Impact of ART in Primary HIV infection on T cell immune exhaustion in Gut-associated lymphoid tissue: Implications for HIV persistence

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Background

- The HIV reservoir is the main barrier to HIV cure
- Gut-associated lymphoid tissue (GALT) harbours the largest anatomical HIV-1 reservoir
- The immune environment in GALT may support viral persistence in the gut
- Early ART in Primary HIV infection limits HIV reservoir and enhances immune recovery in blood
- Immune exhaustion of both CD4 and CD8 T cells may support HIV persistence in the gut
- Novel agents that target immune exhaustion - ? potential targets for HIV cure strategies

What is T Cell exhaustion

- T cell exhaustion is a state of T cell dysfunction that arises during many chronic infections and cancer.
- It is associated with poor effector function of T cells

Drivers of exhaustion → **Over-expression of multiple inhibitory receptors** → **Physiological Consequences**

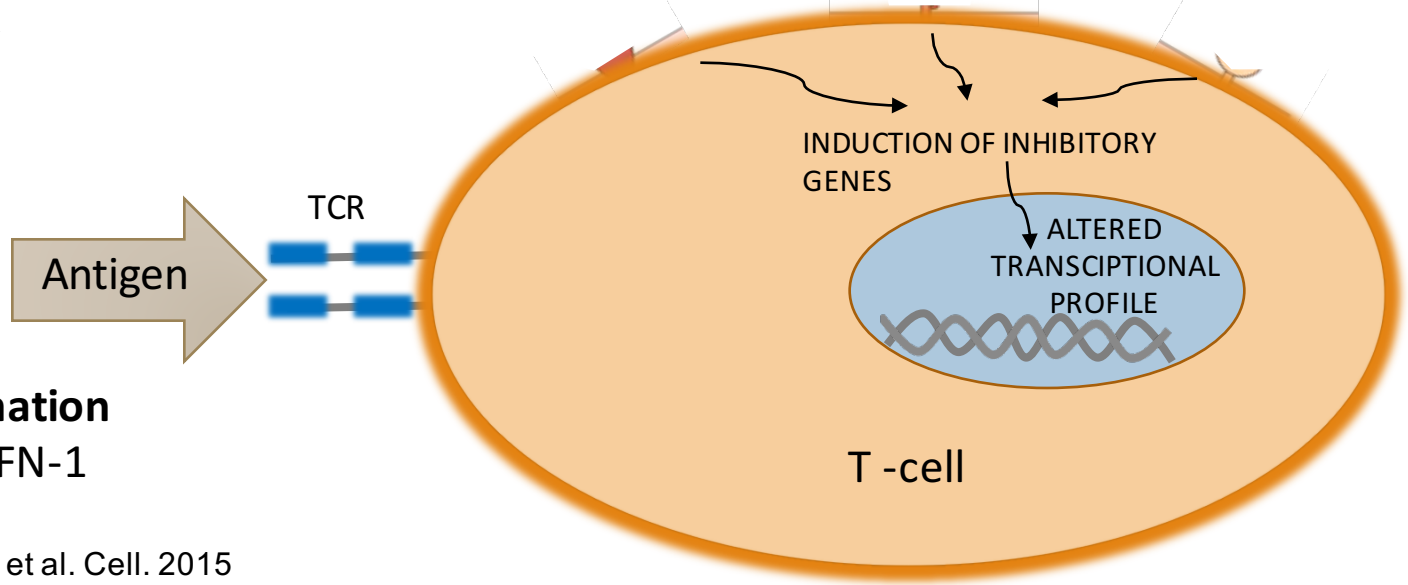
PD-1, CTLA-4, Lag-3, TIGIT, etc

Persistent antigen

- During chronic viral infections, such as HIV, HCV, and EBV

Chronic Inflammation

- IL-6, IL-21 & IFN-1

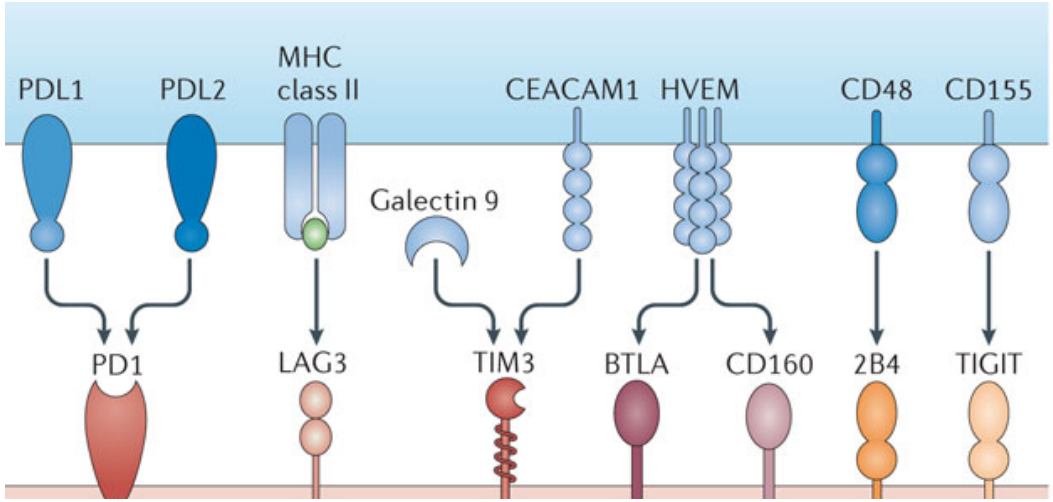


- **Exhaustion is characterized by a progressive loss of T cell function**
- Limited proliferative capacity
- Reduced cytokine production
- **Poor control of infection**

Adapted from Pauken, K et al. Cell. 2015

T-cell exhaustion and HIV

- Exhausted CD4+T-cells are enriched for HIV-1 DNA in peripheral blood[1,2]
- T cell exhaustion limits antiviral function of CD8+ T cells[3,4]
- CD4+ and CD8+ T-cell exhaustion predicts disease progression and viral rebound on treatment cessation[3,4,5]



1. Fromentin, R. et al Plos Pathogens 2016
2. Banga, R. et al Nature medicine 2016
3. Hoffmann, F. et al. Plos Pathogens 2016
4. Chew, G. et al. Plos Pathogens 2016
5. Hurst, J. et al. Nature Communications 2015

Aims

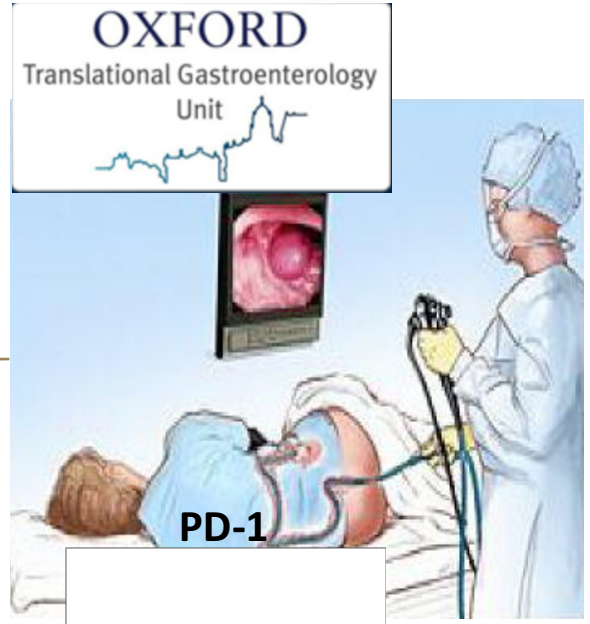
- Characterise the markers of immune function & exhaustion markers in Gut-Associated Lymphoid Tissue (GALT) of individuals commenced on ART in PHI compared with controls
- Examine the relationship between immune exhaustion in tissue and peripheral blood

Methods

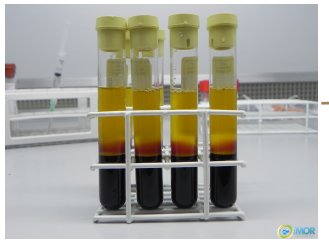
Clinical Cohorts

HEATHER gut sub-study
St Mary's Hospital
London
HIV+ samples

Translational Gastroenterology Unit (TGU) Oxford
HIV- samples



Clinical Samples



- ✓ HIV-1 infection
- ✓ Started on ART in PHI
- ✓ On ART for >1 year
- ✓ No hx of IBD

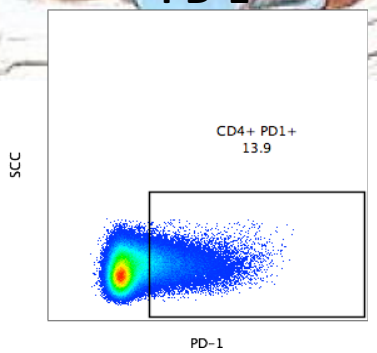
↔

- ✓ Individuals undergoing routine endoscopy at JR Hospital Oxford
- ✓ No known hx of HIV infection or IBD

Laboratory Methods

nd biopsies used to assess
nune function (exhaustion
markers) on CD4 & CD8 cells
using Flow Cytometry

CD4/CD8 & Exhaustion Markers: PD-1, Tim-3, TIGIT



Results

Baseline characteristics	HEATHER gut sub-study HIV+ samples	TGU HIV- samples
Median age in years		9 (55-70)
Male	13	9
Median days from PHI t	8 (20-	-
Median months on ART	6 (19-	-
Median CD4 count at tir	7 (634	-
Median CD4/CD8 ratio	0 (0.6-	-
Median nadir CD4 count	0 (320	-
ART 3rd Agent: PI	1 (8)	-
NNRTI	3 (25)	
INSTI	8 (67)	

26 individuals were included in this analysis

HEATHER gut sub-study
St Mary's Hospital
London
HIV+ samples

n=13

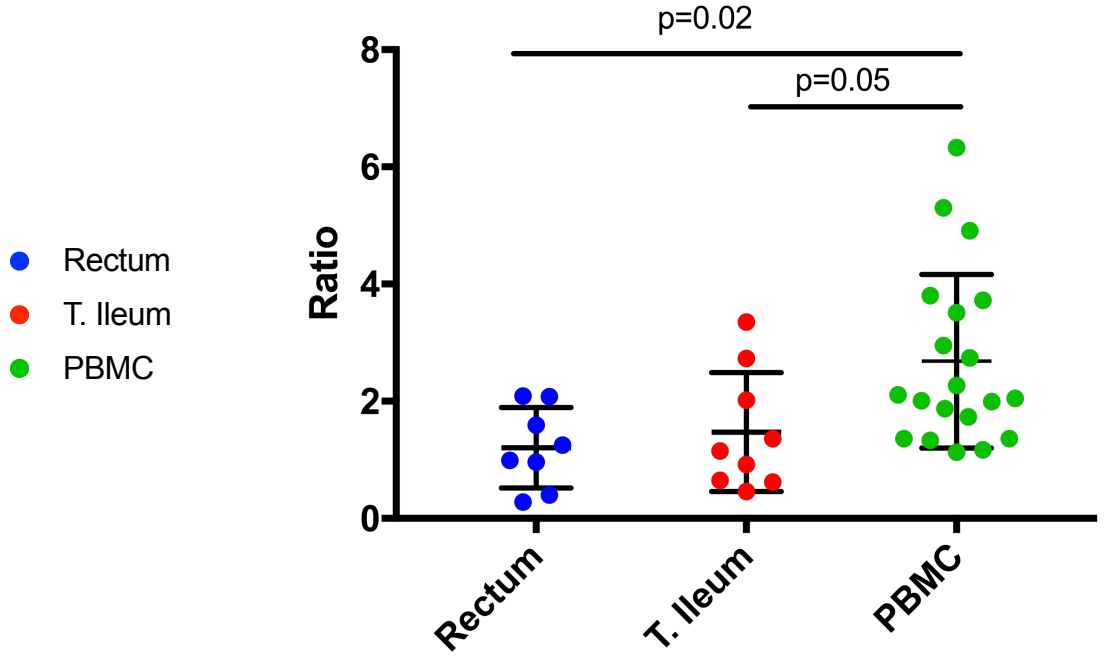
Translational
Gastroenterology Unit
(TGU) Oxford
HIV- samples

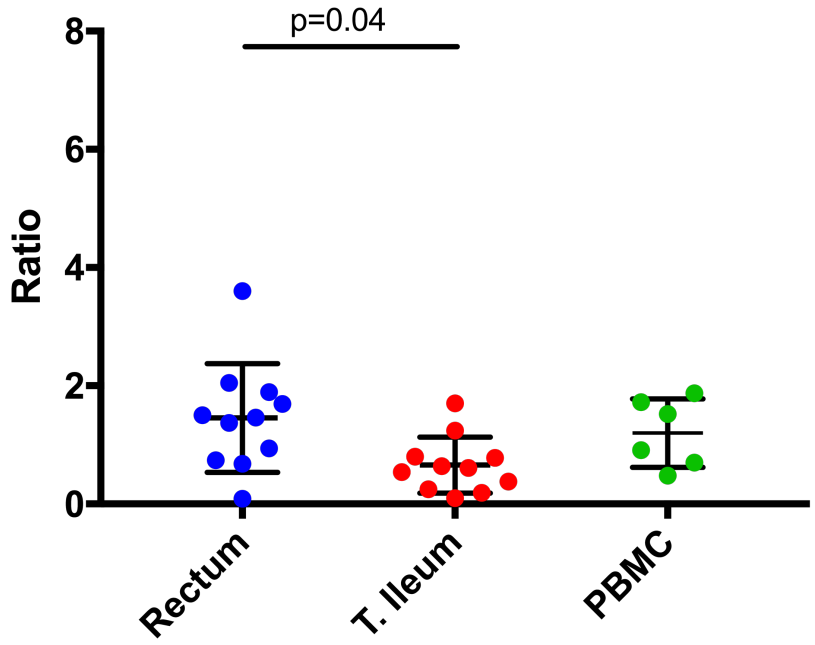
n=13

Immune recovery –
CD4/CD8 by Anatomical Site

HIV- CONTROLS

HIV POSITIVE

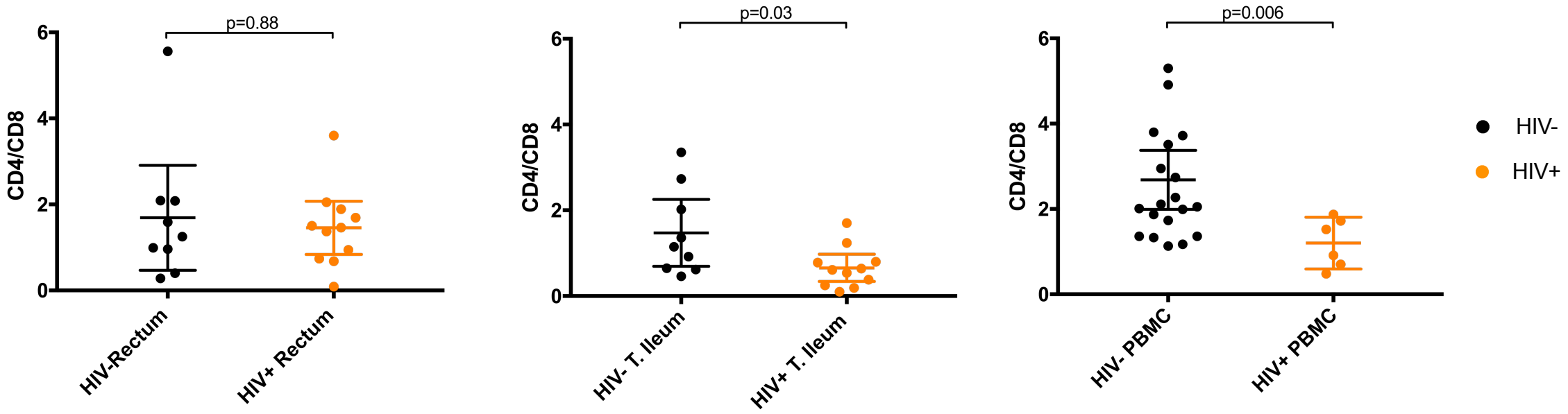




p values calculated using Kruskal-Wallis test. Corrected for multiple Comparisons

Differences in CD4/CD8 recovery by HIV Status

In treated PHI, CD4/CD8 ratio in rectal HIV+GALT is comparable with controls, however, CD4/CD8 remains significantly lower in terminal ileum GALT

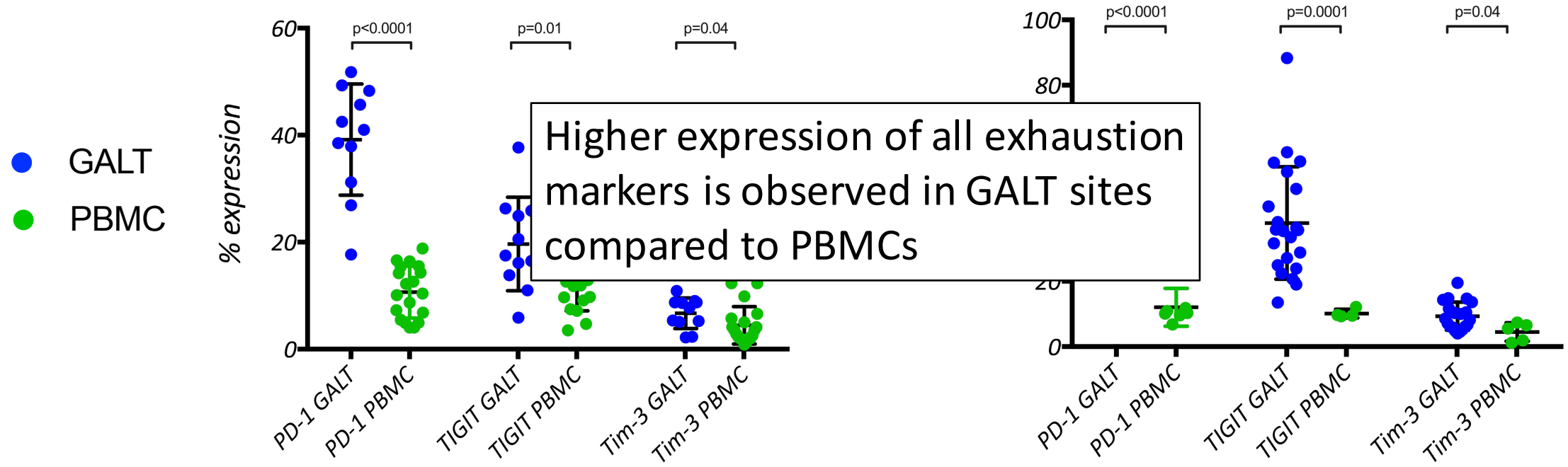


p values calculated using Mann-Whitney test

Immune exhaustion
on CD4+ T-cells across anatomical sites

HIV- CONTROLS

HIV POSITIVE

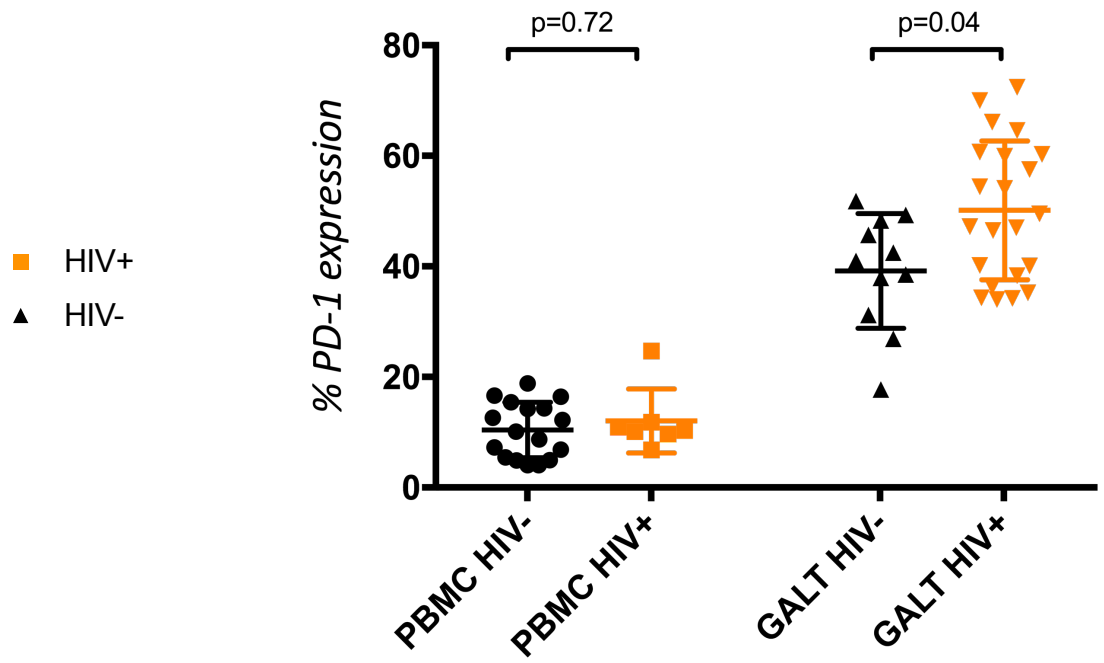


Higher expression of all exhaustion markers is observed in GALT sites compared to PBMCs

p values calculated using Mann-Whitney test

PD-1 expression on CD4+ T-cells

PD-1

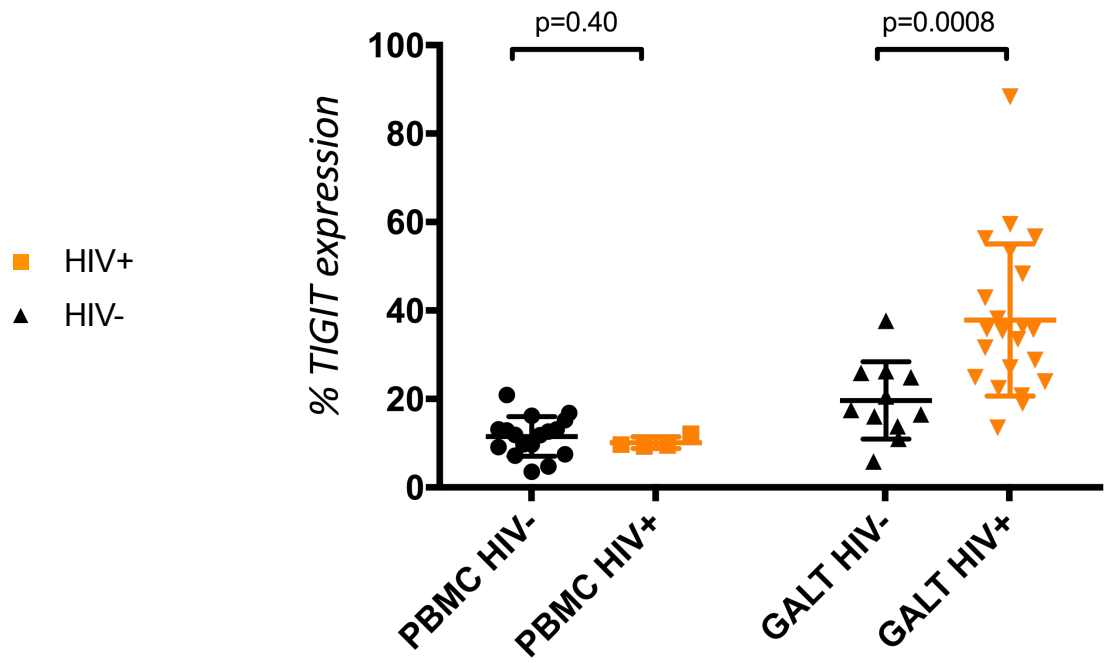


- No difference in PD-1 expression on CD4+ T-cells between HIV+ and HIV- PBMCs
- Higher PD-1 expression in HIV GALT compared to HIV- controls

p values calculated using Mann-Whitney test

TIGIT expression on CD4+ T-cells

TIGIT

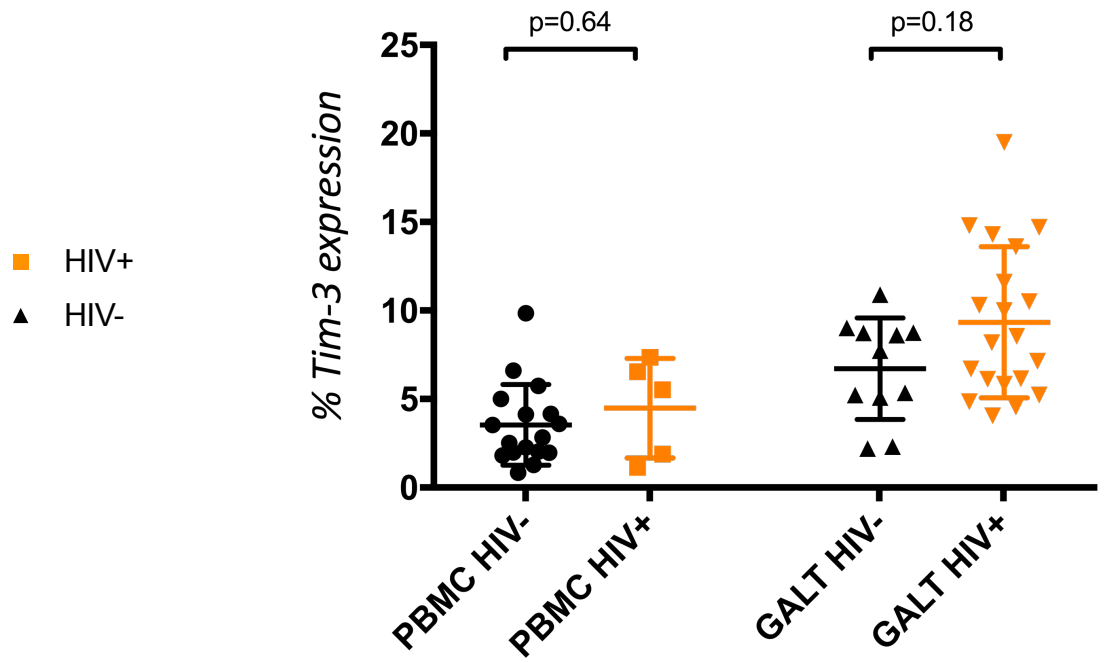


- No difference in TIGIT expression on CD4+ T-cells between HIV+ and HIV- PBMCs
- Higher TIGIT expression in HIV GALT compared to HIV- controls

p values calculated using Mann-Whitney test

Tim-3 expression on CD4+ T-cells

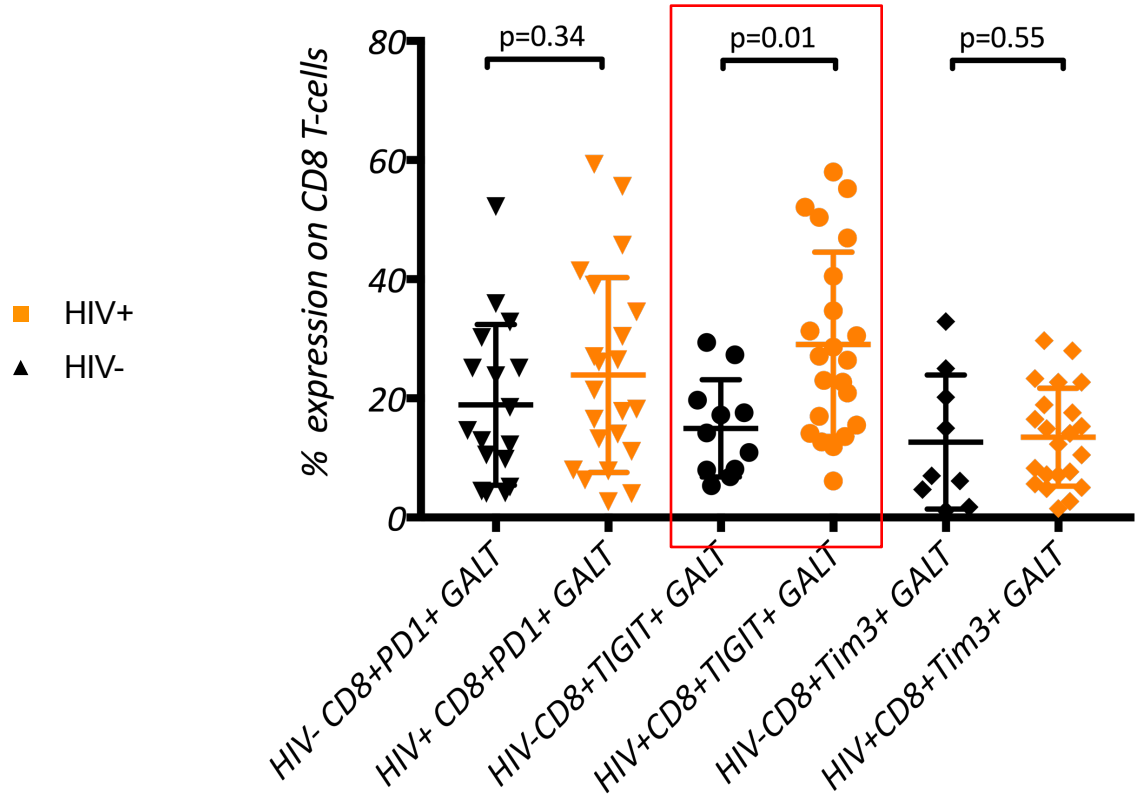
TIM-3



- No difference in Tim-3 expression on CD4+ T-cells in PBMCs or GALT between HIV+ and HIV- samples

p values calculated using Mann-Whitney test

Immune exhaustion
on CD8+ T-cells in GALT

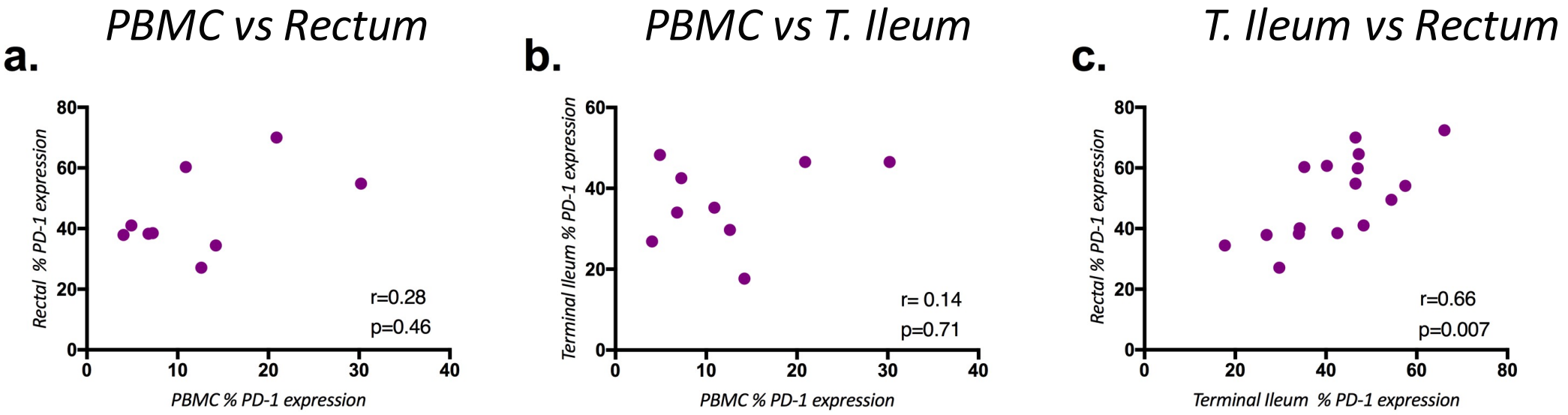


- CD8+TIGIT expression was higher in HIV+ GALT compared to HIV- GALT (p=0.01).
- The greatest difference observed in the TI (p=0.04) *data not shown*
- No differences were noted for PD-1 and Tim-3 expression

p values calculated using Mann-Whitney test

Exhaustion markers expression in peripheral blood does not reflect immune exhaustion in GALT

PD-1 expression in blood does not correlate with expression in GALT



Data for TIGIT andTim-3 not shown

r= spearman's rho

Summary

- Early ART results in restoration of CD4/CD8 in rectal tissue but not in the terminal ileum
- Greater expression of exhaustion markers is seen in gut tissue compared to peripheral blood
- Greater exhaustion marker expression was observed in HIV+ gut tissue compared to controls , despite early ART.
- No difference were seen in exhaustion marker expression in peripheral blood between HIV+ and HIV- participants

Conclusions

- Differential expression of immune exhaustion markers on T-cells by anatomical compartment may reflect and support differential levels of HIV persistence in GALT despite early ART initiation in PHI.

- Measurement of immune exhaustion markers in blood does not reflect expression in GALT and highlights the importance of tissue sampling in HIV cure studies.

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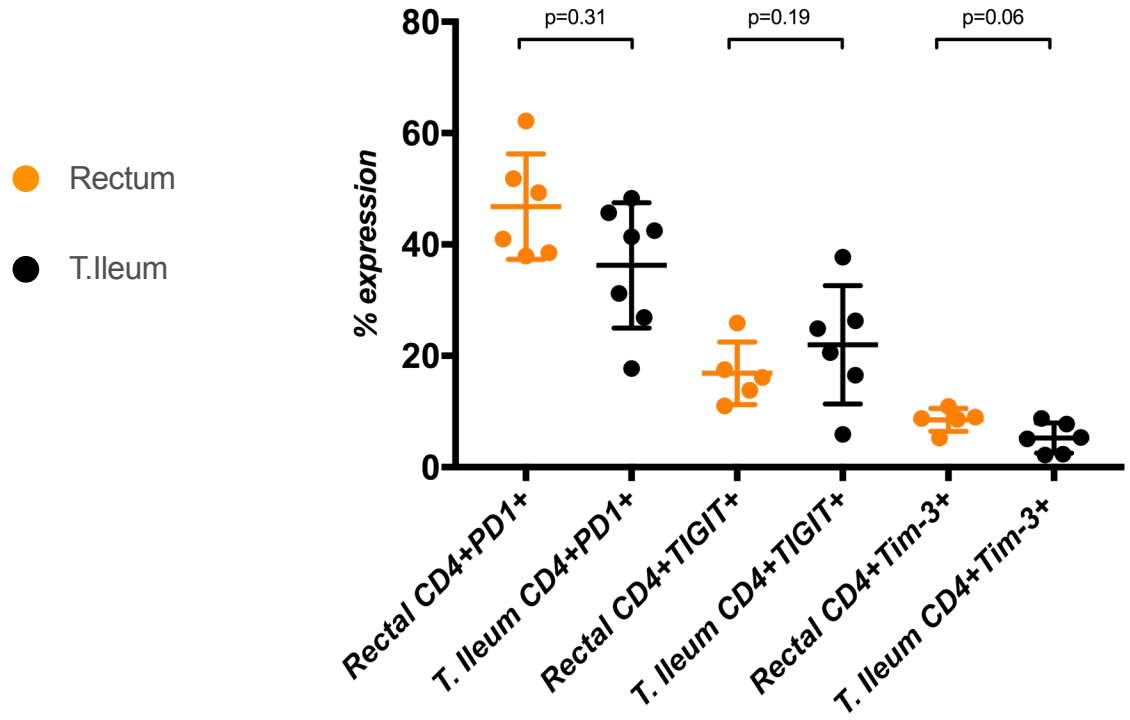
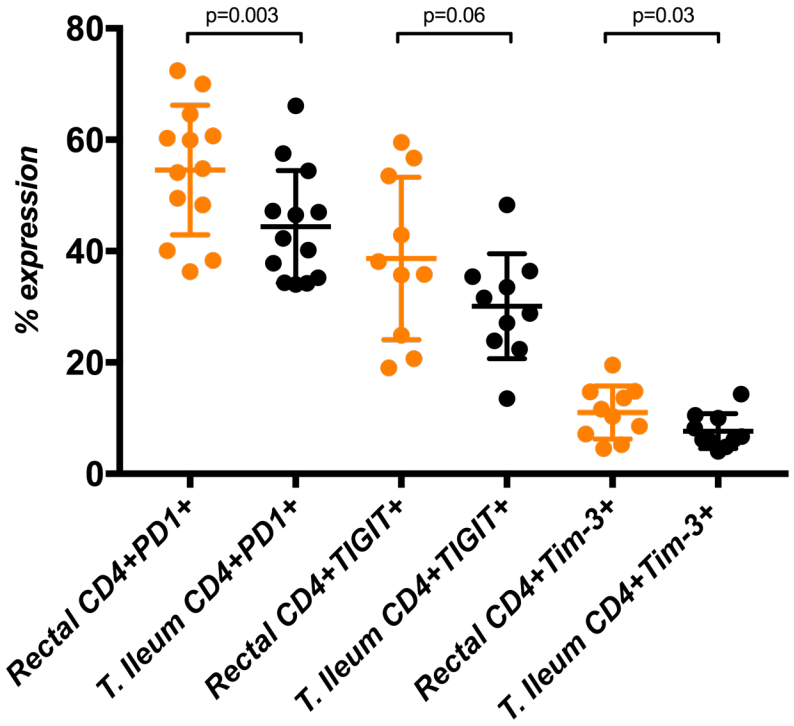
Back-up Slides

Expression of exhaustion markers on CD4+ T cells in Gut associated lymphoid tissue (GALT)

HIV negative GALT

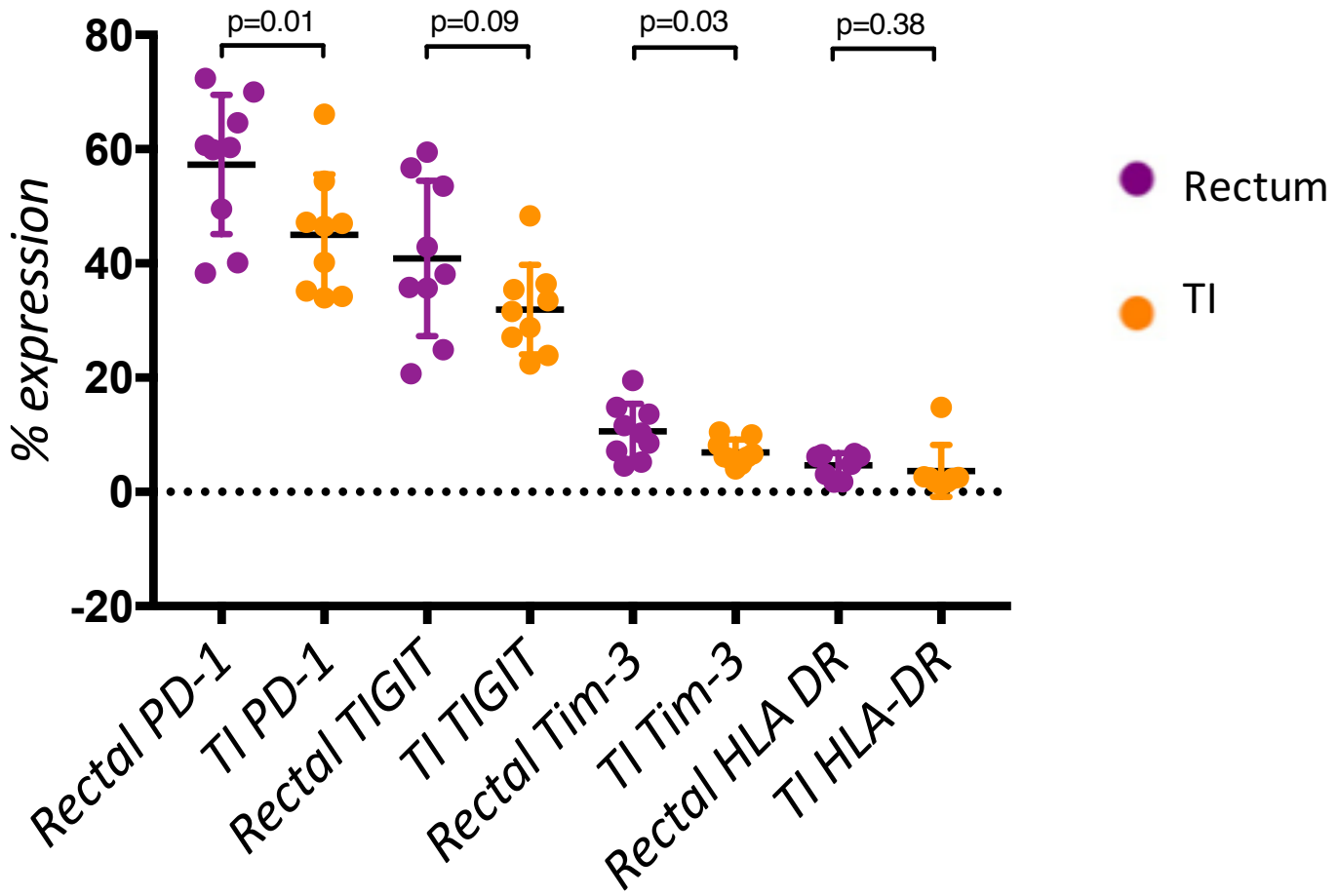


HIV positive GALT



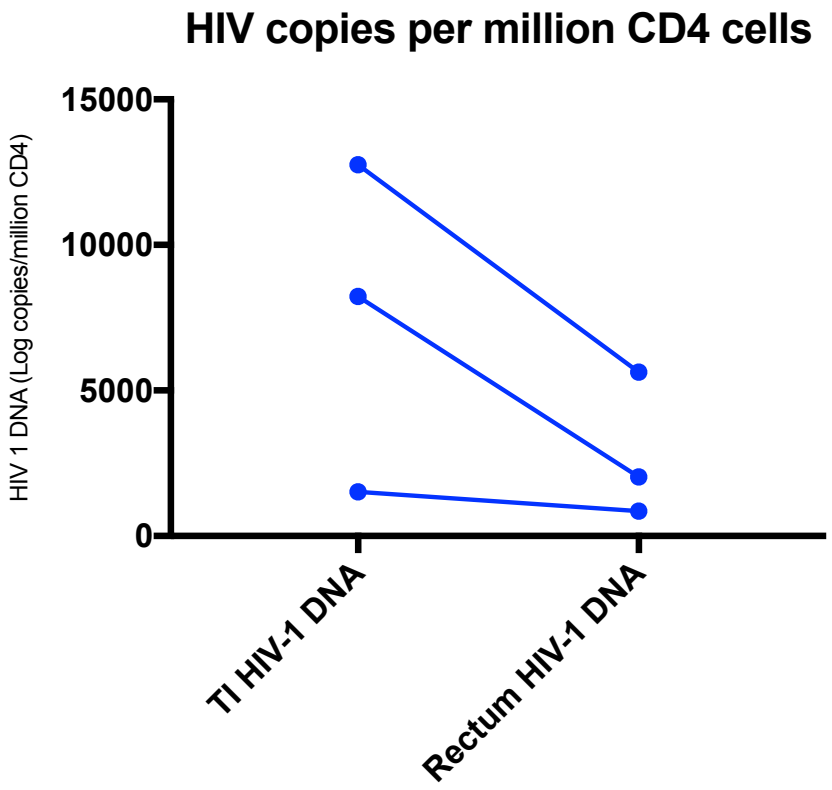
p values calculated using Wilcoxon matched-pairs signed rank test

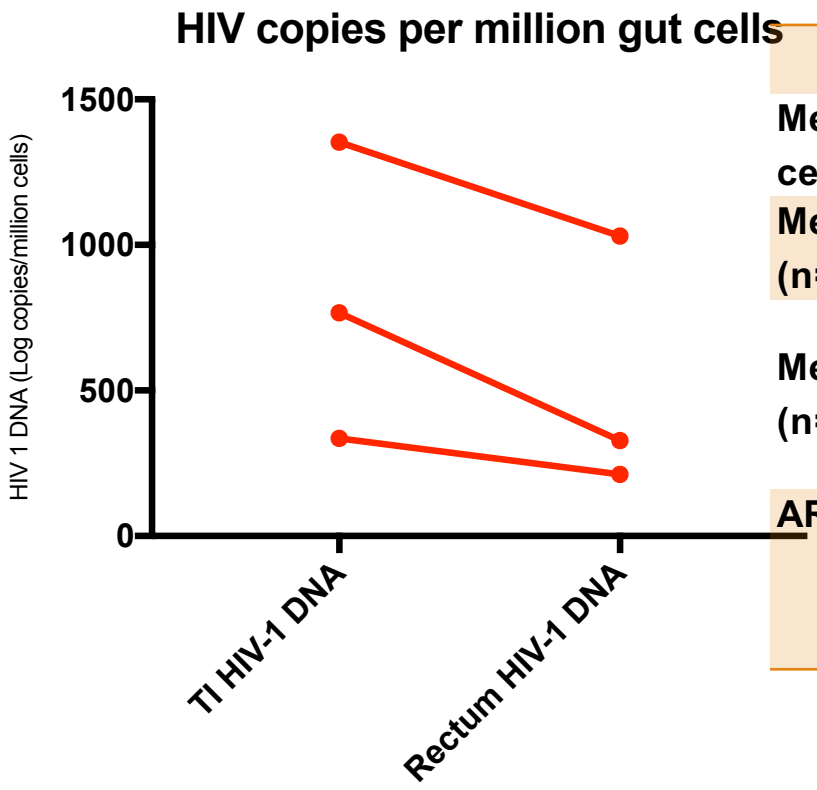
Higher expression of exhaustion markers on CD4 T-cells in rectal GALT compared with TI in HIV infected GALT



Wilcoxon test used to calculate p values, error bars represent mean and SD

Initial HIV-1 DNA results





HEATHE

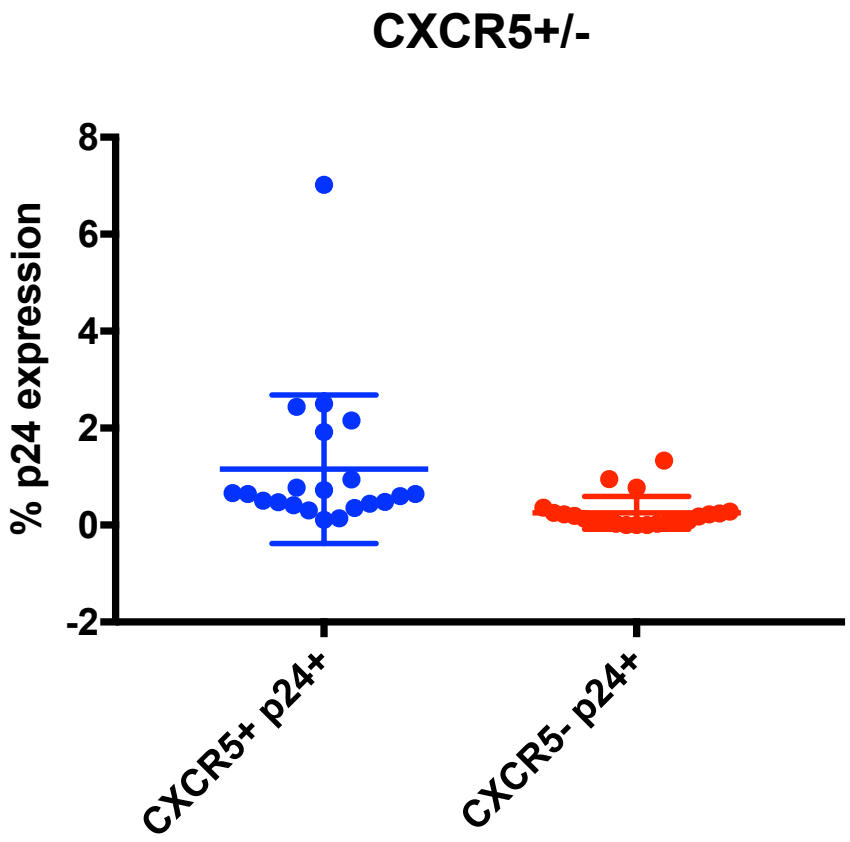
Median PBMC HIV-1 DNA
cells

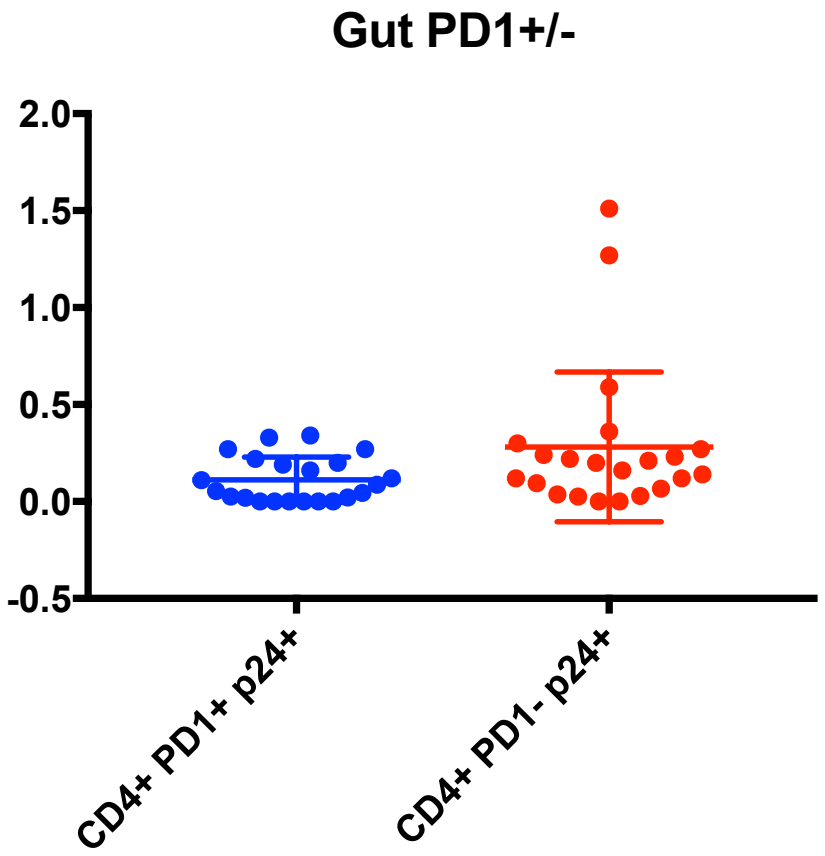
Median Rectal HIV-1 DNA
(n=3)

Median T. Ileum HIV-1 DNA
(n=3)

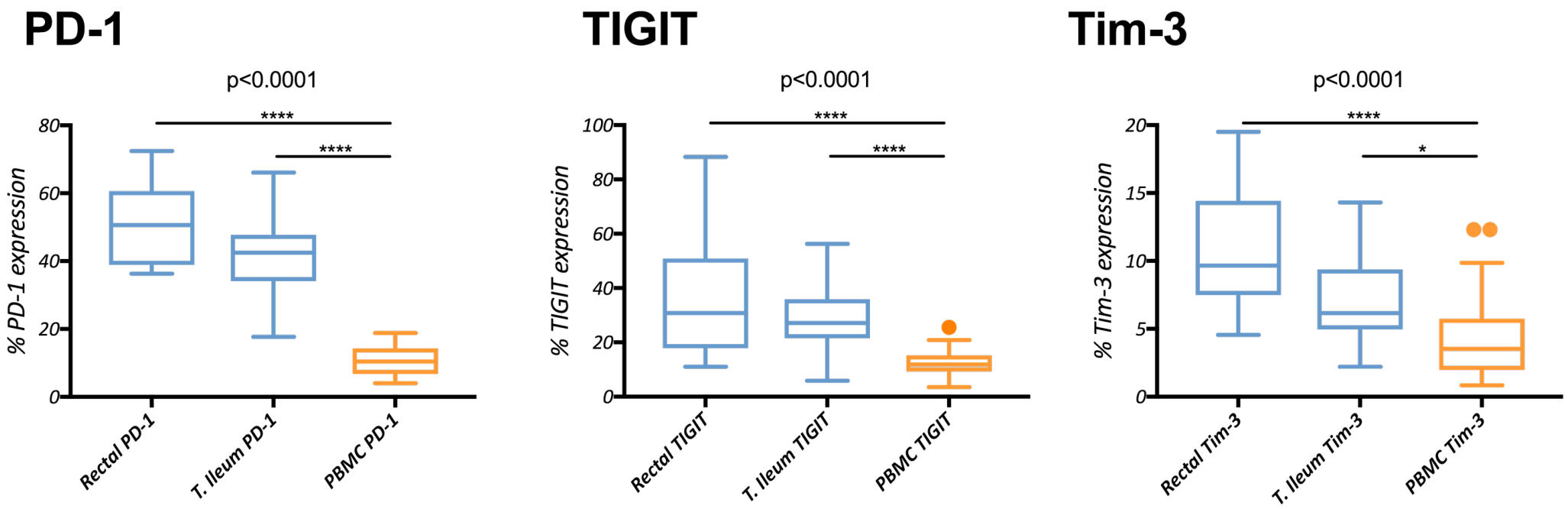
ART 3rd Agent: PI
NNRTI
INSTI

P24 expression by CD4+ T cell type





Exhaustion marker expression on CD4+ T-cells in terminal ileum and rectum compared to PBMC



Expression of all exhaustion markers is higher in each GALT site compared to PBMCs

p values calculated using Kruskal-Wallis test. Corrrected for multiple comparisons

Combined HIV= and HIV- samples

P24 expression by anatomical site