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Alterations in the balance of Th1 cells to Th17 and Th22 cells in HIV-1/HCV co-infection is associated with immune activation, microbial translocation and liver fibrosis

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Background

- HIV/HCV: more rapid & frequent progression to liver fibrosis¹
- Mechanisms driving liver fibrosis likely multiple & complex
- Increased microbial translocation may drive liver fibrosis:
 - promoting systemic immune activation²⁻⁴
 - LPS signalling via TLR4 in the liver⁵
- Th17⁶ & Th22⁷ cells:
 - Integral to maintaining the immune integrity of the gut mucosa
 - Depletion in HIV may lead to increased microbial translocation
- CD4 T cell subsets have interdependent relationships
 - Ratios more important than proportions in determining immune control
 - Th17 & Th1 cells have reciprocal relationship

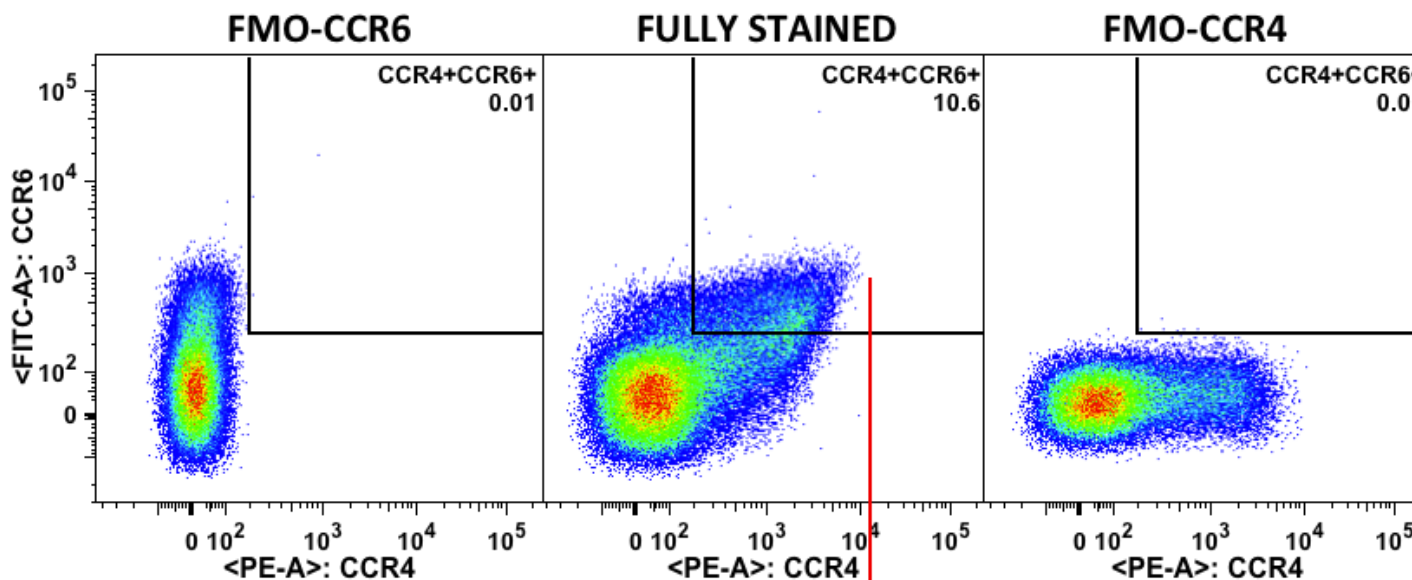
Hypothesis

Alterations in Th1, Th17 and Th22 cells in HIV-1 infection are associated with microbial translocation and immune activation and the rapid development of fibrotic liver disease in HIV-1/HCV co-infection

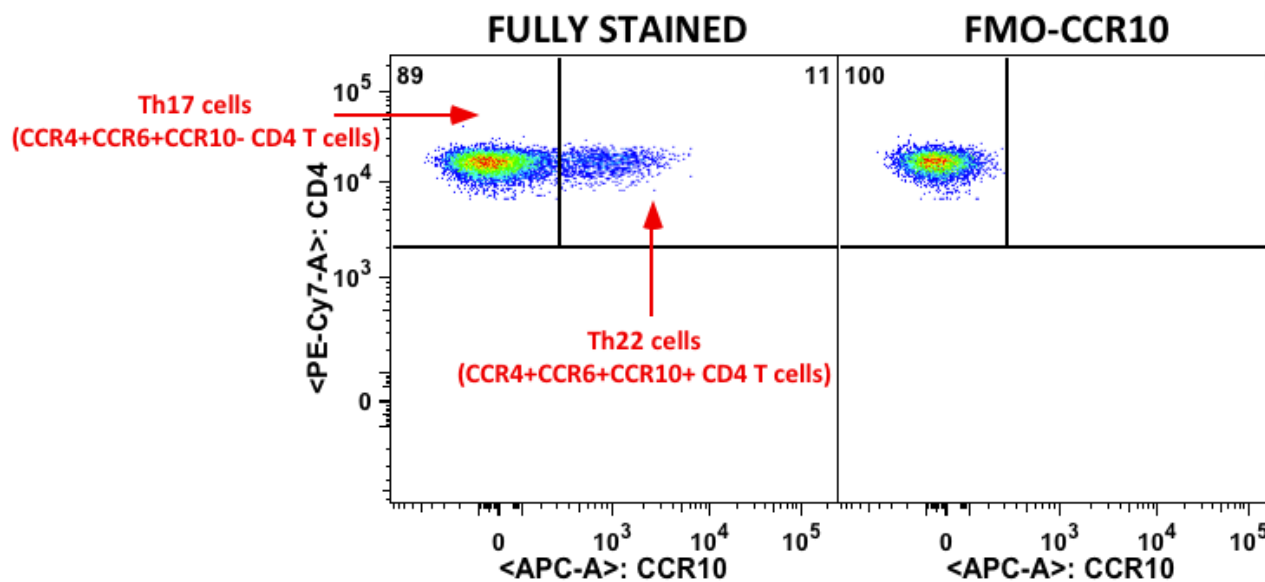
Methods (1)

- Cross-sectional study
- Groups:
 1. HC
 2. HCV
 3. HIV ART
 4. HIV NAÏVE
 5. HCV HIV ART
 6. HCV HIV NAÏVE
- Blood samples:
 - 6 colour flow cytometry to determine CD4 T cell subsets frequencies:
 1. Th1 (CXCR3+CCR5+)
 2. Treg (CD25+CD127lo)
 3. Th17 (CCR4+CCR6+CCR10-)
 4. Th22 (CCR4+CCR6+CCR10+)

CD4 T cells



CCR4+CCR6+ CD4 T cells



Methods (3)

- Blood samples (continued):
 - ELISAs on serum
 - Neopterin (marker of immune activation)
 - LBP (markers of microbial translocation)
- FibroScan
- Statistical analysis: (Graphpad Prism version 5.0)
 - Categorical variables: Chi-squared analysis / Fisher's exact test
 - Continuous variables: Kuskal Wallis test with Dunn's post test
(to correct for multiple comparisons)
 - Correlations: Spearman's rank correlation coefficient
($r = +/- 0.5000$ taken as significant)

Baseline demographic and clinical data

TOTAL (n=101)	HC (n=16)	HCV (n=21)	HIV ART (n=16)	HIV NAÏVE (n=20)	HCV HIV ART (n=18)	HCV HIV NAÏVE (n=10)
AGE in years Median (IQR)	40.5 (12.35)	52.4 (8.39)	47.7 (11.44)	40.3 (8.09)	44.9 (6.39)	43.7 (7.81)
GENDER Male: n (%)	15 (94)	16 (76)	16 (100)	16 (80)	17 (94)	9 (90)
ETHNICITY White: n (%) Hispanic: n (%) Black: n (%)	15 (94) 0(0) 0(0)	13 (62) 3 (14) 1 (5)	13 (81) 2 (13) 1 (6)	16 (80) 1 (5) 2 (10)	14 (77) 3 (17) 0 (0)	7 (70) 3 (30) 0 (0)
CD4 %: Median (IQR) cells/µl: Median (IQR)	48.2 (42.1-52.1) 777 (561-950)	45.1 (35.4-56.1) 781 (501-1081)	37.7 (29.2-42.5) 619 (542-880)	15.7 (13.5-20.7) 283 (169-338)	35.2 (13.5-20.7) 646 (519-919)	27.8 (17.4-32.5) 434 (369-709)
CD4:CD8 Median (IQR)	1.72 (1.51-2.75)	1.99 (1.14-3.19)	0.96 (0.64-1.33)	0.25 (0.19-0.39)	0.80 (0.57-1.22)	0.46 (0.36-0.74)
Years since HIV diagnosis Median (IQR)	NA	NA	12.7 (8.3-16.2)	5.1 (2.8-9.0)	9.6 (7.4-15.5)	4.9 (2.8-9.4)
Years on ART Median (IQR)	NA	NA	8.0 (3.2-11.3)	NA	9.6 (3.7-11.6)	NA
Years since HCV diagnosis Median (IQR)	NA	11.0 (7.5-16.0)	NA	NA	8.0 (5.0-10.3)	4.5 (2.4-6.0)
HCV Genotype 1: n (%) 2: n (%) 3: n (%) 4: n (%)	NA	15 (71) 0 (0) 1 (5) 5 (24)	NA	NA	17 (94) 0 (0) 0 (0) 1 (6)	8 (80) 1 (10) 1 (10) 0 (0)

P < 0.001

P < 0.01

P < 0.001

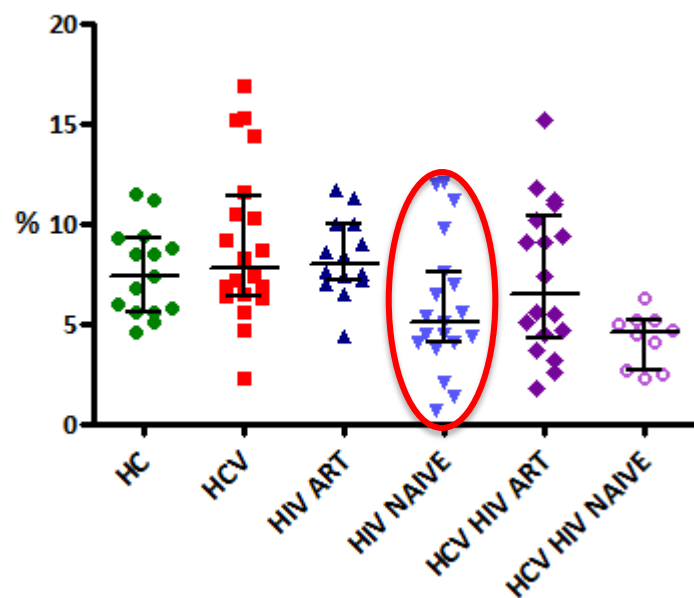
P < 0.001

P < 0.001

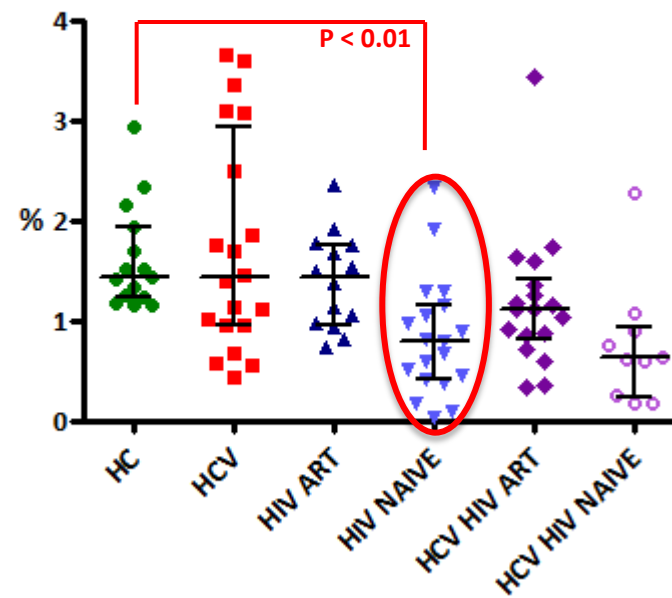
P < 0.001

Frequency of Th17 & Th22 cells

Frequency (%) of Th17 (CCR4+CCR6+CCR10-) cells



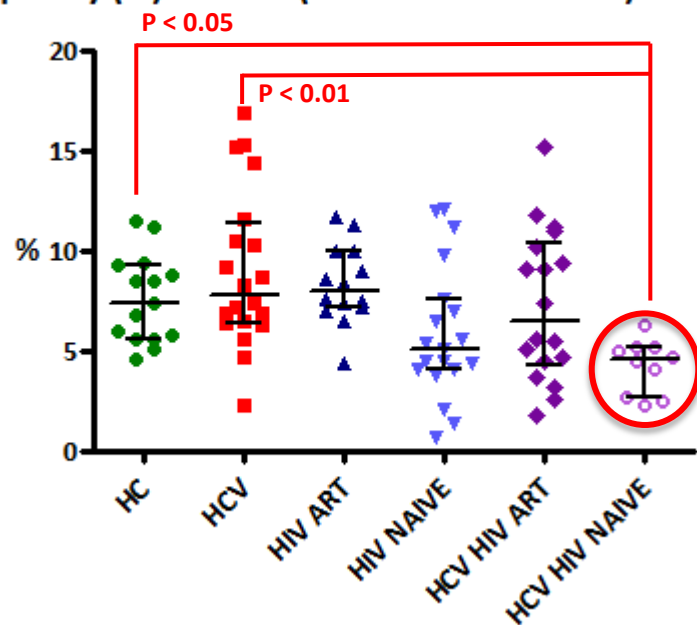
Frequency (%) of Th22 (CCR4+CCR6+CCR10+) cells



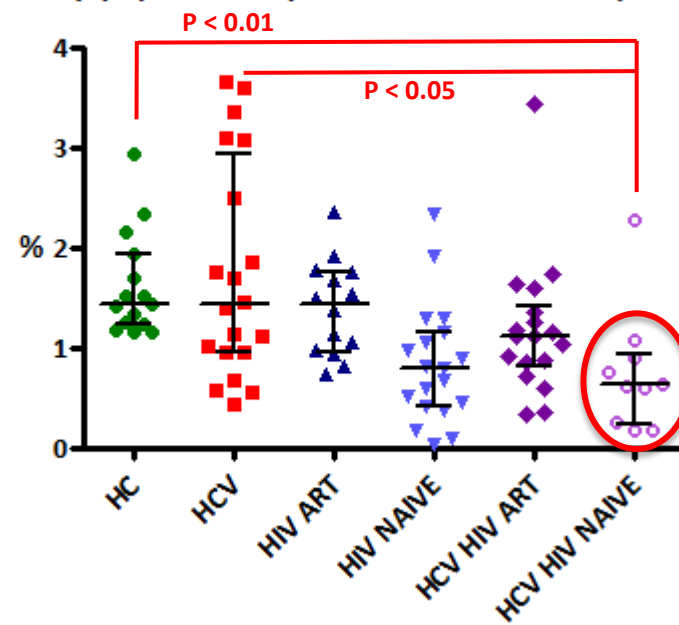
- No difference in Th17 or Th22 cells in HCV mono-infection
- Trend to reduced Th17 cells in HIV mono-infection
 - Confirmed with functional tests: IL-21+CD4 T cells
- Th22 cells depleted in HIV mono-infection
 - Confirmed with functional tests: IL-22+CD4 T cells

Frequency of Th17 & Th22 cells

Frequency (%) of Th17 (CCR4+CCR6+CCR10-) cells

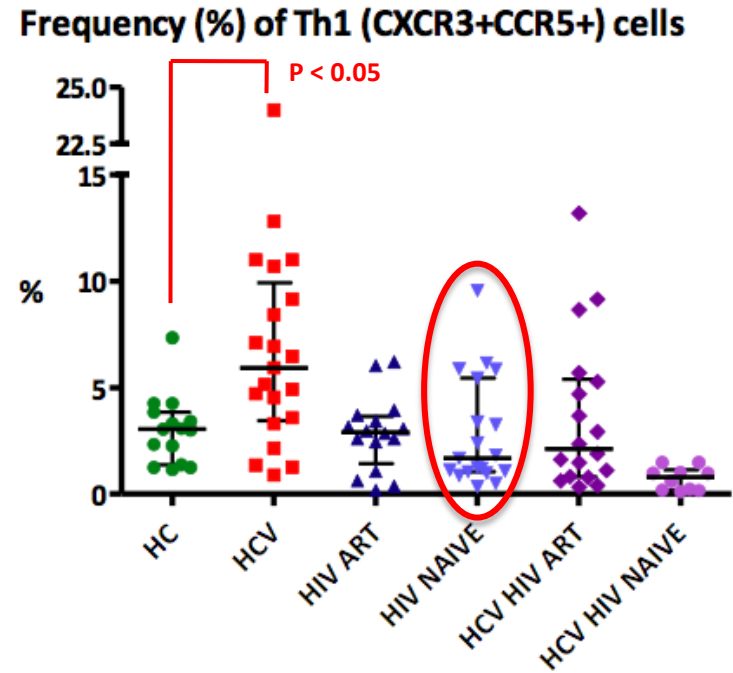
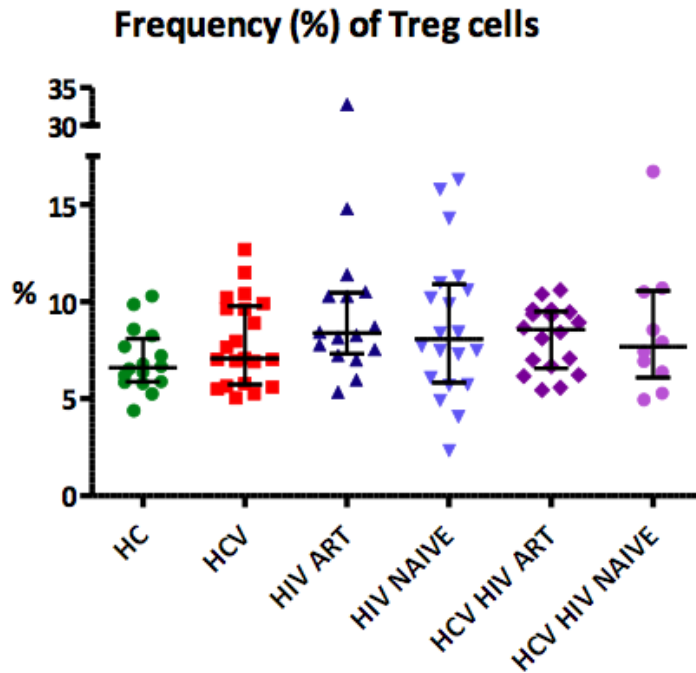


Frequency (%) of Th22 (CCR4+CCR6+CCR10+) cells



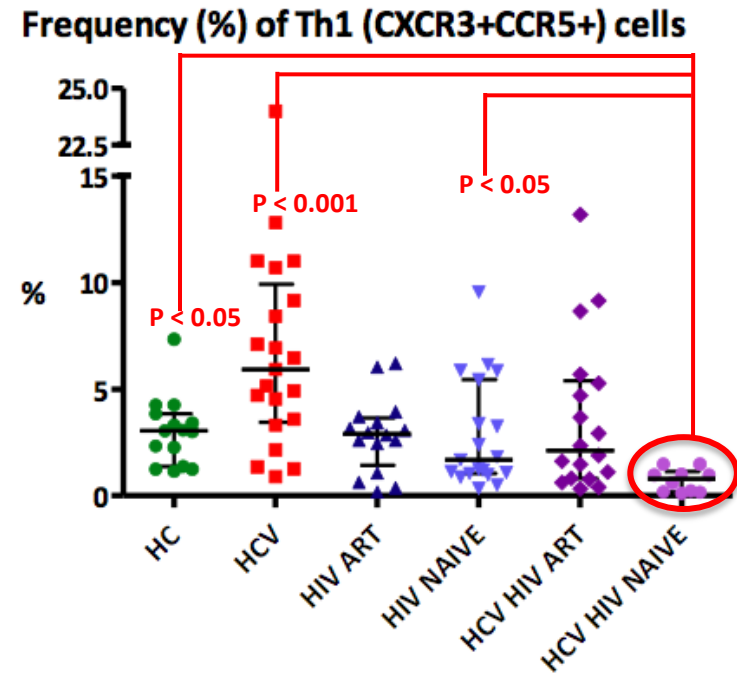
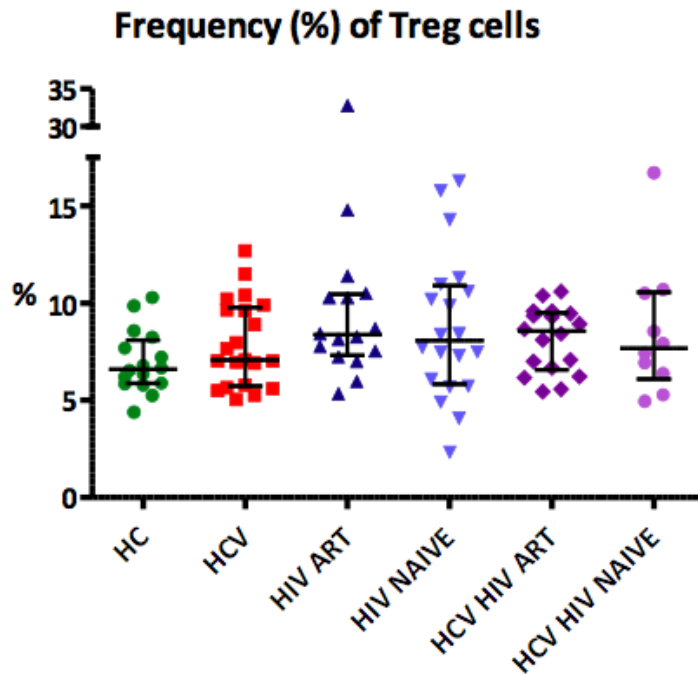
- Th17 and Th22 cells depleted in HIV/HCV co-infection
 - Confirmed with functional tests: IL-21+CD4 T cells & IL-22+CD4 T cells

Frequency of Treg & Th1 cells



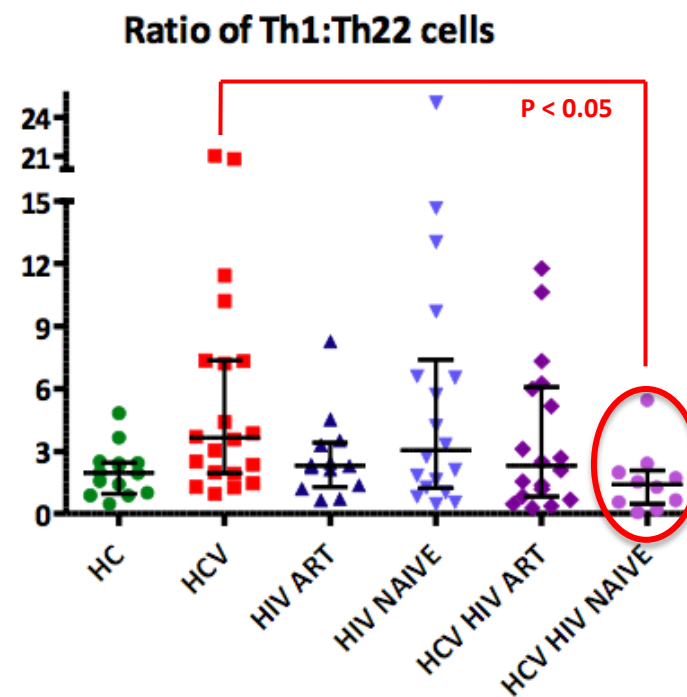
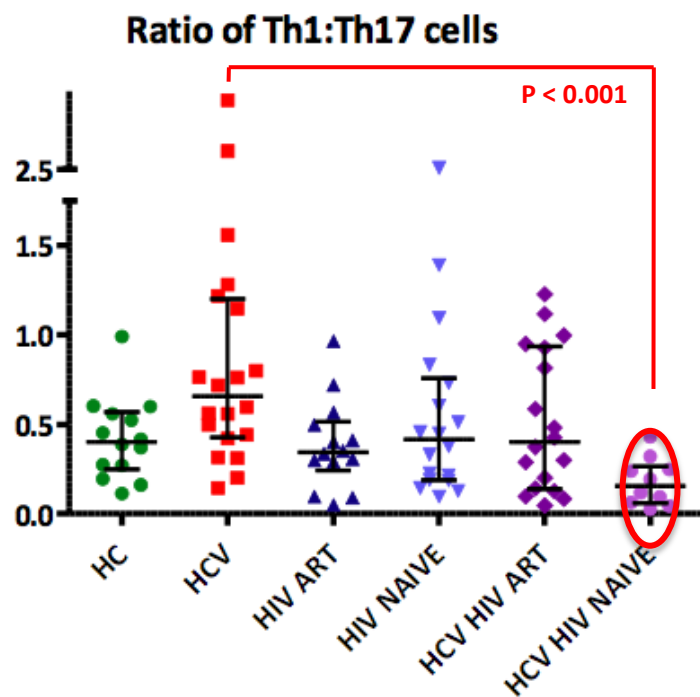
- No difference between groups in Treg cells
- Th1 cells increased in HCV
- Th1 cells unchanged in HIV

Frequency of Treg & Th1 cells



- Marked depletion of Th1 cells in HIV/HCV co-infection
 - Striking compared to HCV mono-infection
 - Deletion compared to HIV mono-infection

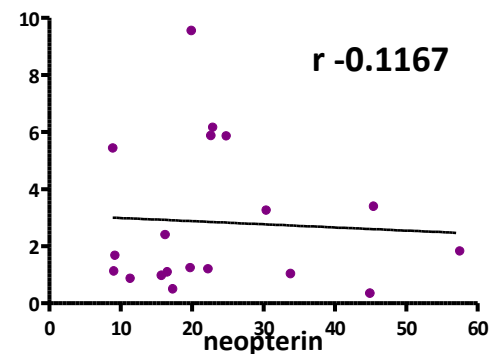
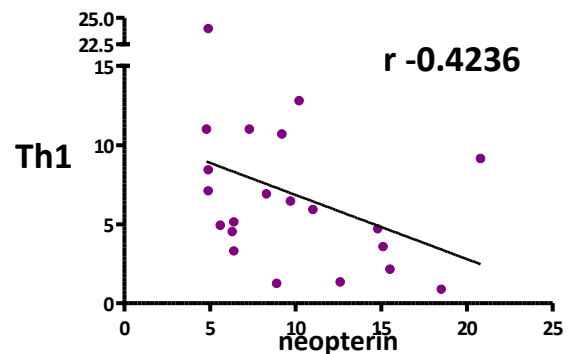
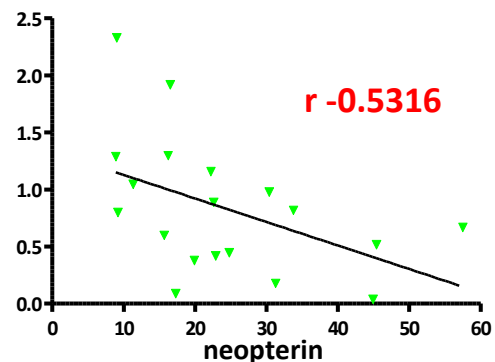
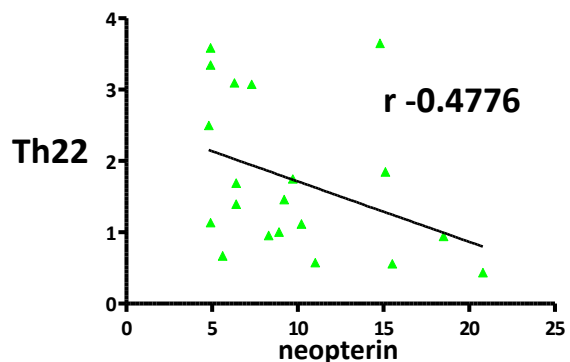
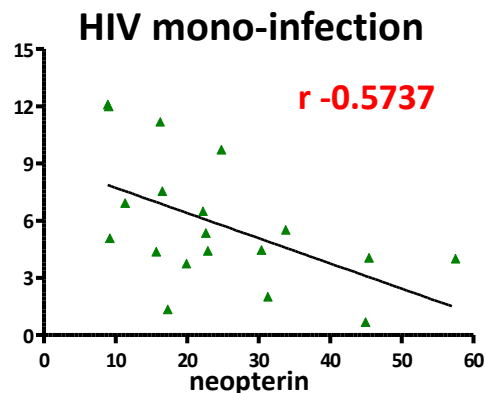
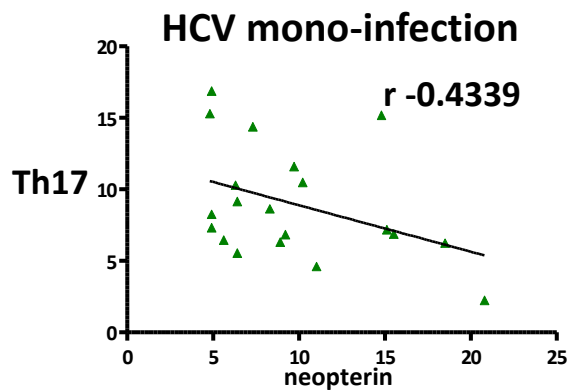
Shifts in CD4 T cell subsets



- HIV/HCV co-infection
reduced Th1:Th22 & Th1:Th17 cell ratio

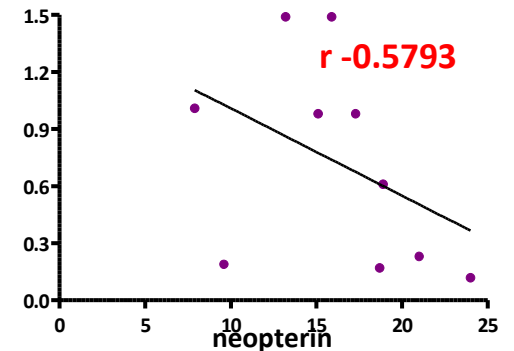
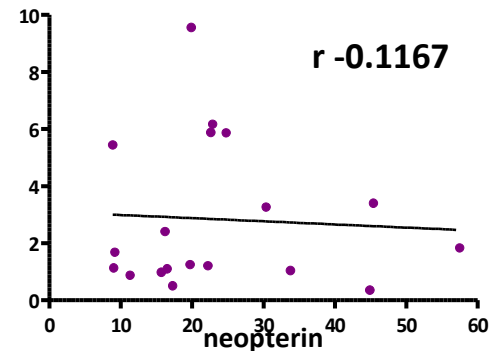
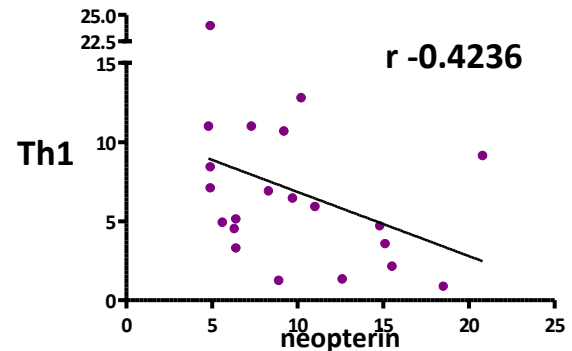
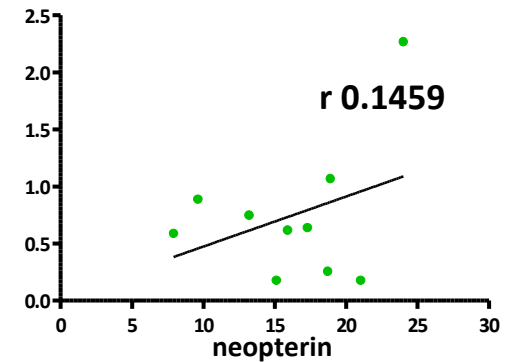
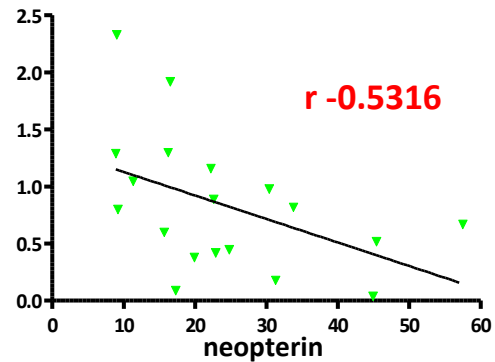
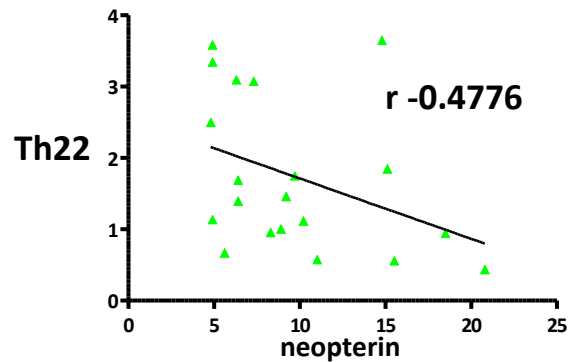
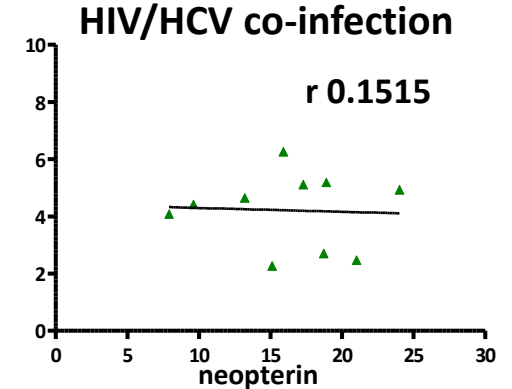
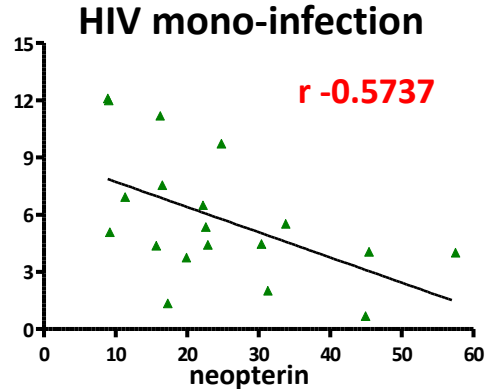
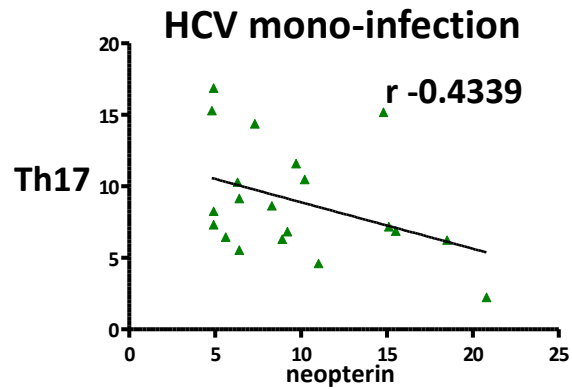
Correlations:

immune activation & CD4 T cell subsets



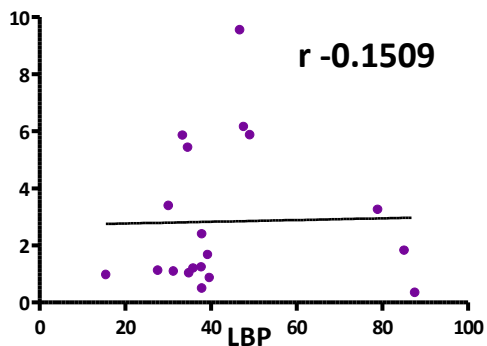
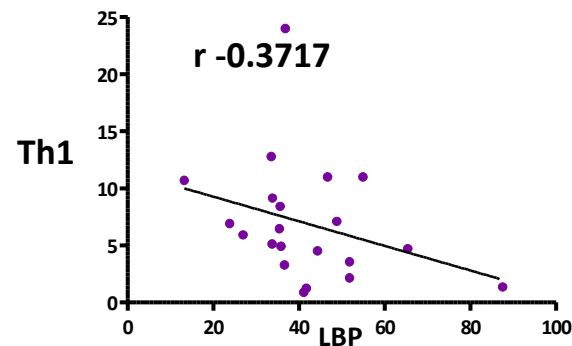
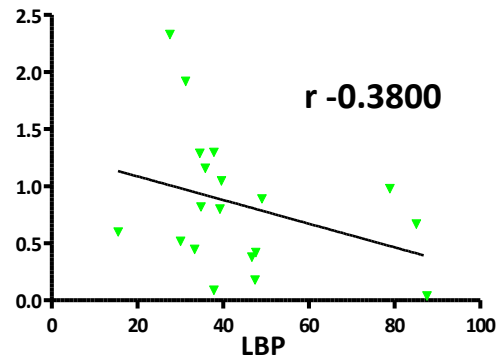
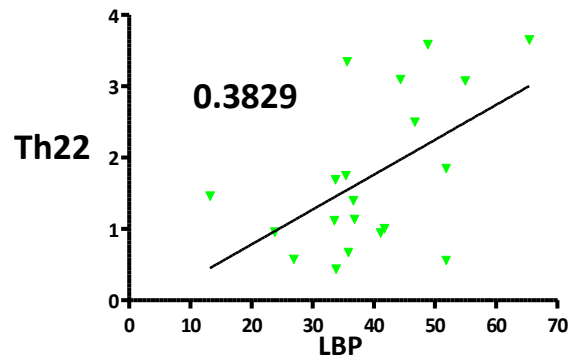
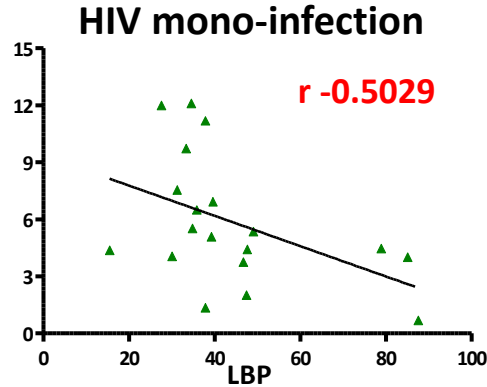
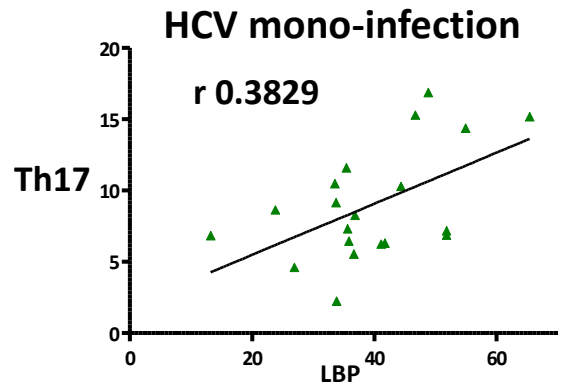
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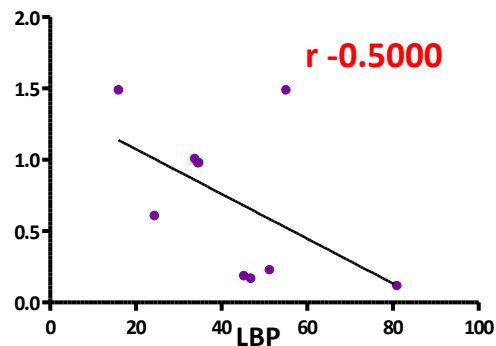
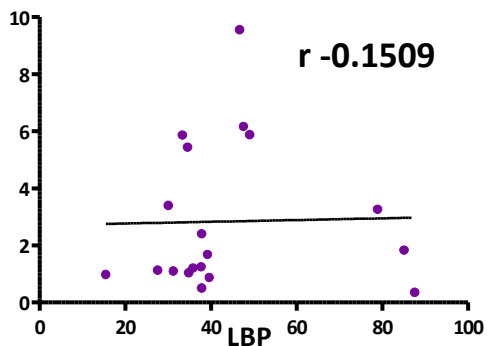
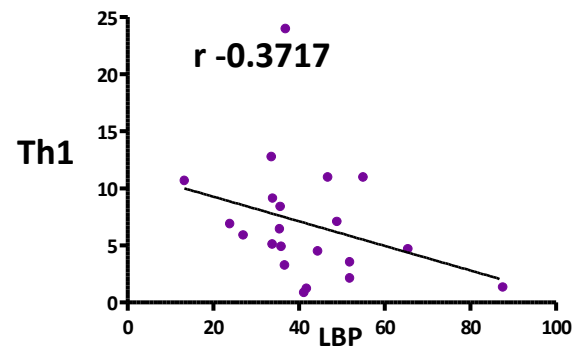
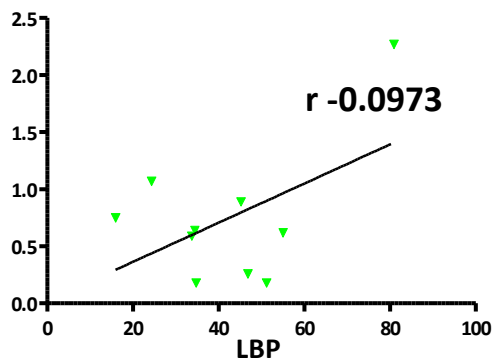
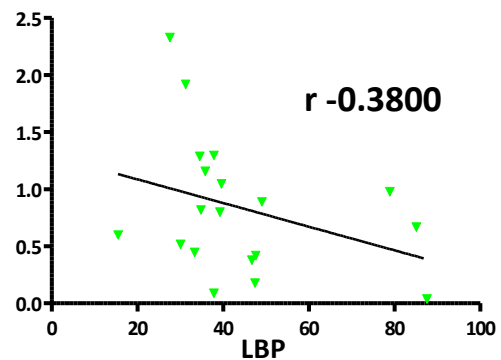
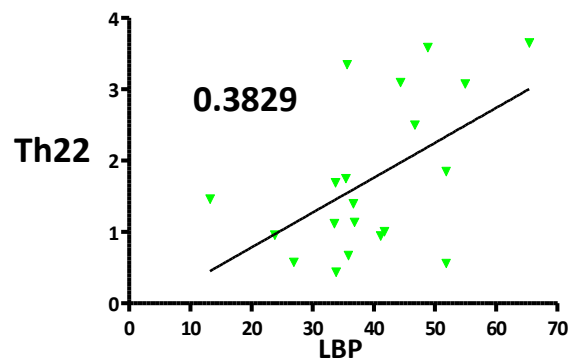
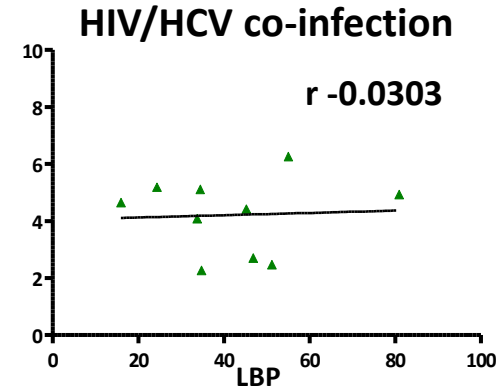
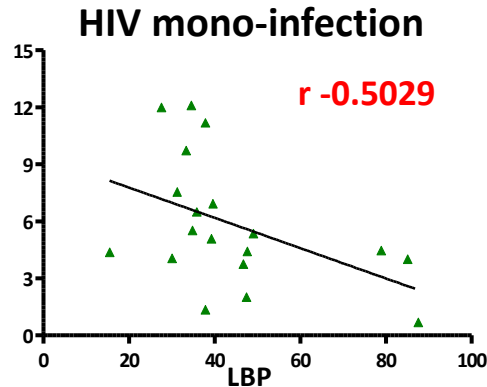
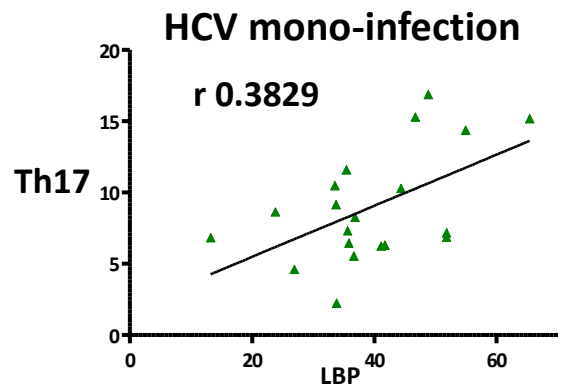
Correlations:

microbial translocation & CD4 T cell subsets



Correlations:

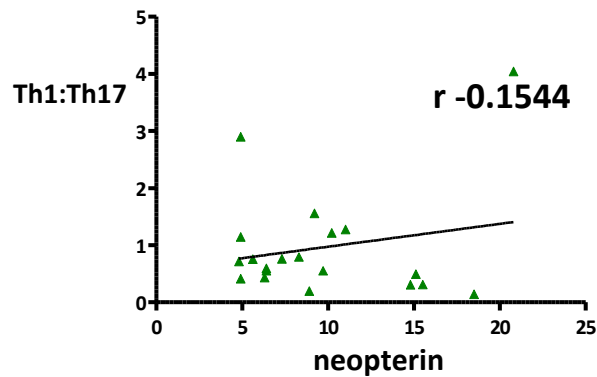
microbial translocation & CD4 T cell subsets



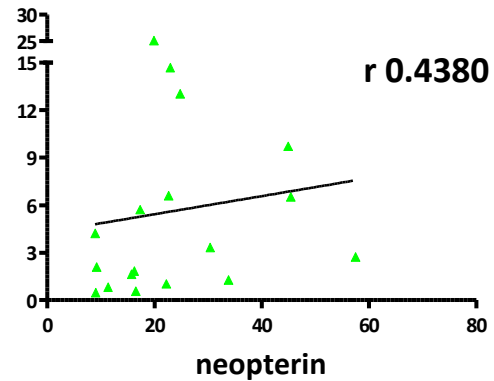
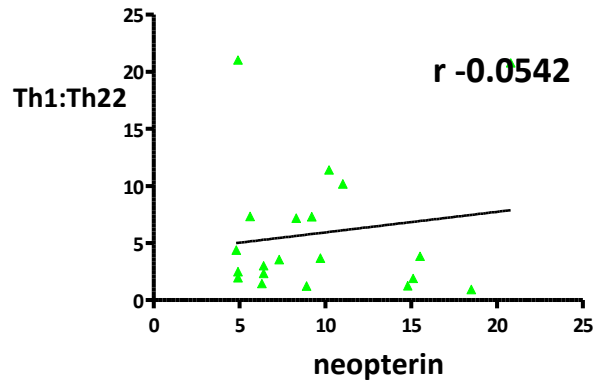
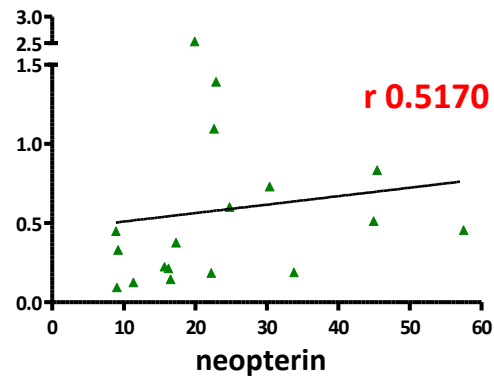
Correlations:

immune activation & CD4 T cell shifts

HCV mono-infection



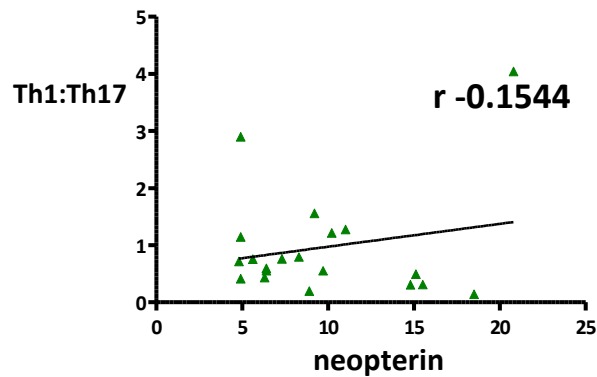
HIV mono-infection



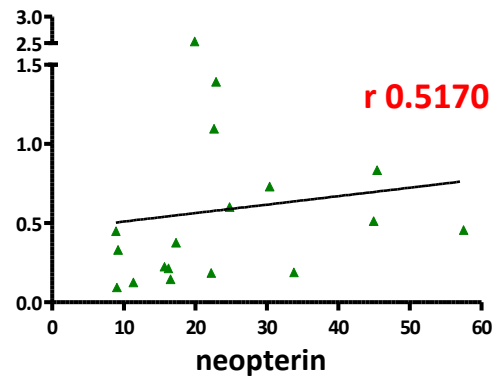
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immune activation & CD4 T cell shifts

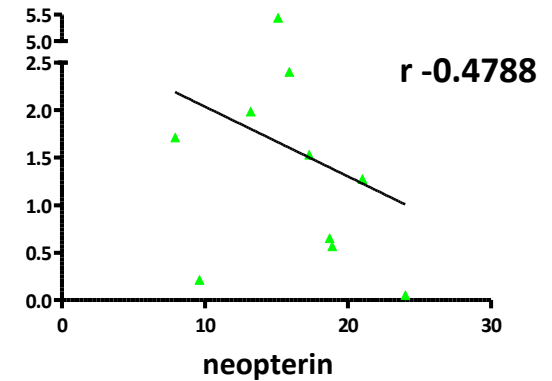
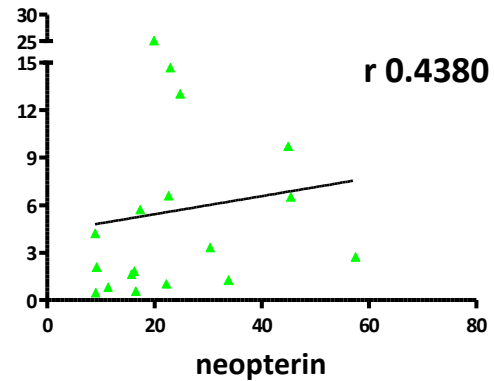
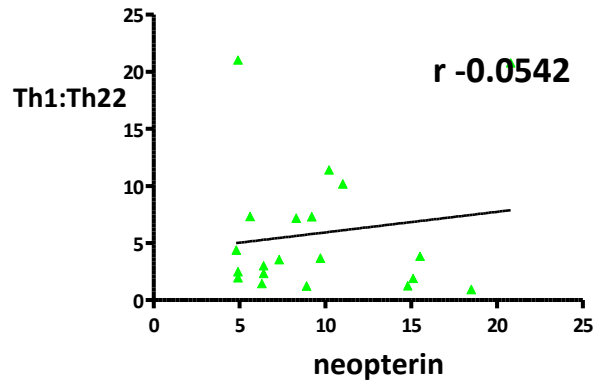
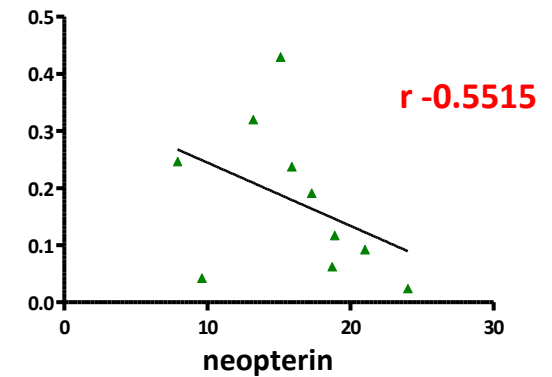
HCV mono-infection



HIV mono-infection



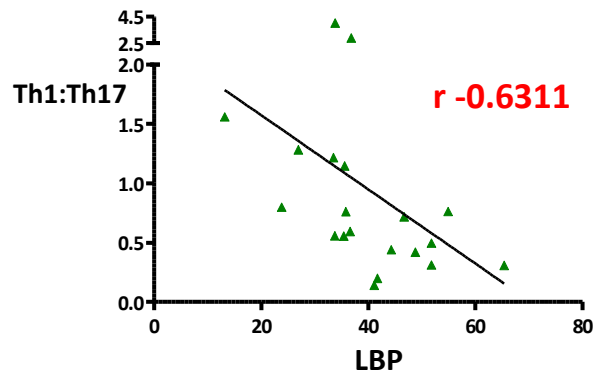
HIV/HCV co-infection



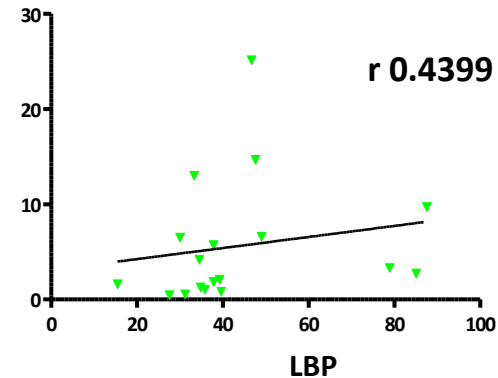
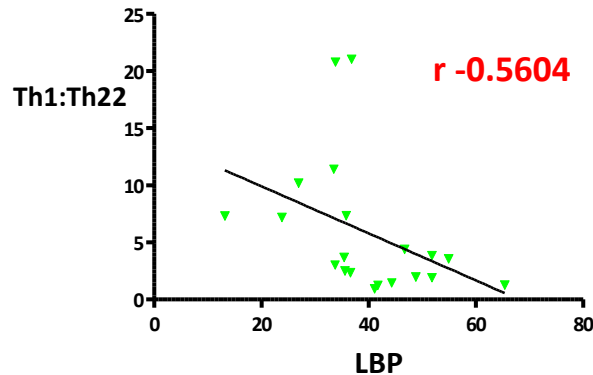
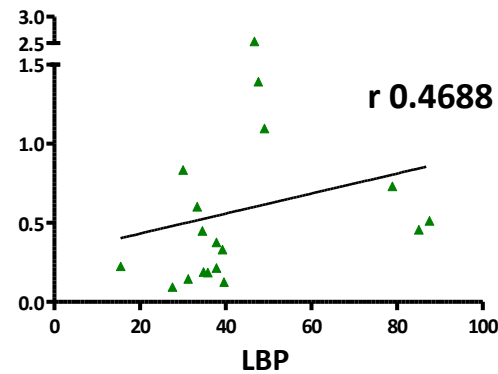
Correlations:

microbial translocation & CD4 T cell shifts

HCV mono-infection



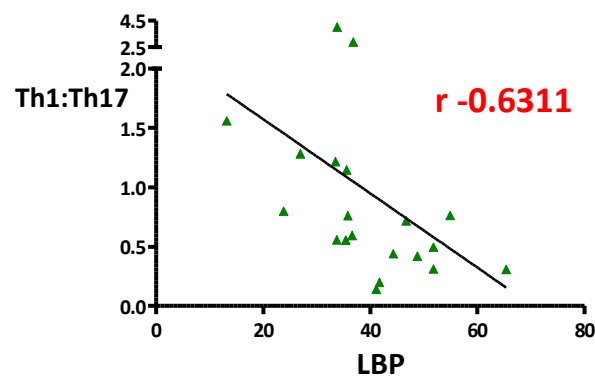
HIV mono-infection



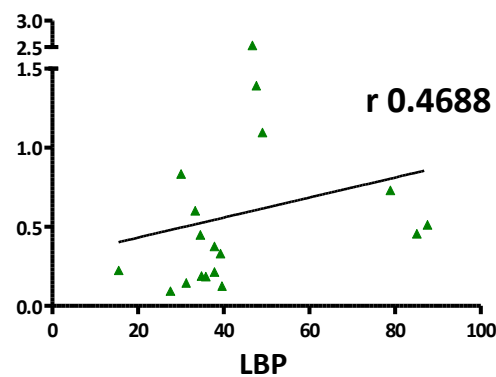
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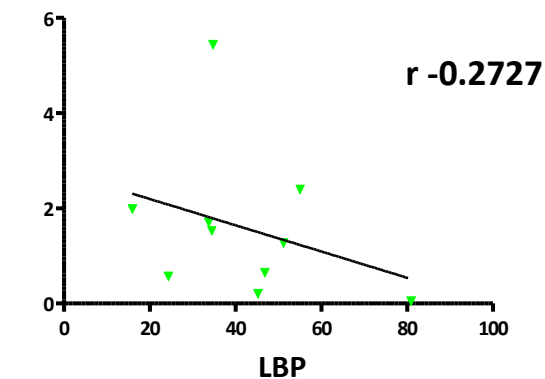
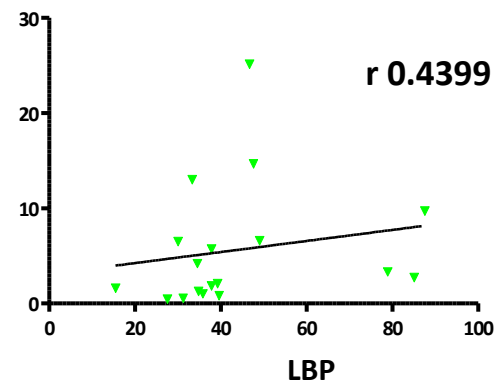
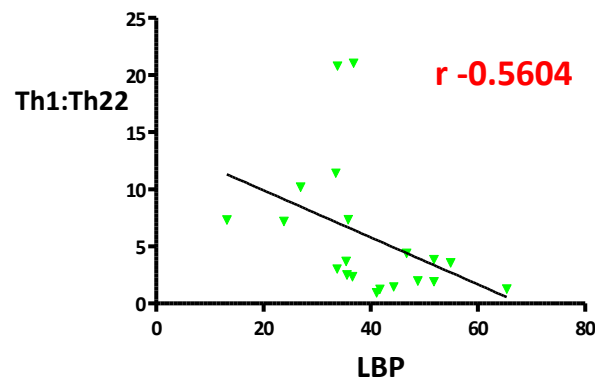
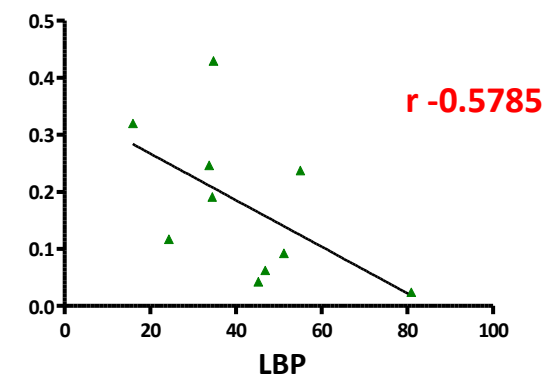
HCV mono-infection



HIV mono-infection



HIV/HCV co-infection

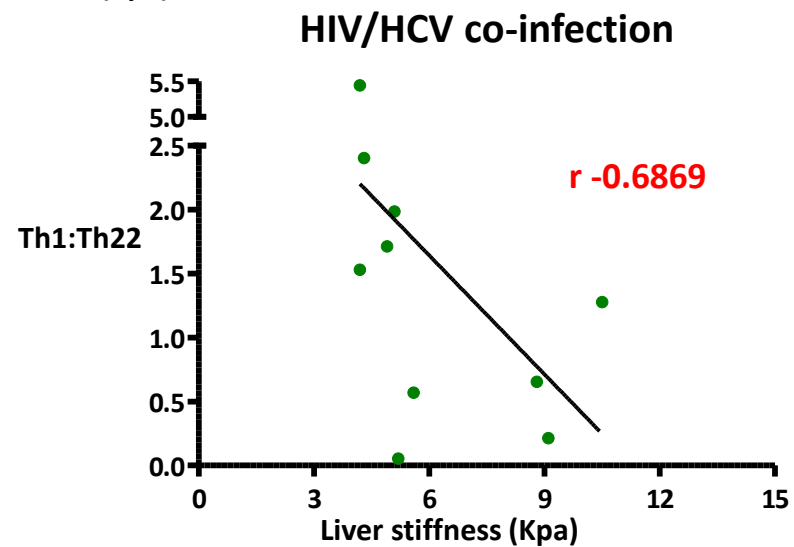
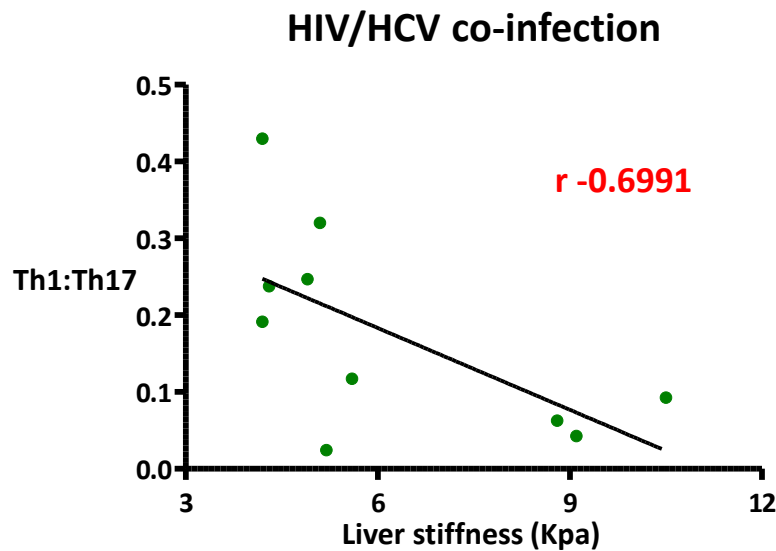
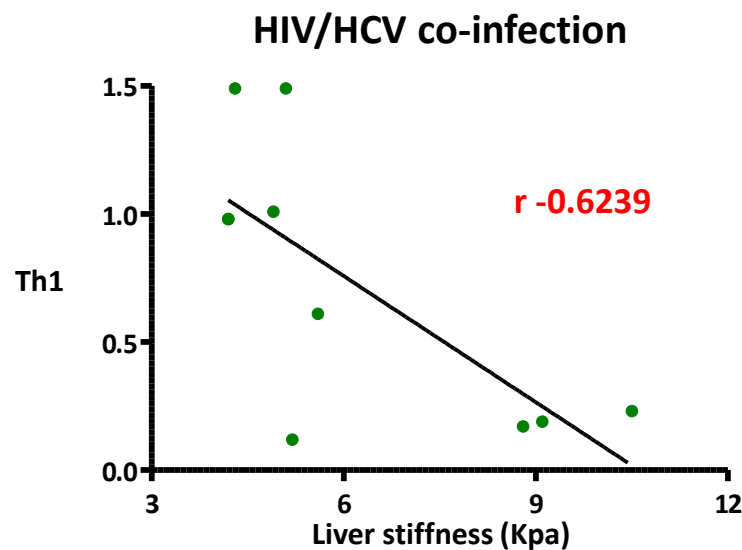


Correlations: Liver stiffness

**In HCV & HIV groups immune
activation was positively
associated with liver stiffness**

Correlations: Liver stiffness

In HCV & HIV groups immune activation was positively associated with liver stiffness



Conclusions

HCV mono-infection

- preferential expansion of Th1 cells
- Shifts towards Th1 cells associated with reduced levels of microbial translocation

HIV mono-infection

- preferential depletion of Th17 and Th22 cells
- Depleted Th17 cells associated with increased levels of immune activation and microbial translocation

HIV/HCV co-infection

- depletion of Th1, Th17 & Th22 cells
- AND
- Shifts away from Th1 cells towards Th17 and Th22 cells
 - Shifts away from Th1 cells associated with increased levels of microbial translocation, immune activation and liver stiffness.

In HIV mono-infection a preferential reduction in Th17 cells may lead to increased levels of microbial translocation & immune activation.

In HIV/HCV co-infection an additional lack of Th1 cell expansion with alterations in the balance of Th1 to Th17 cells may contribute toward development of liver fibrosis through secretion of pro-inflammatory cytokines

Limitations

- Cross-sectional – causality cannot be determined
- Accuracy of markers used for microbial translocation
- Liver fibrosis assessment: use of transient elastography rather than biopsy
- Lack of paired gut or liver samples

Acknowledgements

Imperial College
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Peter Kelleher
Sally Ann Clark
Melanie Hart
Louise Greathead
Rebecca Metcalf
Adriano Boasso

Brian Gazzard
Mark Nelson
Mike Anderson

Grants



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INNSBRUCK


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369 Fulham Road
London SW10 9NH

British HIV Association
BHIVA

Dietmar Fuchs

Patients & Staff



British HIV Association
BHIVA



**19th Annual Conference of the
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16–19 April 2013

Manchester Central Convention Complex