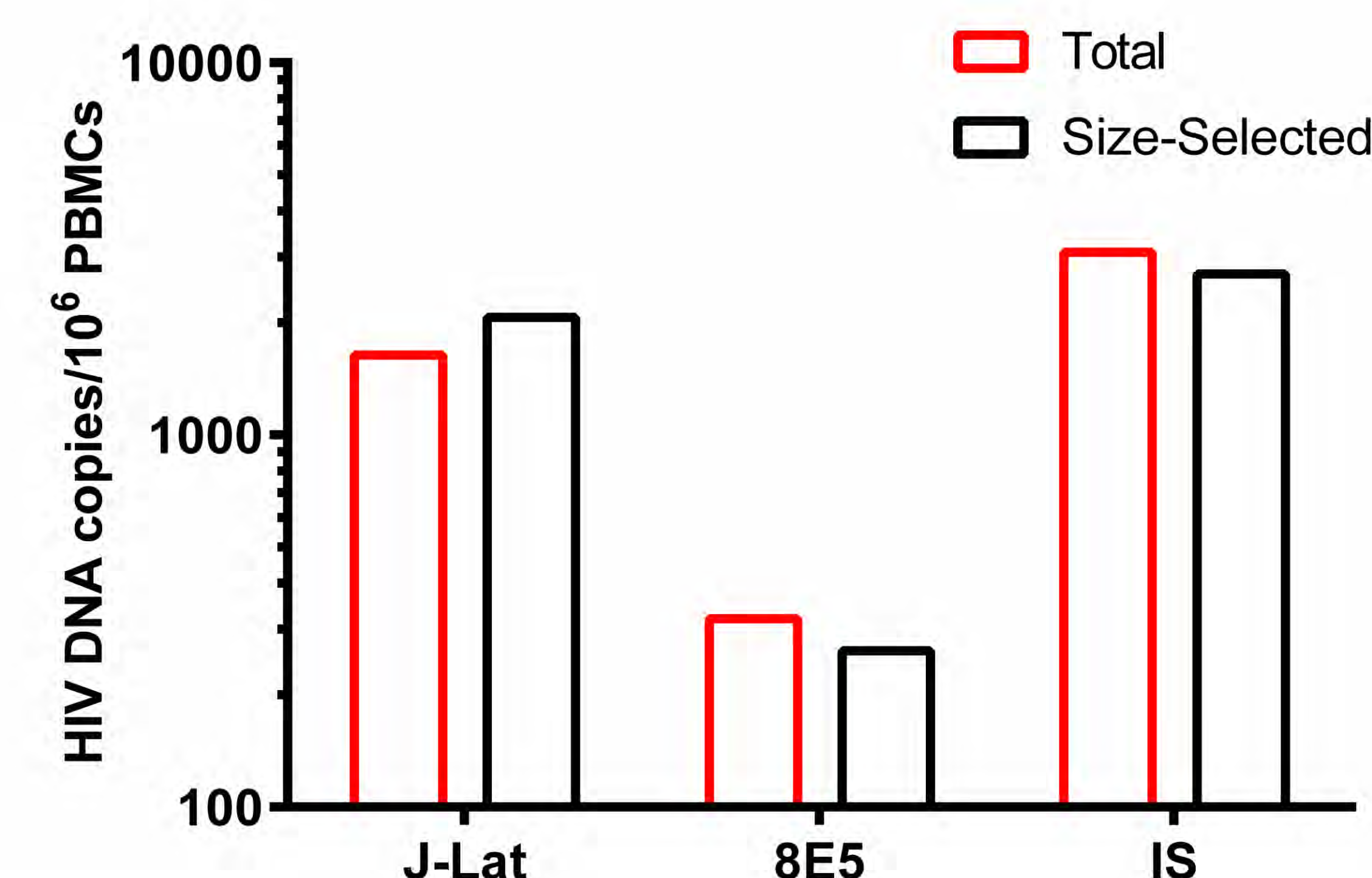
- BluePippin eliminated 99% of unintegrated HIV DNA species spiked into HIV-negative DNA.

Correlation between HIDE and Alu-gag assays



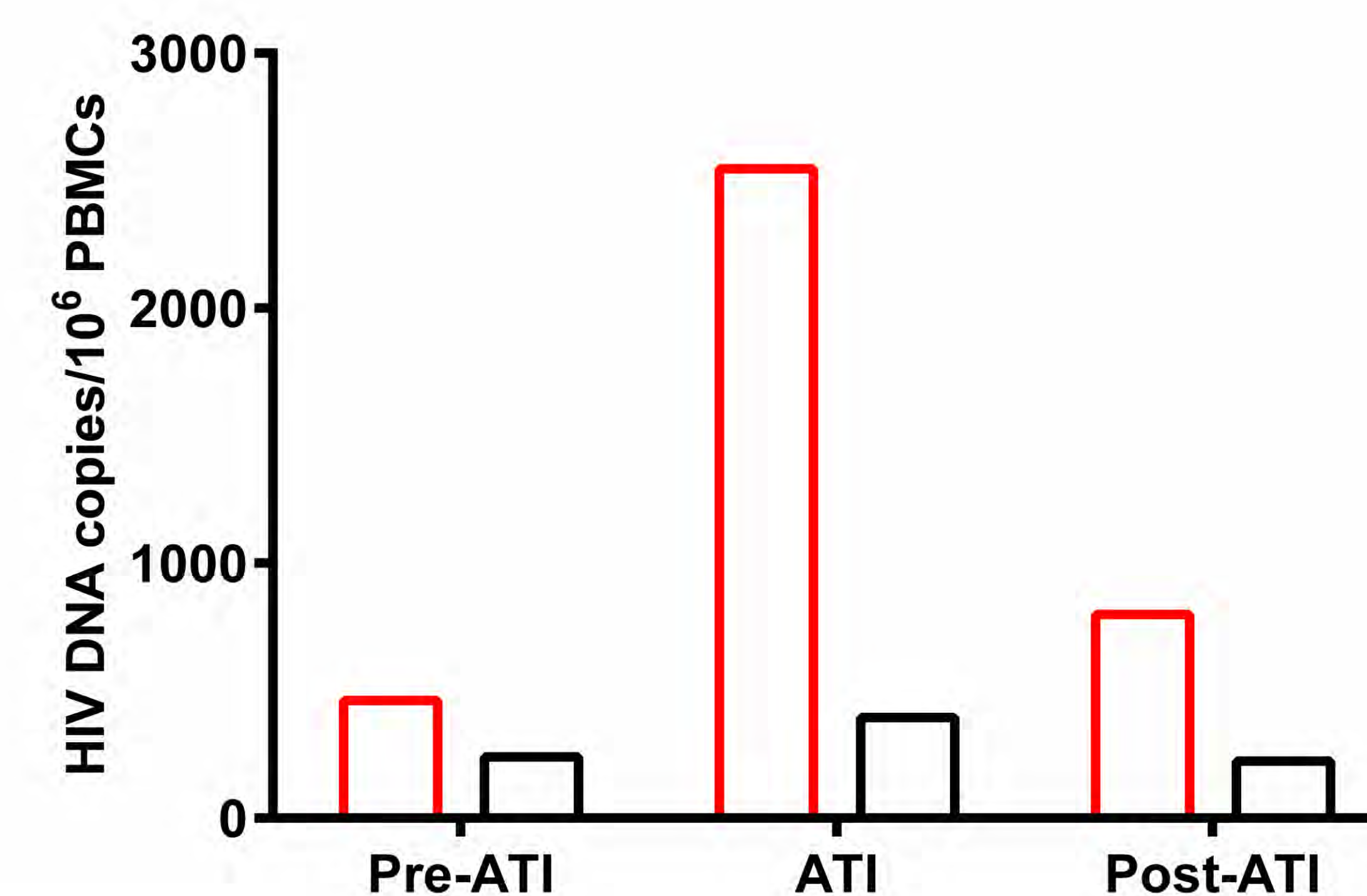
- Integrated HIV DNA levels by the HIDE assay significantly correlated with measurements by the traditional Alu-gag assay in both cell lines and participant samples.

Levels of Integrated HIV DNA before and after HIDE



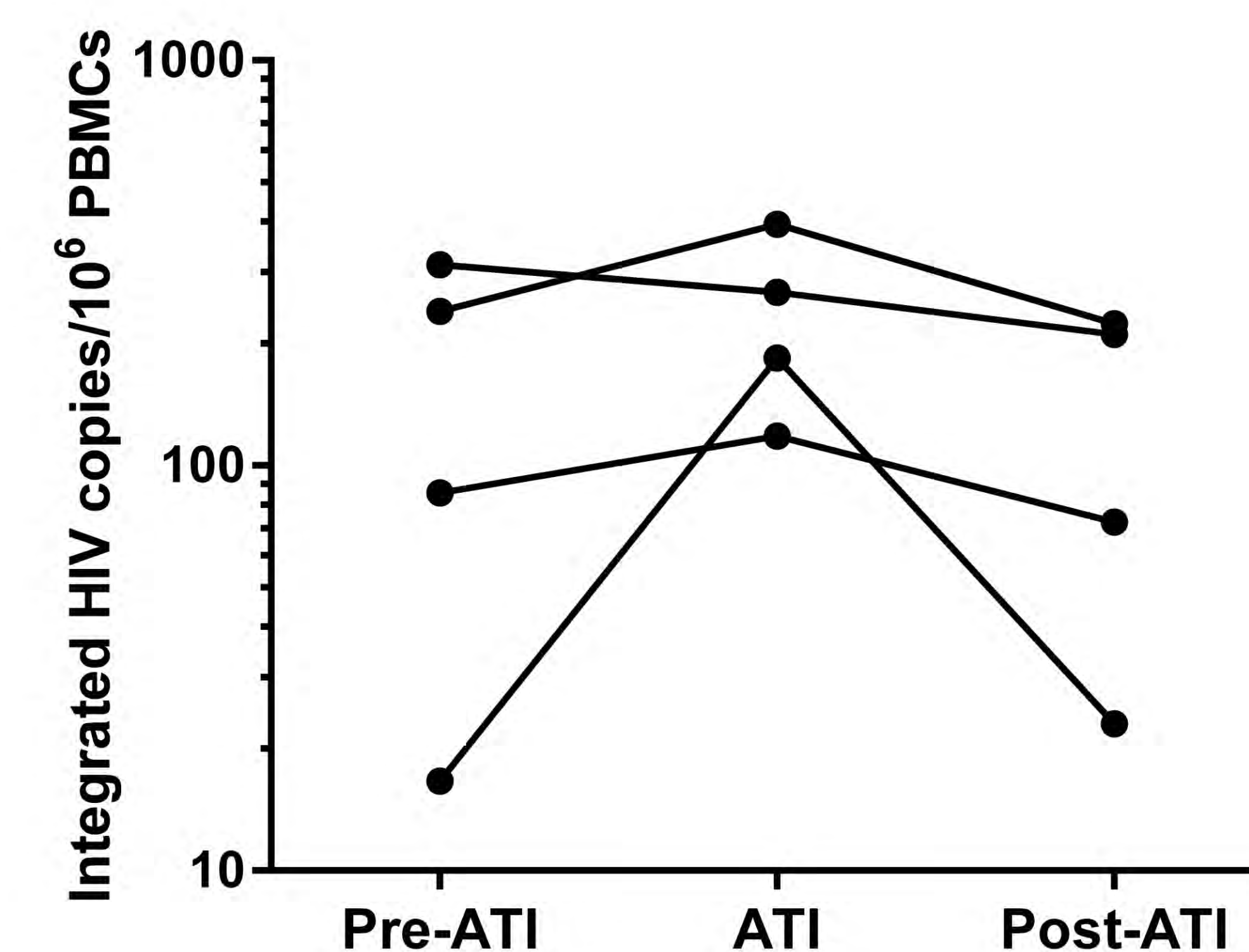
- Levels of HIV DNA did not change after size-selection for genomic DNA in cell lines containing integrated, replication-incompetent HIV DNA (mean ratio total:size-selected 1.02).

Total and Integrated HIV DNA Levels during ATI



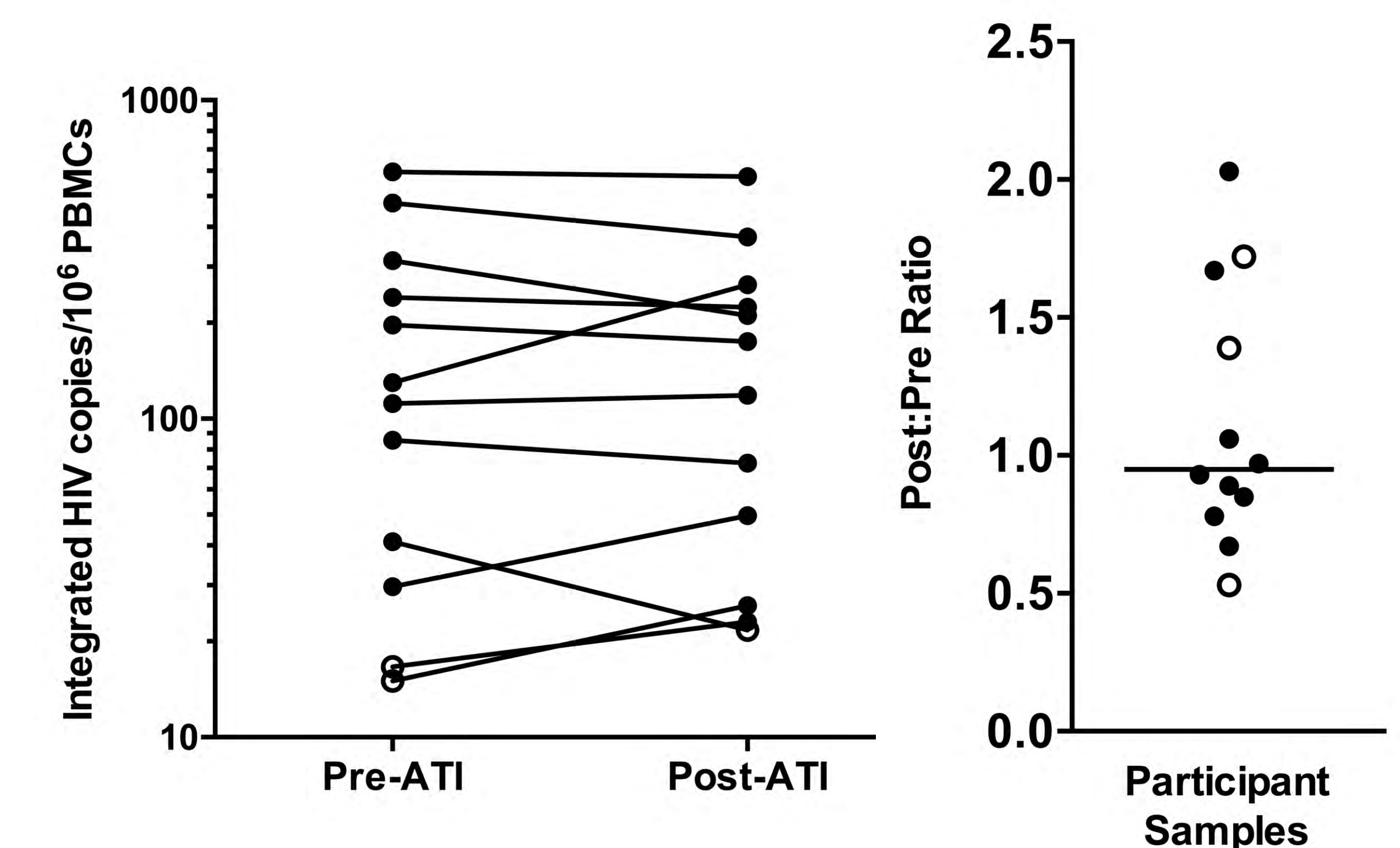
- Levels of HIV DNA were higher in total (red) vs. size-selected (black) DNA across all timepoints, especially during the treatment interruption (representative participant shown).

Levels of Integrated HIV DNA during ATI



- Levels of integrated HIV DNA increased at varying degrees amongst participants with samples available during ATI, but returned to pre-ATI levels after ART resumption.

Levels of Integrated DNA after ART Resumption



- There was no significant difference between the pre- and post-ATI time points in levels of HIV integrated DNA (median difference -15 copies/10⁶ PBMCs, Wilcoxon Signed Rank P=0.34). The median ratio of post:pre-ATI HIV DNA levels was 0.95 (Q1, Q3: 0.8, 1.6). Open circles denote below LOD.

Conclusions

- The HIDE assay measures integrated HIV DNA levels using an automated size-selection system that is less sample and labor-intensive than current assays.
- Despite an increase in integrated DNA levels during the ATI, the viral reservoir subsequently was reduced to pre-ATI levels after approximately 6 months of ART.

Implications

- The HIDE assay is a useful technique that can be applied to quantify levels of total integrated HIV DNA.
- Short-term ATI can be conducted without causing a significant, long-term effect on the size of the total proviral reservoir.

Acknowledgements

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References

- Lada S, VanBelzen J, Ignacio C, Strain M, O'Doherty U, Richman DD. Novel Assay to Measure Integrated HIV DNA in PBMC from ART-Suppressed Persons. [Abstract 300] Conference on Retroviruses and Opportunistic Infections (CROI) 13-16 February 2017.