CTL RESPONSES ARE NOT ASSOCIATED WITH DECAY OF INTACT PROVIRUSES OR HIV RNA ON ART

398

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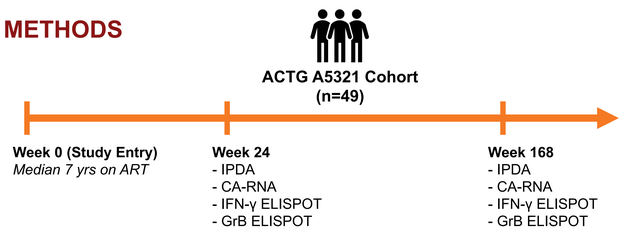
BACKGROUND

- In PLWH on long-term ART:
  - Magnitudes of HIV-specific T-cells targeting Nef – but not other HIV proteins – are positively associated with HIV DNA levels (Thomas *et al*, PLOS Pathogens, 2017)
  - Changes in Nef-specific responses over time are positively associated with HIV DNA levels – higher DNA, less decay in Nef-specific responses (Stevenson & Ward *et al*, JCI Insight, 2021)
  - Nef-specific T-cells disproportionately exhibit a cytotoxic profile as measured by *ex vivo* granzyme B production (Stevenson & Ward *et al*, JCI Insight, 2021)
- Results above suggest ongoing antigen stimulation of HIV-specific T-cells in PLWH on ART – especially Nef-specific T-cells
  - **It is not known if HIV-specific T-cells impact measures of either proviral persistence or expression**

Hypothesis: Decay of intact proviral DNA and CA-RNA levels on ART will be associated with HIV-specific cytotoxic T-cell (CTL) responses

METHODS



- 49 participants from the ACTG A5321 cohort on suppressive ART (plasma HIV RNA <40 copies/mL) were studied at weeks 24 and 168 post-study entry (*median 7 years on ART at entry*)
- HIV DNA and CA-RNA were measured by droplet digital PCR (IPDA for DNA, 5' unspliced and 3' total poly(A) for RNA)
- T-cell responses were measured by IFN-γ and granzyme B [GrB] ELISPOT to each HIV gene product

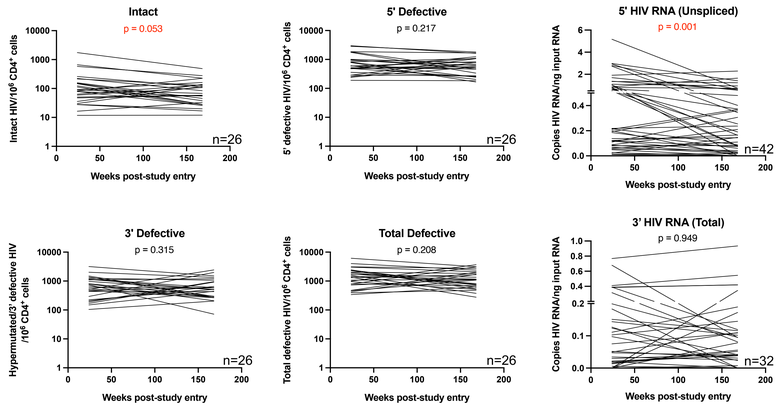
RESULTS

1. Participant characteristics

Table 1. Characteristics of longitudinal substudy participants

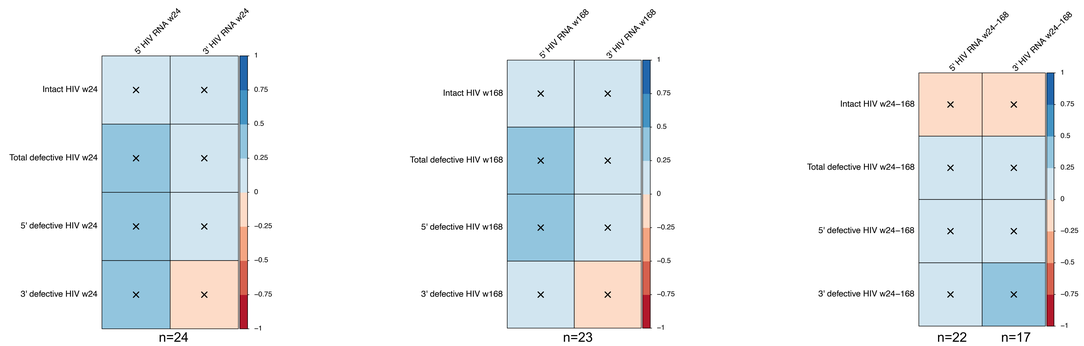
Variable	Median (Range) or No. (%)
Age at A5321 entry (years)	48 (23-74)
Years on ART at A5321 entry	6.6 (4.2-14.8)
Sex	
Female	11 (22.45%)
Male	38 (77.55%)
Race/Ethnicity	
American Indian/Alaskan Native	1 (2.04%)
Black (non-Hispanic)	5 (10.20%)
Hispanic (regardless of Race)	16 (32.65%)
White (non-Hispanic)	27 (55.10%)

2. Intact HIV DNA and 5' HIV CA-RNA decay on ART



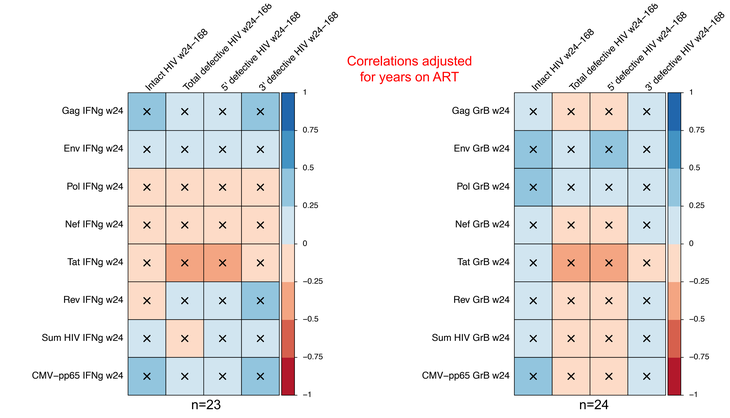
- Intact HIV DNA, but not defective HIV DNA species, exhibited a decreasing trend from week 24 to week 168, which approached significance
- 5' HIV RNA (unspliced), but not 3' HIV RNA, decreased significantly from week 24 to week 168
- *Note: sample size difference due to IPDA failures and sample availability limitations*

3. IPDA measures are not associated with HIV CA-RNA levels



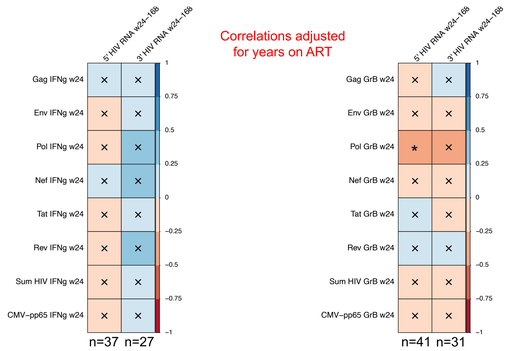
- IPDA measures at weeks 24 and 168 were not cross-sectionally associated with CA-RNA levels
- Changes in IPDA measures from weeks 24 to 168 were not associated with changes in CA-RNA levels

4. Magnitudes of HIV-specific T-cell responses are not associated with changes in IPDA measures



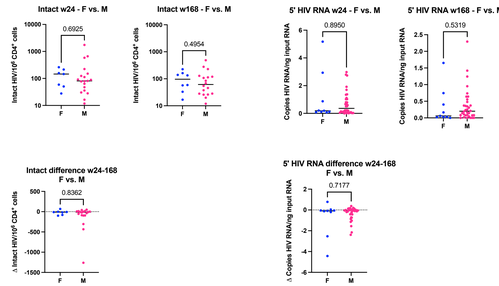
- Magnitudes of IFN-γ- and GrB-producing HIV-specific T-cell responses were not associated with changes in HIV DNA levels by IPDA from weeks 24 to 168
  - Including after controlling for differences in time on ART

5. Magnitudes of HIV-specific T-cell responses are not associated with changes in CA-RNA levels



- Magnitudes of IFN-γ- and GrB-producing HIV-specific T-cell responses were not associated with changes in CA-RNA levels from weeks 24 to 168 – except for Pol-specific GrB responses, but the unadjusted correlation was not significant (not shown)

6. No apparent sex-specific differences in IPDA measures or CA-RNA levels



CONCLUSIONS

- Contrary to our hypothesis, no associations were observed between decay of intact HIV DNA or CA-RNA with HIV-specific T-cell responses after long-term ART
  - Including with cytotoxic function (granzyme B)
  - Including after controlling for time on ART
  - **Findings suggest a possible limited role for CTLs in reservoir decay after multiple years of suppressive ART**
- Other unmeasured parameters may be important:
  - Variation in susceptibility of reservoir cells to CTL killing?
  - Genomic context of provirus – likely to be expressed?
  - Other immune responses (NK cells) or other parameters of CTLs?
- What is the relationship between CTLs and HIV persistence measures earlier on ART?

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