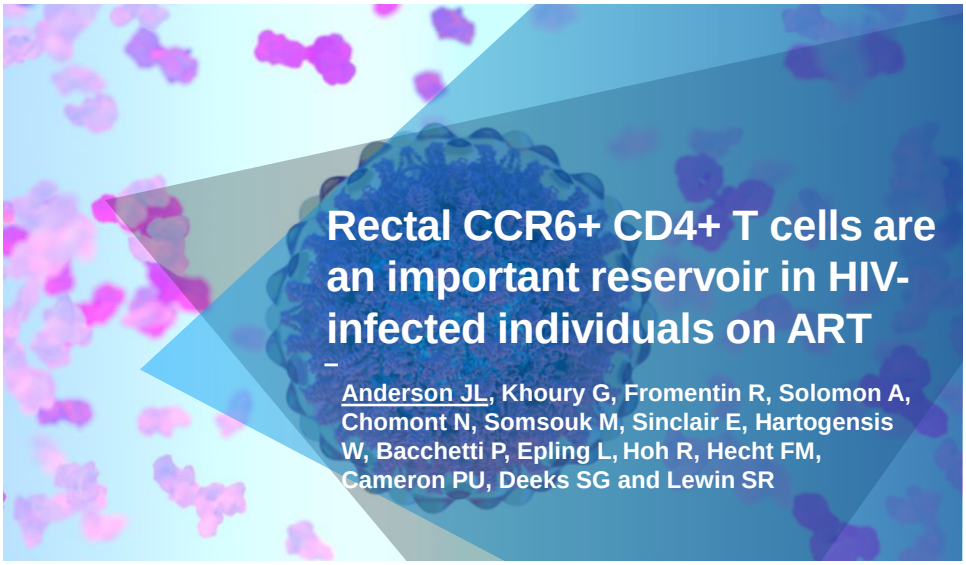


Rectal CCR6+ CD4+ T cells are an important reservoir in HIV-infected individuals on ART

Anderson JL, Khoury G, Fromentin R, Solomon A, Chomont N, Somsouk M, Sinclair E, Hartogensis W, Bacchetti P, Epling L, Hoh R, Hecht FM, Cameron PU, Deeks SG and Lewin SR

Doherty Institute
A joint venture between The University of Melbourne and The Royal Melbourne Hospital

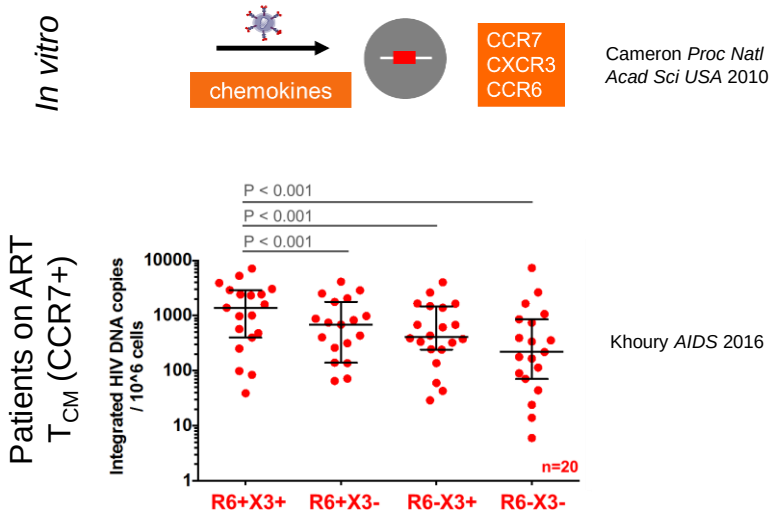
HIV/AIDS Research Community Acknowledgement

We are extremely grateful to people living with HIV who generously participated in this research via donation of essential blood, lymph node and rectal tissue.

Disclosure of Interest

SL and JA collaborate with Infinity Pharmaceuticals and Merck Pharmaceuticals to test drugs to purge the latent HIV reservoir unrelated to this study.

Chemokine receptors (CKR) influence persistent HIV reservoir in blood memory CD4+ T cells



CKR and their chemokines may also influence HIV persistence in tissue sites

- HIV infection enriched in immune tissues like **gut** & **LN** due to:
  - lower antiretroviral drug levels[1]
  - reduced access CD8+ T cells[2]
  - increased susceptibility T cell subsets[3] *eg*. LN T_{FH} cells, **CKR?**

Receptor	Chemokine	Site
CCR6	**CCL20**	**Mucosa**
CCR7	**CCL19** **CCL21**	**LN**
CXCR3	**CXCL9** **CXCL10** **CXCL11**	**Inflammatory**

[1] Fletcher *PNAS* 2014, Yukl *AIDS* 2010, Estes *Nat Med* 2017; [2] Connick *J Immunol* 2007, 2014; [3] Chun *JID* 2008, Yukl *JID* 2013, Yukl *PlosOne* 2015, Banga *NatMed* 2016; Gosselin *JImmunol* 2010, El Hed *JID* 2010, Monteiro *JImmunol* 2011, Khoury *AIDS* 2016

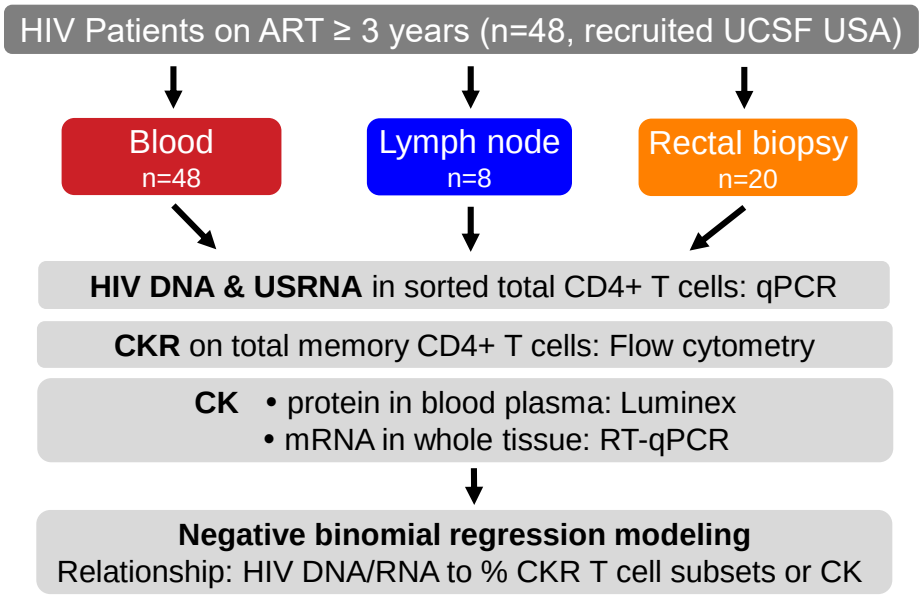
HYPOTHESIS:

Expression of specific CKR (CCR6, CCR7, CXCR3) on memory CD4+ T cells & their chemokines are associated with HIV persistence in **gut** & **LN** tissues in individuals on ART

AIMS:

1. Examine if the expression of CKR on memory CD4+ T cells & their chemokines are related to HIV reservoir size in **rectum** & **LN** of individuals on ART
2. Examine if subsets of **rectal** memory CD4+ T cells expressing CCR6 &/or CXCR3 CKR preferentially harbour persistent HIV in individuals on ART

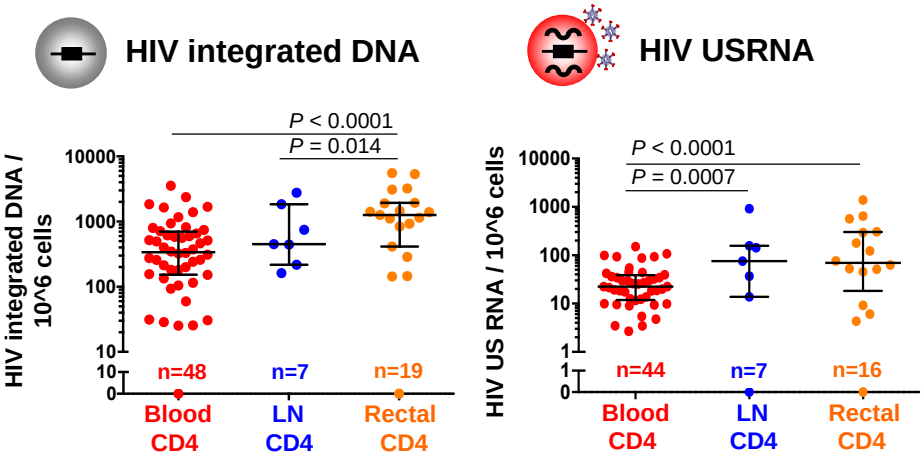
AIM 1: Study Design



Participant demographics

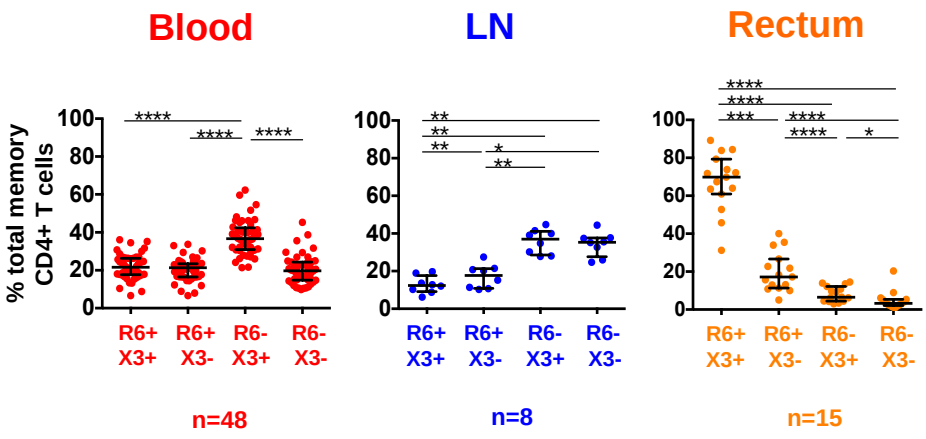
PARAMETER		BLOOD n = 48	LN n = 8	RECTUM n = 20
Age, years		57	58	58
Gender, n (%):	Male	46 (96%)	8	20
	Female	1 (2%)	0	0
	Transgen.	1 (2%)	0	0
ART, years		8.5	10.9	11.8
Viral Load, copies / ml		< 40	< 40	< 40
CD4 T-cells: Nadir, cells/µl		216	134	137
Current, cells/µl		684	549	639
Current %		32	25	30
CD8 T-cells: Current, cells/µl		914	1069	988
CD4/CD8 T-cell count		0.77	0.55	0.67

HIV integrated DNA & US RNA elevated in rectal CD4+ T cells versus blood



* **Consistent with:** Chun *JID* 2008, Yukl *JID* 2010, Yukl *JID* 2013

Rectal tissue enriched in CCR6+ CXCR3+ memory CD4+ T cells



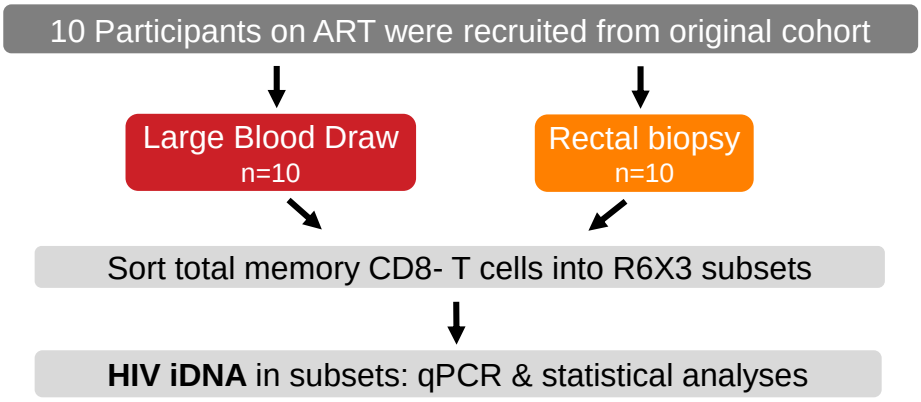
Wilcoxon matched-pairs signed rank
**** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05

Persistent HIV DNA & RNA associated with CD4+ T cell subsets expressing CCR6/CXCR3 in rectum

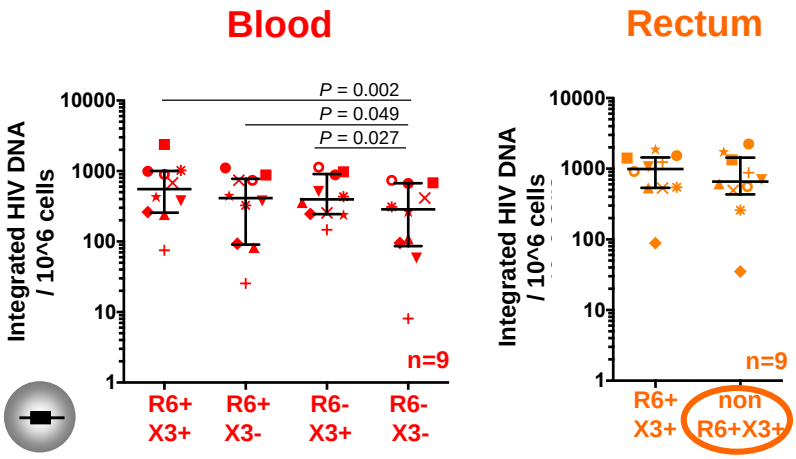
TISSUE	CKR	HIV RESERVOIR		
		iDNA	CA-US RNA	CA-US RNA: iDNA
LN	R6+X3+	0.96 (0.73 to 1.26) *p=0.79*	0.70 (0.44 to 1.09) *p=0.11*	0.84 (0.67 to 1.06) *p=0.15*
	R6+X3-	0.95 (0.84 to 1.07) *p=0.41*	**0.84 (0.71 to 1.00) *p=0.046* ***	0.90 (0.80 to 1.02) *p=0.11*
	R6-X3+	1.01 (0.92 to 1.12) *p=0.79*	1.13 (0.97 to 1.31) *p=0.12* *	1.09 (0.98 to 1.20) *p=0.10*
	R6-X3-	1.09 (0.92 to 1.29) *p=0.31*	1.34 (0.92 to 1.95) *p=0.13*	1.06 (0.89 to 1.26) *p=0.50*

Negative Binomial Regression Modeling
Fold change in HIV reservoir outcome per unit increase in % CKR memory T cell predictor
* *p<0.05* observed in models adjusted for current &/or nadir CD4 count

AIM 2: Is persistent HIV enriched in specific CCR6/CXCR3 T cell subsets in rectum?

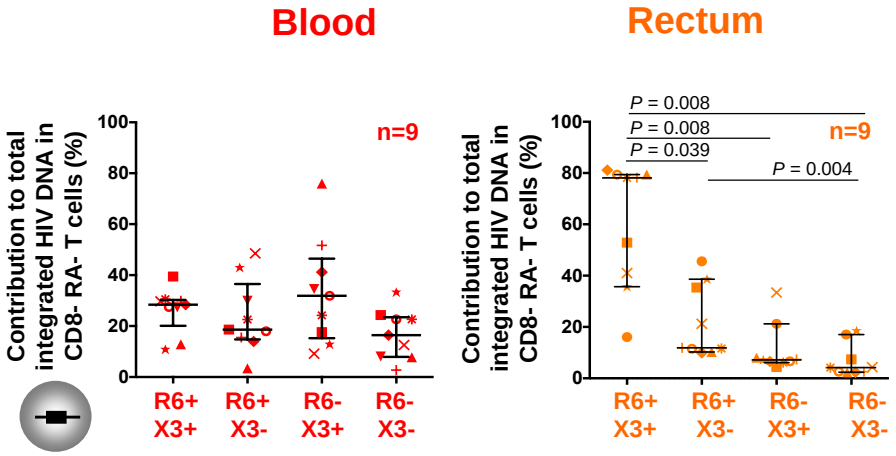


HIV iDNA not significantly enriched in rectal CCR6+CXCR3+ T cells



Wilcoxon matched-pairs signed rank
**** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05

CCR6+CXCR3+ T cells are a major contributor to the total HIV iDNA reservoir in rectum



Mann-Whitney test
**** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05

Conclusions and Implications

1. **Rectum** is a significant site of persistent HIV with high DNA and RNA
2. **Rectal** CCR6+CXCR3+ T cells **not** enriched in HIV DNA vs pooled remaining T cell subsets but still a **major contributor** to the total HIV DNA reservoir in rectum due to the high frequency of these cells
3. **Rectal** HIV DNA and RNA **positive** association with % CCR6+CXCR3- T cells but **negative** association % CCR6+CXCR3+ T cells
   - as HIV DNA enriched in **blood** CCR6+CXCR3+ T cells, defective trafficking of CCR6+CXCR3+ cells between blood and rectum?
4. **As LN** have a **different** distribution of T cell subsets and chemokines to rectum, **tissue specific mechanisms** may account for HIV persistence in these two sites
5. **Future studies** will focus on interventions that interfere with trafficking of CCR6+ T cells to the GI tract, including anti-CCL20 (GSK)

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Delaney AIDS Research Enterprise DARE to find a cure

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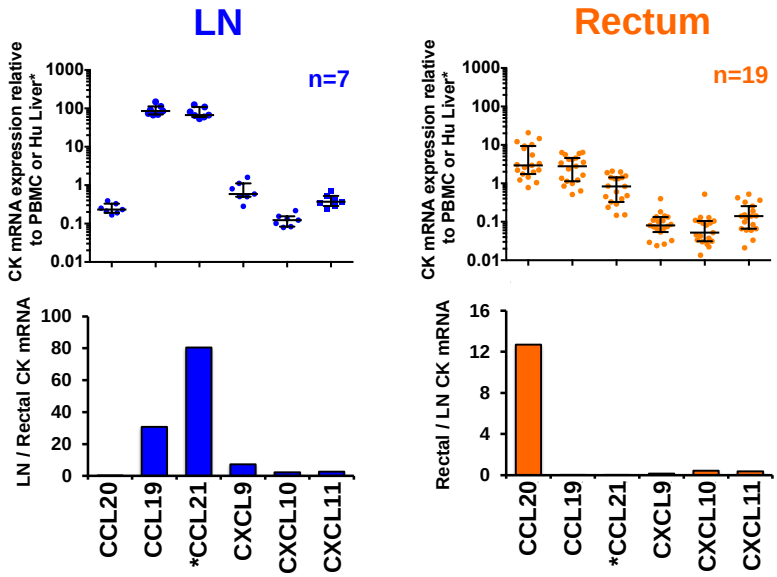
Australian Government National Health and Medical Research Council NHMRC

Rectal tissue enriched with CCL20 mRNA while LN tissue enriched with CCL19 & CCL21 mRNA



LN: HIV Reservoir related to expression of CCL5, CCL19, CCL21 or CXCL13 CK mRNA

TISSUE	CK	HIV RESERVOIR		
		iDNA	CA-US RNA	CA-US RNA: iDNA
LN	CCL5	**0.20 (0.05 to 0.84) *p=0.028*** *	0.15 (0.02 to 1.22) *p=0.076*	0.71 (0.06 to 9.15) *p=0.79*
	CCL20	0.30 (0.09 to 1.00) *p=0.050* *	0.26 (0.04 to 1.74) *p=0.17*	0.97 (0.18 to 5.29) *p=0.97*
	CCL19	**0.15 (0.05 to 0.50) *p=0.0017*** *	**0.05 (0.01 to 0.30) *p=0.0011*** *	0.37 (0.07 to 2.09) *p=0.26*
	CCL21	0.33 (0.10 to 1.07) *p=0.064* *	**0.10 (0.01 to 0.73) *p=0.024*** *	0.49 (0.10 to 2.29) *p=0.36*
	CXCL9	0.88 (0.22 to 3.54) *p=0.86*	5.10 (0.40 to 64.39) *p=0.21*	2.20 (0.79 to 6.09) *p=0.13*
	CXCL10	0.88 (0.13 to 6.10) *p=0.89*	1.48 (0.06 to 34.51) *p=0.81*	1.48 (0.29 to 7.53) *p=0.63*
	CXCL11	0.50 (0.12 to 2.07) *p=0.34*	0.71 (0.05 to 9.73) *p=0.80*	2.26 (0.50 to 10.23) *p=0.29*
	CXCL12	0.64 (0.36 to 1.14) *p=0.13*	0.45 (0.17 to 1.16) *p=0.10*	0.81 (0.39 to 1.68) *p=0.56*
	CXCL13	1.29 (0.57 to 2.95) *p=0.54*	**3.94 (1.11 to 14.04) *p=0.034*** *	2.02 (0.72 to 5.69) *p=0.18*

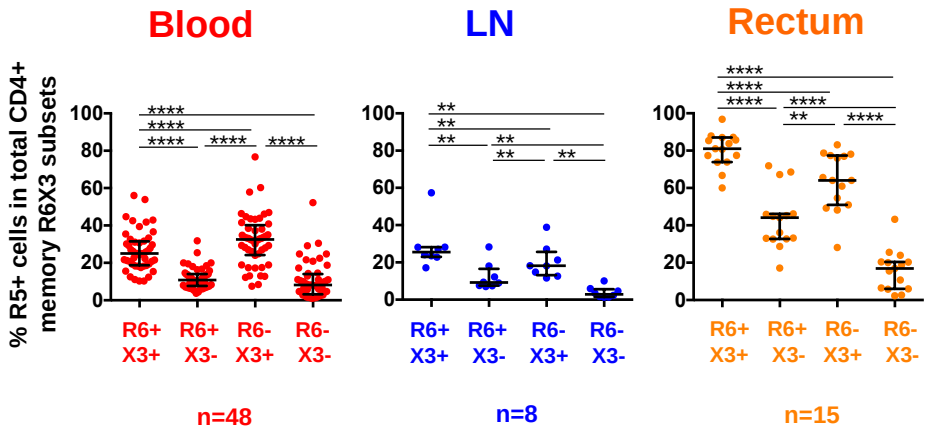
Negative Binomial Regression Modeling
Fold change in HIV reservoir outcome per 2 fold increase in CK mRNA predictor
* *p<0.05* observed in models adjusted for current or nadir CD4 count

RECTUM: HIV Reservoir not related to current expression of chemokine mRNA

TISSUE	CK	HIV RESERVOIR		
		iDNA	CA-US RNA	CA-US RNA: iDNA
RECTUM	CCL5	1.07 (0.52 to 2.20) *p=0.85*	1.81 (0.50 to 6.61) *p=0.37*	1.35 (0.51 to 3.58) *p=0.55*
	CCL20	0.97 (0.68 to 1.37) *p=0.85*	0.92 (0.41 to 2.05) *p=0.83*	1.05 (0.67 to 1.66) *p=0.83*
	CCL19	1.15 (0.84 to 1.58) *p=0.37*	1.25 (0.63 to 2.52) *p=0.52*	1.07 (0.65 to 1.76) *p=0.78*
	CCL21	0.83 (0.61 to 1.12) *p=0.22*	0.92 (0.52 to 1.65) *p=0.79*	1.07 (0.69 to 1.66) *p=0.77*
	CXCL9	1.19 (0.76 to 1.88) *p=0.44*	1.54 (0.82 to 2.90) *p=0.18*	1.29 (0.65 to 2.57) *p=0.47*
	CXCL10	1.25 (0.85 to 1.82) *p=0.26*	1.95 (0.98 to 3.89) *p=0.057*	1.38 (0.77 to 2.48) *p=0.28*
	CXCL11	1.24 (0.90 to 1.72) *p=0.19*	1.68 (0.97 to 2.90) *p=0.063*	1.29 (0.80 to 2.08) *p=0.30*
	CXCL12	0.86 (0.34 to 2.14) *p=0.74*	0.28 (0.03 to 2.49) *p=0.25*	0.64 (0.20 to 2.07) *p=0.46*
	CXCL13	1.05 (0.81 to 1.35) *p=0.73*	1.21 (0.79 to 1.85) *p=0.39*	1.12 (0.80 to 1.56) *p=0.51*

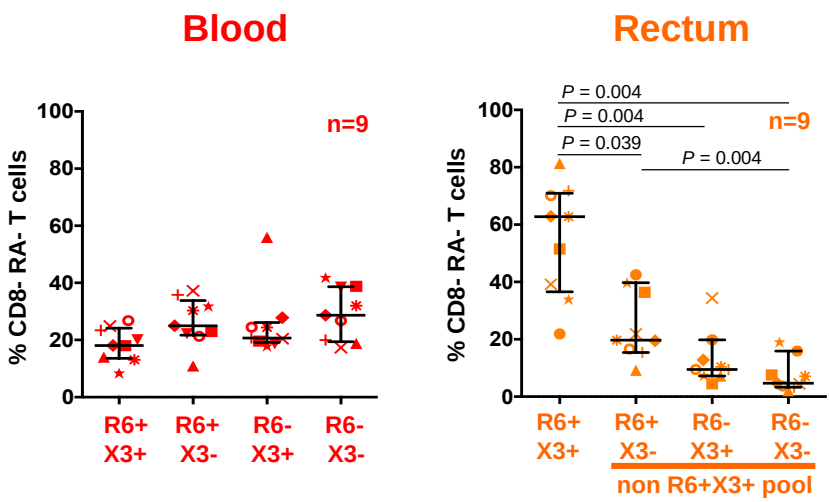
Negative Binomial Regression Modeling
Fold change in HIV reservoir outcome per 2 fold increase in CK mRNA predictor
∗ *p<0.05* observed in models adjusted for current &/or nadir CD4 count

Rectal CCR6+ CXCR3+ memory CD4+ T cells also enriched in CCR5



Wilcoxon matched-pairs signed rank
****** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05**

Rectal tissue enriched in CCR6+ CXCR3+ CD8- total memory T cells



Wilcoxon matched-pairs signed rank
**** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05

Calculating relative contribution of a T cell subset to total HIV iDNA reservoir in all T cell subsets

1. Determine relative pool of HIV infected cells per T cell subset

(HIV iDNA per 10^6 cells in T cell subset) x (subset frequency) / 100

ie. assess for each T cell subset: R6+X3+, R6+X3-, R6-X3+ or R6-X3-

2. Determine sum of relative pool of infected cells in ALL T cell subsets

ie. add individual relative pool of infected cells for **4 R6X3 subsets**

3. Determine relative contribution of T cell subset to total HIV iDNA in all subsets

$$\left(\text{Relative pool of HIV infected cells per T cell subset}\right) / \left(\text{Sum of relative pool of HIV infected cells for ALL T cell subsets}\right) \times 100$$