Weill Cornell Medicine
Jones Lab

REACH For the Cure
Research Enterprise to Advance a Cure for HIV

No Associations Between Magnitudes of HIV-Specific CTL Responses on Stable ART and Subsequent Decay of Intact Proviruses or Cell-Associated HIV mRNA

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Session 4: Immunology of HIV Persistence

Conflicts of interest

- None to disclose

ACTG A5321 cohort

- Decay of HIV-1 Reservoirs in Participants on Long-Term Antiretroviral Therapy: The ACTG HIV Reservoirs Cohort (AHRC) Study
  - Chronic HIV infection before ART initiation
  - Virally suppressed prior to study entry and throughout study period

Table 1. Characteristics of longitudinal sub-study participants - continuous variables

Variable	Median	Range		Missing	
		Lower	Upper	n	%
Age at A5321 entry (years)	48	23	74	0	0.00%
Years on ART at A5321 entry	6.6	4.2	14.8	0	0.00%
HIV CA-DNA at A5321 entry (cps/10^6 CD4+ T-cells)	515.7	5.2	5494.0	0	0.00%
HIV CA-RNA at A5321 entry (cps/10^6 CD4+ T-cells)	24.2	13.6	898.9	2	4.08%
HIV plasma RNA via iSCA at A5321 entry (cps/mL)[A]	0.4	0.4	8.8	3	6.12%
%PD-1+ CD4+ cells at A5321 entry	36.75%	1.20%	83.10%	5	10.20%
%PD-1+ CD8+ cells at A5321 entry	35.40%	0.70%	84.90%	5	10.20%
Pre-ART plasma HIV-1 RNA (log_{10}cps/mL)	4.6	2.3	5.9	0	0.00%
Pre-ART CD4+ T-cell count (cells/mm^3)	287.5	15.5	708.5	0	0.00%

[A]iSCA assay limit of detection 0.4 copies HIV per mL plasma

Table 2. Characteristics of longitudinal sub-study participants - categorical variables

Variable	n	%
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%
iSCA qualifier at A5321 entry[A]		
Undetectable	22	44.90%
Detectable	27	55.10%

[A]iSCA assay limit of detection 0.4 copies HIV per mL plasma

ACTG A5321 cohort

Week 0 (Study Entry)
Median 7 yrs on ART

Week 24
- IPDA
- CA-RNA
- IFN-γ ELISPOT
- GrB ELISPOT

Week 168
- IPDA
- CA-RNA
- IFN-γ ELISPOT
- GrB ELISPOT

- N=49 participants included in a longitudinal sub-study
  - Measures across time points assayed in batch for each participant
- HIV DNA levels measured by the intact proviral DNA assay (IPDA)
- HIV cell-associated (CA)-RNA levels measured by droplet digital PCR
  - 5' unspliced CA-RNA
  - 3' total poly(A) CA-RNA
- T-cell responses assessed by IFN-γ and granzyme B (GrB) ELISPOT
  - Assessed using peptide pools covering each HIV gene product and CMV-pp65

HIV-Nef-specific T-cells are associated with HIV DNA on ART

- Nef-specific T-cells – but not T-cells targeting other HIV proteins – are associated with HIV DNA levels on ART

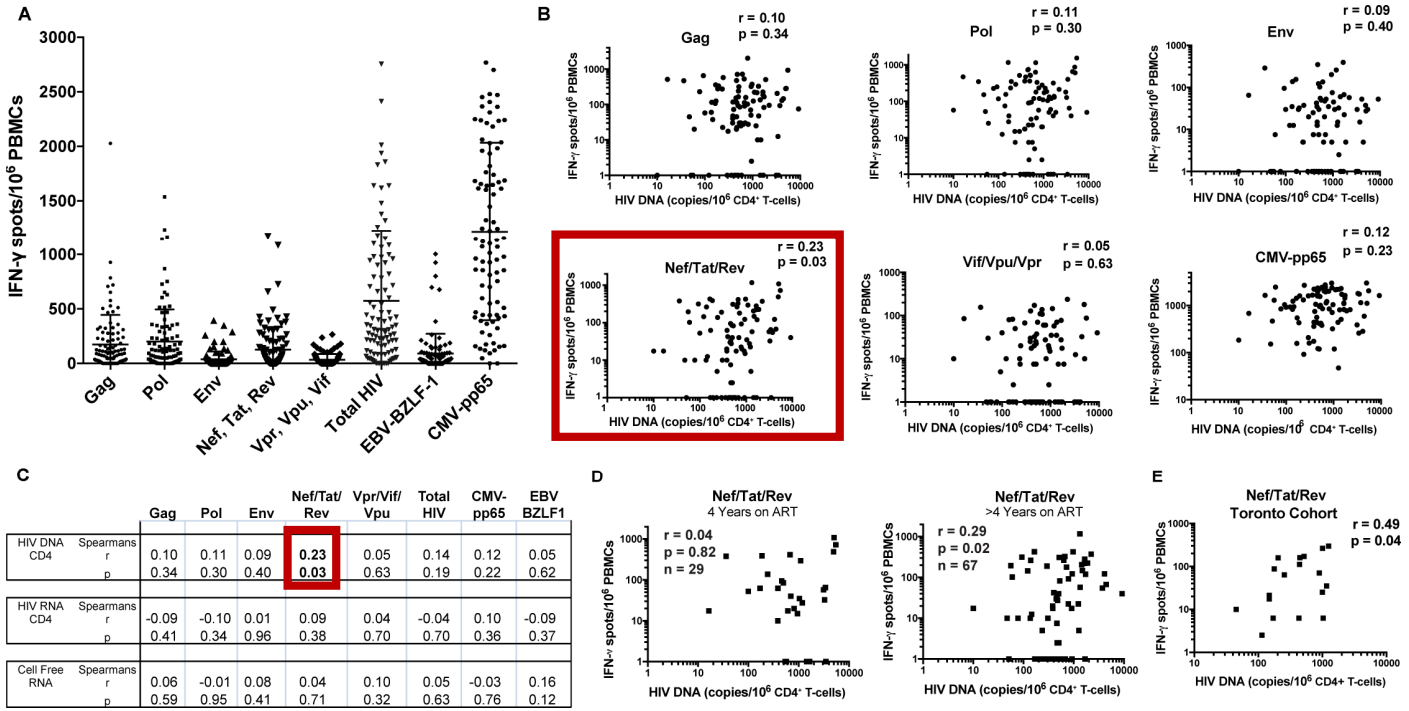
PLOS | PATHOGENS

RESEARCH ARTICLE

T-cell responses targeting HIV Nef uniquely correlate with infected cell frequencies after long-term antiretroviral therapy

Week 0 (Study Entry)
Median 7 yrs on ART
- CA-DNA (qPCR)
- CA-RNA (qPCR)
- Plasma single copy assay (iSCA)
- IFN-γ ELISPOT

n=99



		Gag	Pol	Env	Nef/Tat/ Rev	Vpr/Vif/ Vpu	Total HIV	CMV-pp65	EBV BZLF1
HIV DNA CD4	Spearmans r	0.10	0.11	0.09	**0.23**	0.05	0.14	0.12	0.05
	p	0.34	0.30	0.40	**0.03**	0.63	0.19	0.22	0.62
HIV RNA CD4	Spearmans r	-0.09	-0.10	0.01	0.09	0.04	-0.04	0.10	-0.09
	p	0.41	0.34	0.96	0.38	0.70	0.70	0.36	0.37
Cell Free RNA	Spearmans r	0.06	-0.01	0.08	0.04	0.10	0.05	-0.03	0.16
	p	0.59	0.95	0.41	0.71	0.32	0.63	0.76	0.12

HIV-Nef-specific T-cells are associated with HIV DNA on-ART

- Changes in IFNγ-producing Nef-specific responses from week 24 to week 168 are uniquely positively correlated with HIV DNA levels at study entry

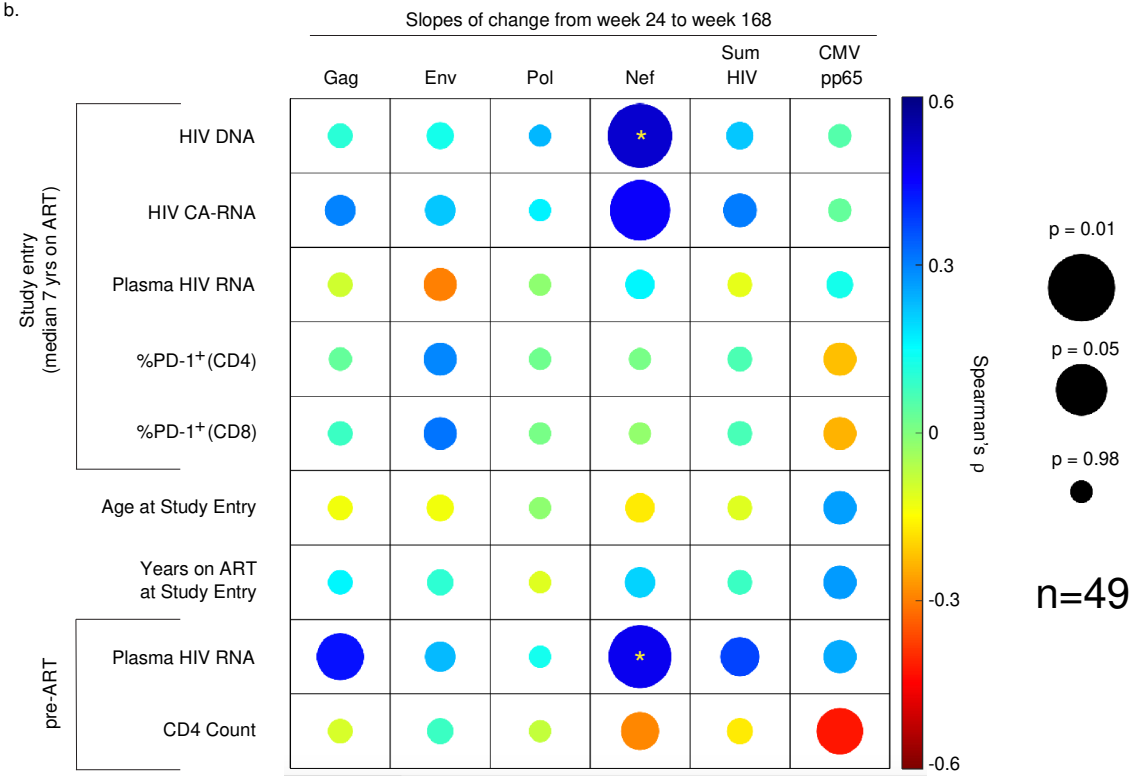
JCI insight

RESEARCH ARTICLE

HIV-specific T cell responses reflect substantive in vivo interactions with antigen despite long-term therapy

Results suggest ongoing antigen stimulation of HIV-specific T-cells on long-term ART

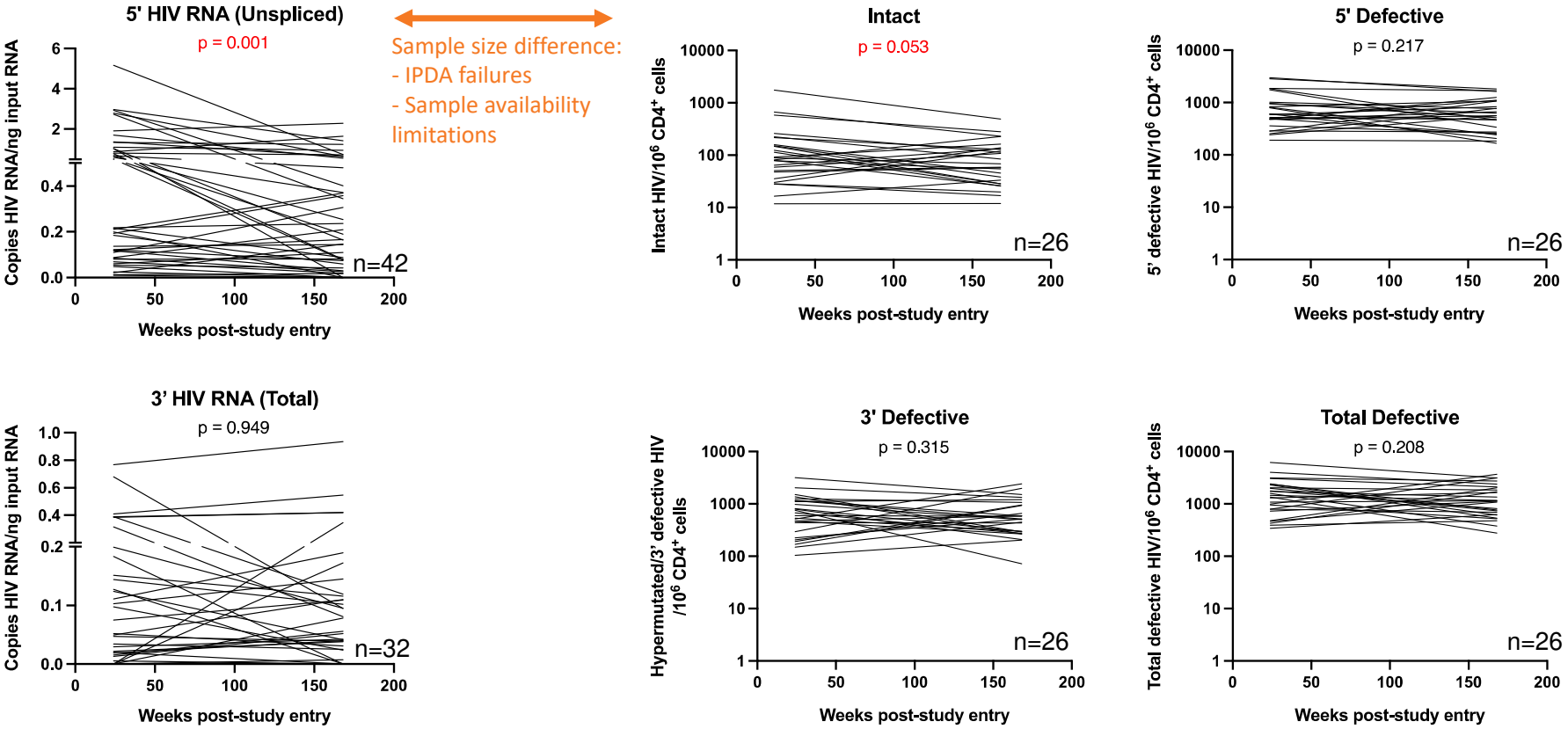
b.



Current study question and hypothesis

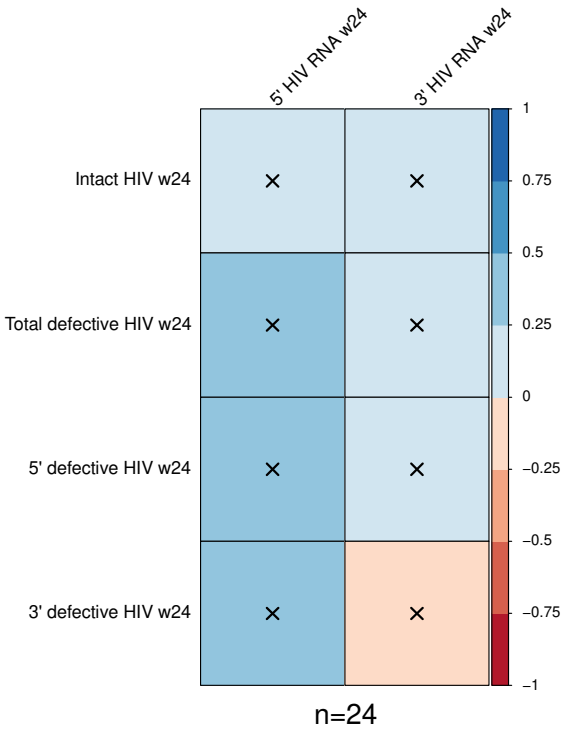
- **Question:** do magnitudes of HIV-specific T cell responses impact measures of proviral persistence (IPDA) or expression (CA-RNA)?
- **Hypothesis:** decay of intact proviral DNA and CA-RNA levels on ART will be associated with HIV-specific T cell responses
  - Nef-specific cytolytic responses (granzyme B-producing) may show stronger associations than other HIV-specific responses

5’ CA-RNA and intact HIV DNA decay on ART

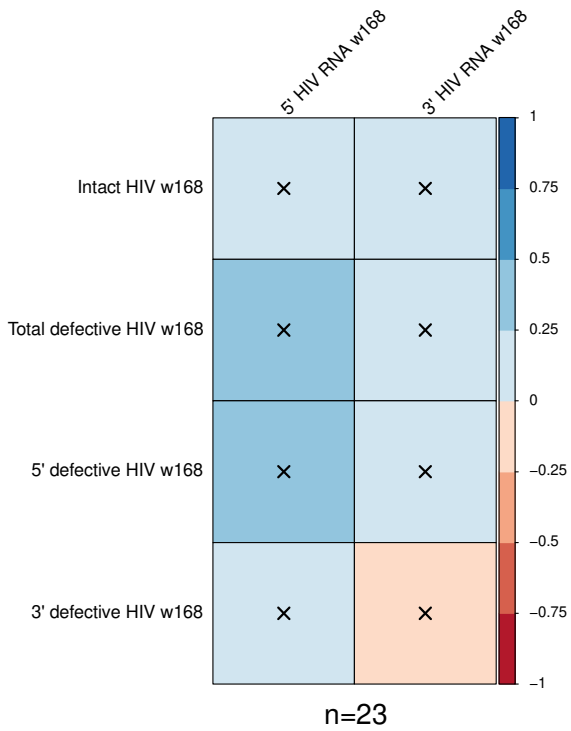


CA-RNA levels are not associated with IPDA levels

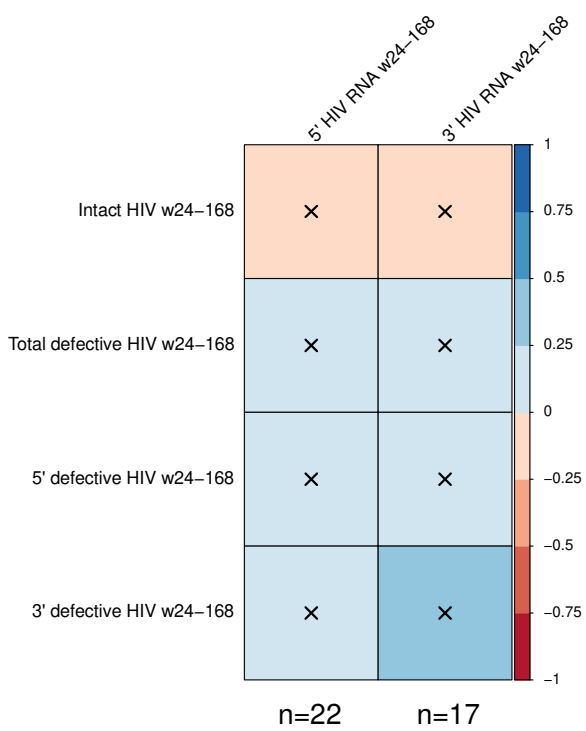
x = n.s.



n=24

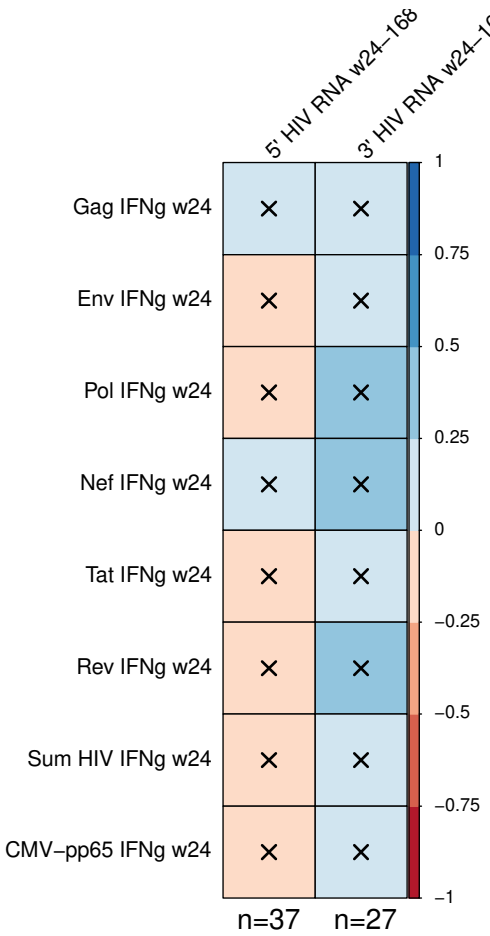


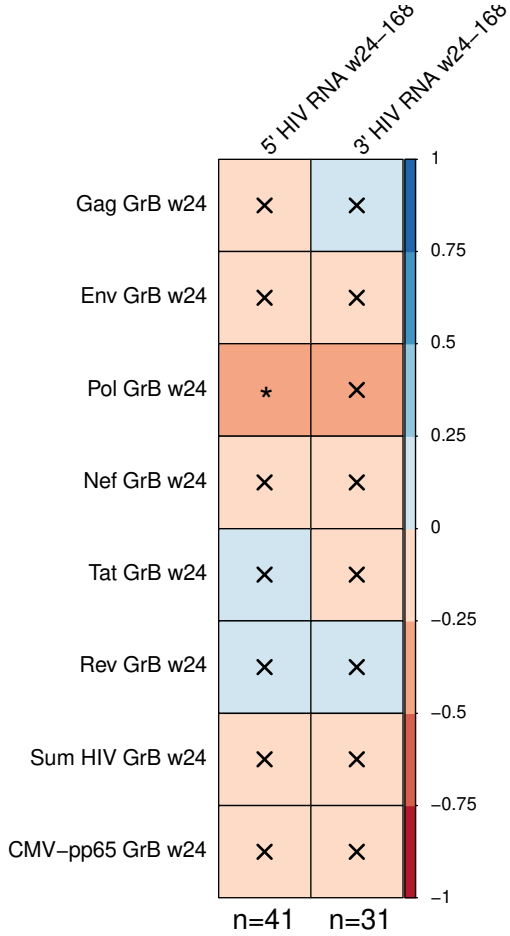
n=23



n=22 n=17

No associations between magnitudes of T-cell responses with changes in CA-RNA levels



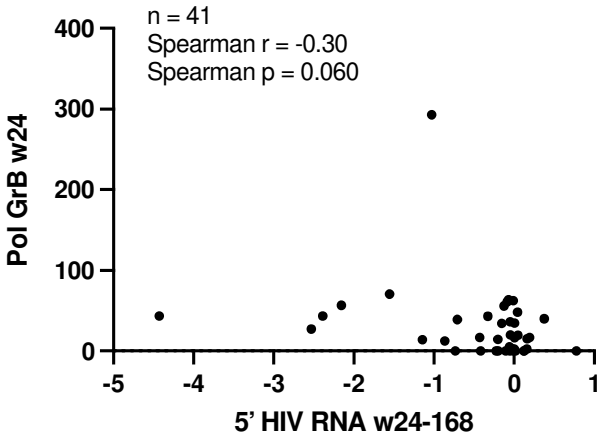


Correlations adjusted for years on ART

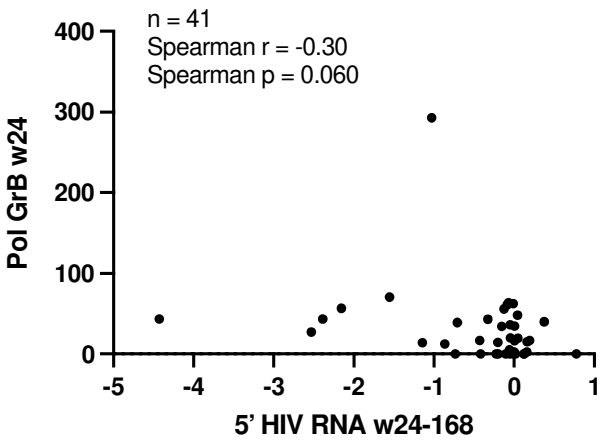
x = n.s.

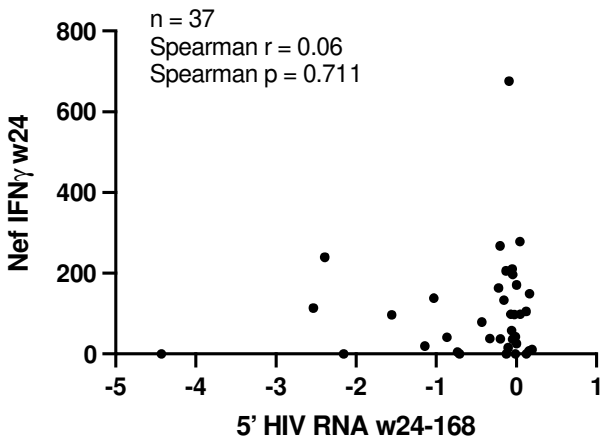
*p<0.05

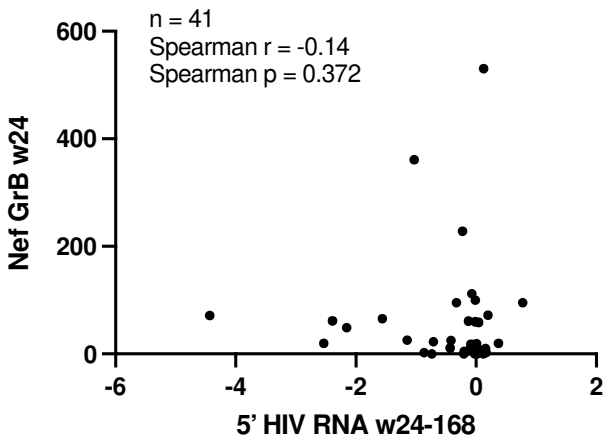
No associations between magnitudes of T-cell responses with changes in CA-RNA levels



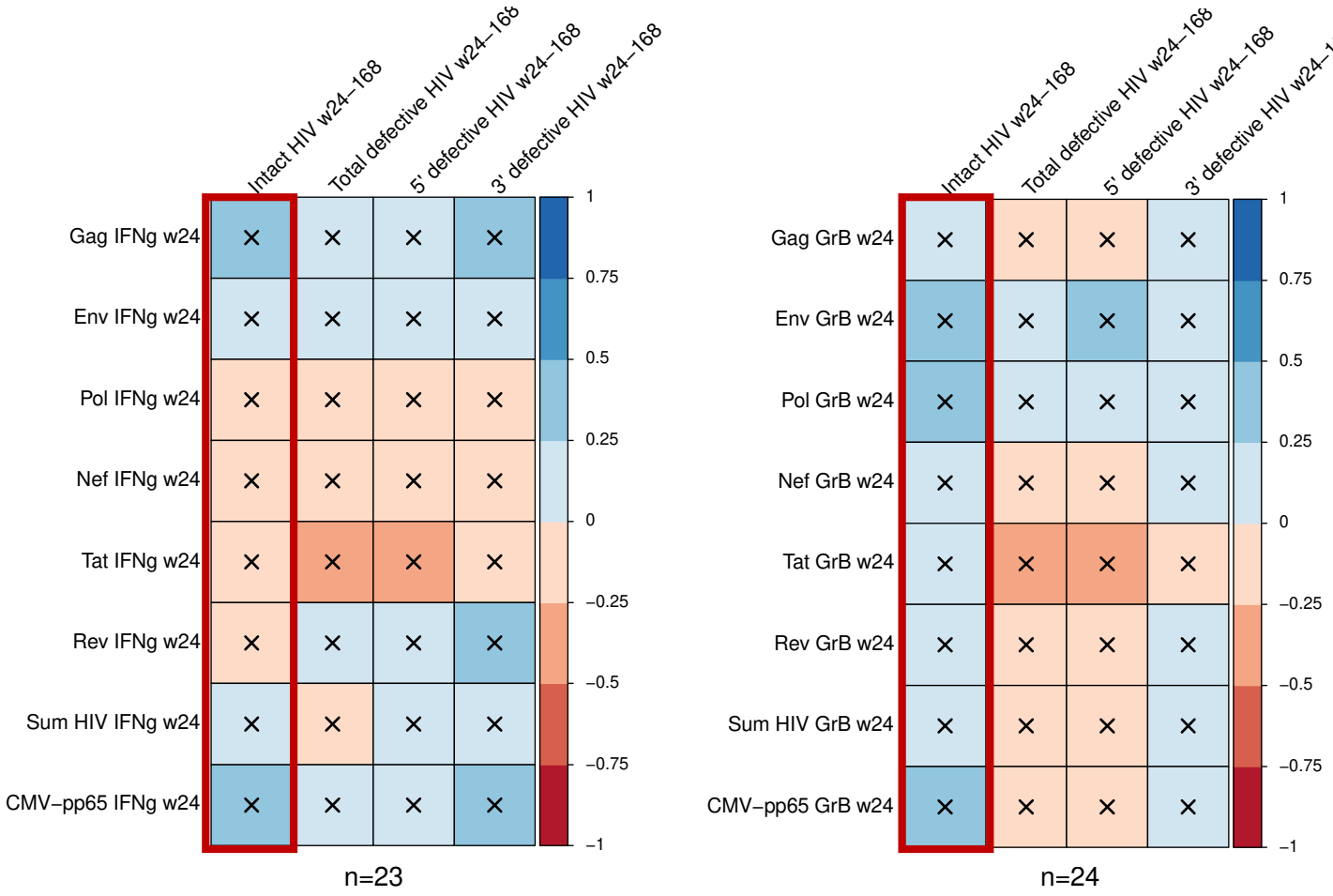
No associations between magnitudes of T-cell responses with changes in CA-RNA levels







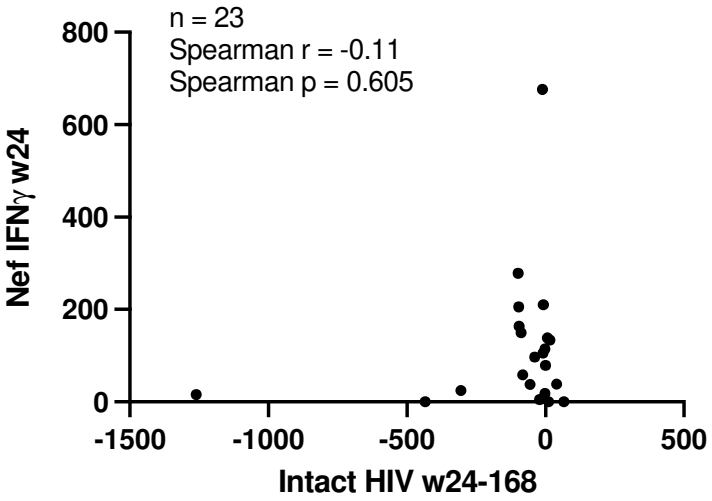
No associations between magnitudes T-cell responses with changes in IPDA levels

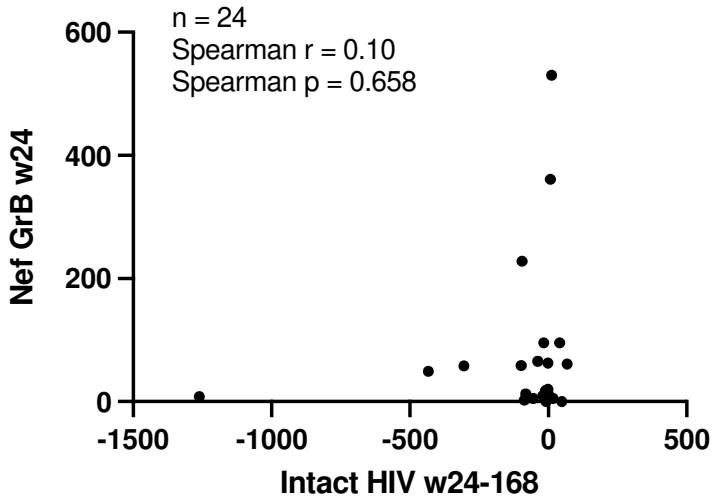


Correlations adjusted for years on ART

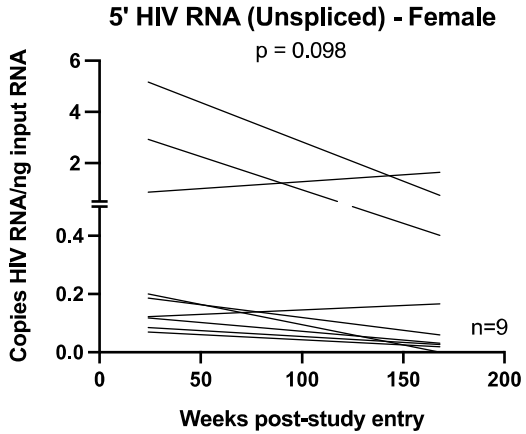
x = n.s.

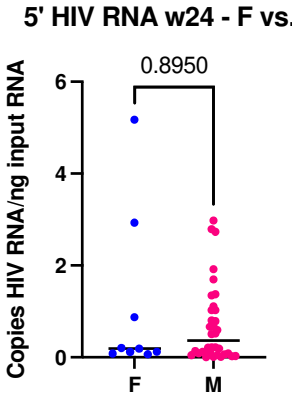
No associations between magnitudes T-cell responses with changes in IPDA levels

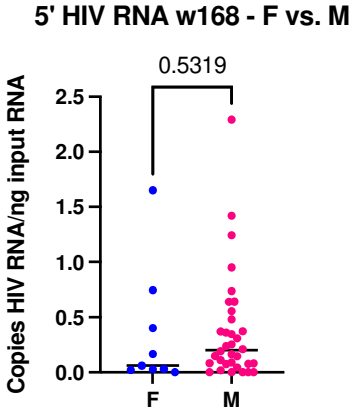


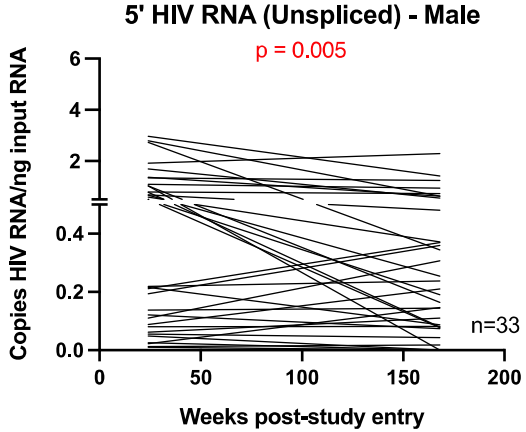


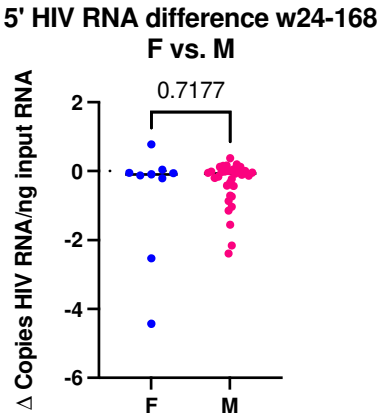
No sex-specific differences in 5’ CA-RNA levels



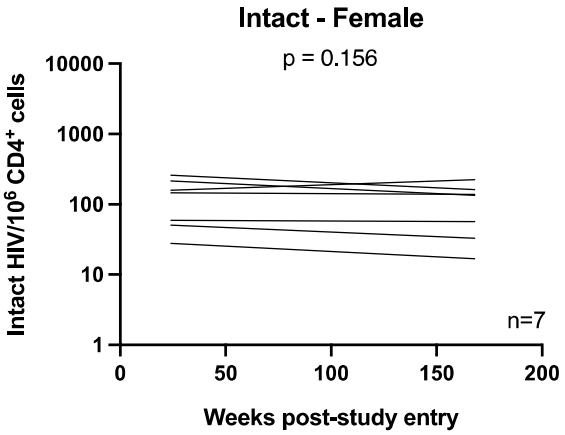


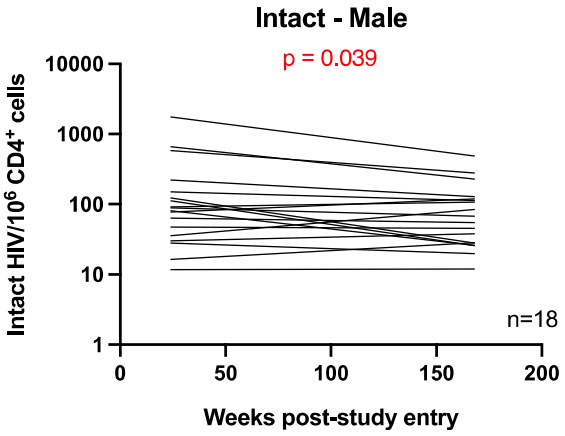


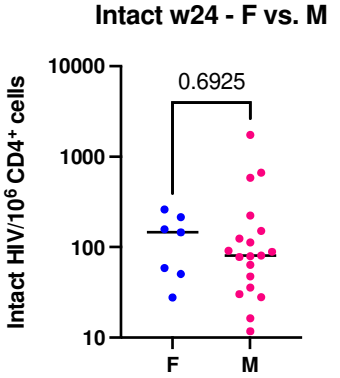


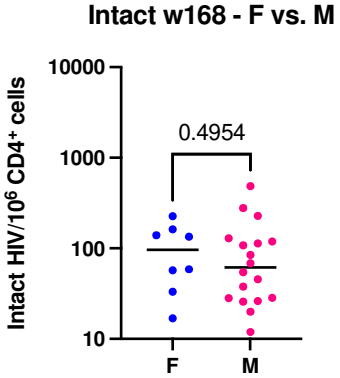


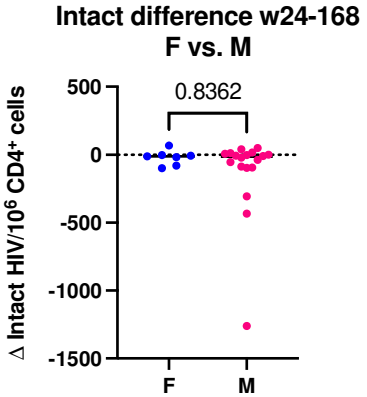
No sex-specific differences in intact HIV DNA levels











Conclusions

- Both intact proviral DNA and 5’ unspliced CA-RNA levels decayed over the 144-week period

- Contrary to our hypothesis, no associations were observed between decay of intact HIV DNA or CA-RNA with HIV-specific T-cell responses
  - 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**

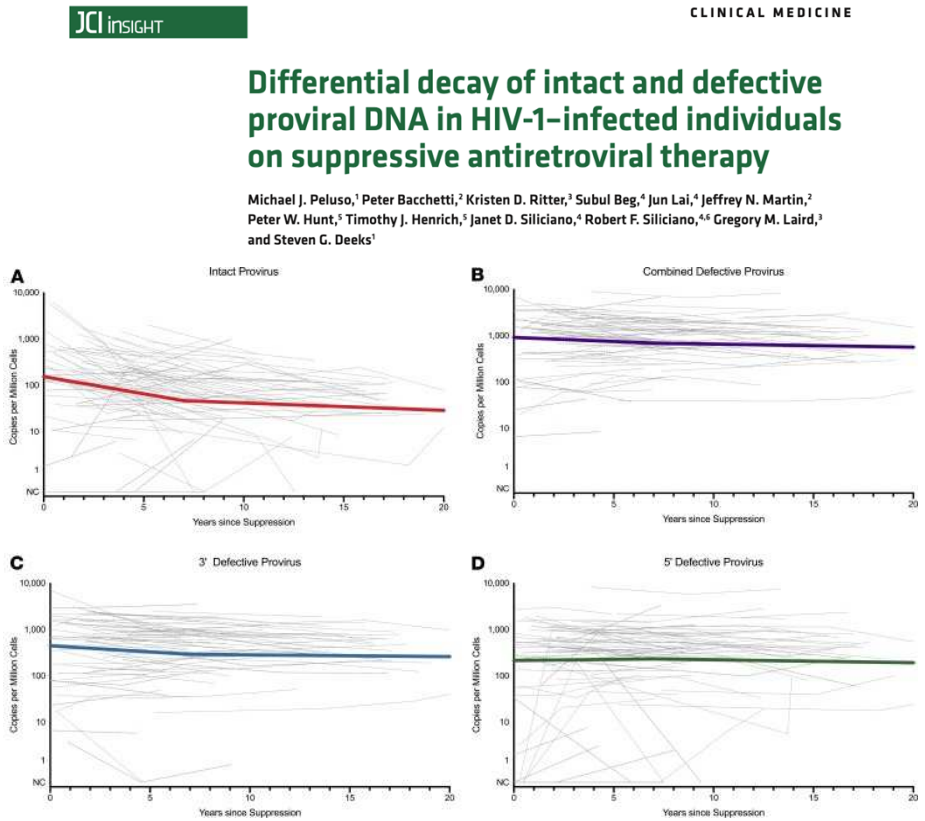
- No apparent sex-specific differences in intact proviral DNA levels or CA-RNA levels
  - Fewer female participants > difficult to make conclusions from limited sample size

Limitations

- Could not assess T-cell responses or HIV persistence measures in tissues
- T-cell responses were measured using consensus HIV peptides, not autologous peptides

Discussion & future directions

- Participants were on ART for a median 7 years at study entry
  - Intact HIV DNA decay is biphasic: slower rate of decay in intact HIV DNA at later ART
    ➡ more minimal association with CTL responses?



Discussion & future directions

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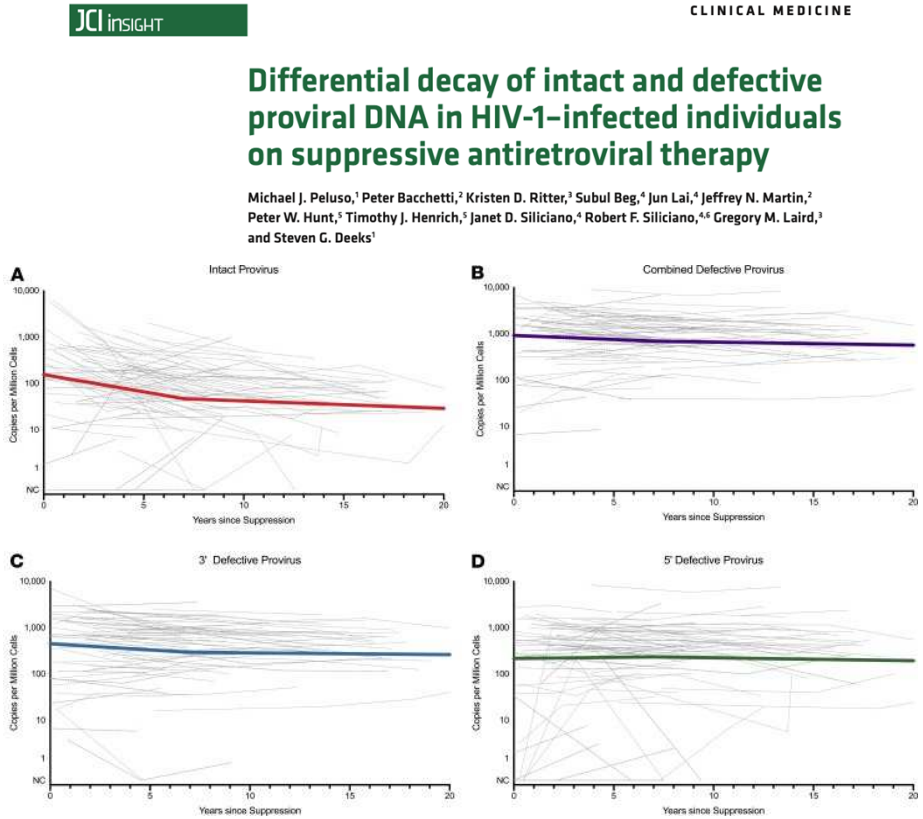
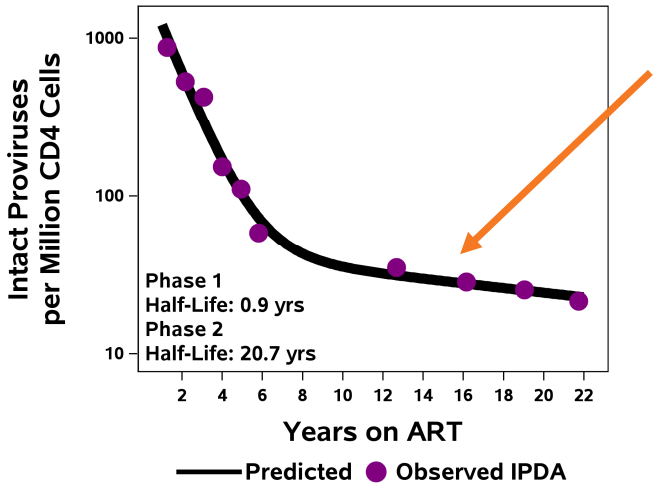




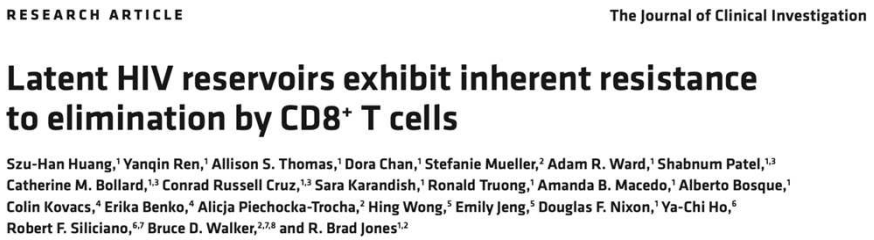
We are looking at this later phase of decay in the A5321 cohort - what is the relationship with T cells at early ART?

PP 8.8: Slowing or Reversal of Decay of Intact Proviruses Over Two Decades of Suppressive ART

J. Cyktor, R. Gandhi, R. Bosch, H. Mar, G. Laird, E. Halvas, L. Brandt, D. Mcmahon, J. Eron, J. Mellors, L. Hovind, S. Riddler, K. Ritter

Discussion & future directions

- Other important unmeasured parameters?
  - Variation in susceptibility of reservoir cells to CTL-mediated killing?



RESEARCH ARTICLE The Journal of Clinical Investigation

Latent HIV reservoirs exhibit inherent resistance to elimination by CD8+ T cells

Szu-Han Huang,[1] Yanqin Ren,[1] Allison S. Thomas,[1] Dora Chan,[1] Stefanie Mueller,[2] Adam R. Ward,[1] Shabnum Patel,[1,3] Catherine M. Bollard,[1,3] Conrad Russell Cruz,[1,3] Sara Karandish,[1] Ronald Truong,[1] Amanda B. Macedo,[1] Alberto Bosque,[1] Colin Kovacs,[4] Erika Benko,[4] Alicja Piechocka-Trocha,[2] Hing Wong,[5] Emily Jeng,[5] Douglas F. Nixon,[1] Ya-Chi Ho,[6] Robert F. Siliciano,[6,7] Bruce D. Walker,[2,7,8] and R. Brad Jones[1,2]

  - Genomic context of provirus (integration site – likely to be expressed)?

Article

Distinct viral reservoirs in individuals with spontaneous control of HIV-1

  - Other immune responses (NK cells, T cell subset analyses)?

Community summary

- **Key question(s)** do HIV-specific T-cell responses, part of the ongoing immune response to HIV, impact measures of HIV persistence in participants on long-term suppressive antiretroviral therapy?

- **Key finding(s)** HIV-specific T-cells, including cytotoxic cells, do not appear to impact levels of persistent HIV after multiple years of ART.

- What are the **next steps?** (**1**) examine the relationship between HIV-specific T-cells and persistent HIV earlier on-ART to determine if there is an impact that may be lost after multiple years on ART, (**2**) study reservoir cell resistance to T-cells to determine if immune resistance affects the relationship between T-cell responses and HIV persistence.

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Weill Cornell Medicine
Jones Lab

Jones Lab

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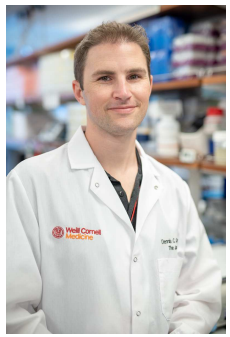
- Talia Mota, PhD
- Itzayana Miller
- Sandra Terry



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AIDS CLINICAL TRIALS GROUP

ACTG A5321 Team

- Ronald J. Bosch
- Joshua C. Cyktor
- Joseph J. Eron
- Rajesh T. Gandhi
- Bernard J. Macatangay
- John W. Mellors
- Deborah K. McMahon
- All other team members

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REACH
For the Cure
Research Enterprise to Advance a Cure for HIV

All A5321 cohort study participants, without whom this research would not be possible