# Impact of Mucosal Immunity and HIV Persistence on CD4/CD8 ratio after ART initiation

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## INTRODUCTION

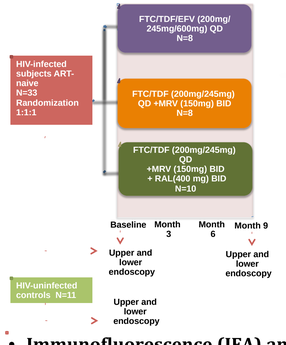
- Persistence of a low CD4/CD8 ratio during ART-mediated HIV suppression correlates with activation of the innate and adaptive immunity, and independently predicts mortality (1,2).
- Persistence of HIV replication in anatomic reservoirs (i.e., the gut-associated lymphoid tissue) and impairment of mucosal immunity likely contribute to chronic immune dysfunction and inflammation during ART (3-5). Conceptually, it could also correlate with the CD4CD8 ratio.
- This study examined the impact of changes in the HIV reservoir, systemic inflammation/bacterial translocation and mucosal immunity following ART initiation on CD4/CD8 ratio.

## METHODS

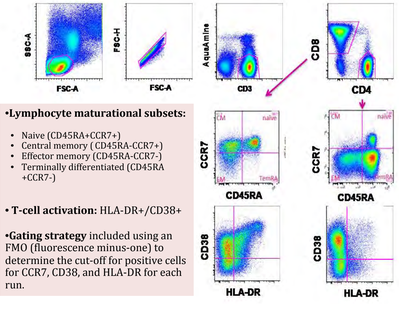
•Peripheral blood, duodenal and rectal biopsies were obtained from 11 HIV- controls and 33 ART nave HIV+ subjects participating in a randomized clinical trial comparing three different ART initiation strategies, at baseline and after 9 months of treatment.

•Tissue was digested into single-cell suspensions for immunofluorescence analysis and flow-citometry measurements.

### Study Design




### Flow citometry analysis on peripheral and mucosal mononuclear cells




•**Lymphocyte maturational subsets:**

- Naive (CD45RA+CCR7+)
- Central memory ( CD45RA-CCR7+)
- Effector memory (CD45RA-CCR7-)
- Terminally differentiated (CD45RA +CCR7-)

• **T-cell activation:** HLA-DR+/CD38+

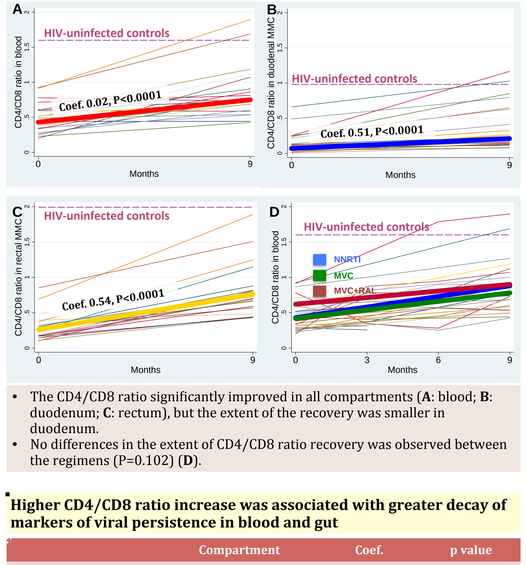
•**Gating strategy** included using an FMO (fluorescence minus-one) to determine the cut-off for positive cells for CCR7, CD38, and HLA-DR for each run.

- **Immunofluorescence (IFA) analysis:** tissue was stained using polyclonal anti-CD3 rabbit and monoclonal anti-CD4 or CD8 mouse serum. Binding of CD3 and CD4 or CD8 receptors were detected simultaneously using Alexafluor 488-labeled polyclonal goat antirabbit IgG (and Alexafluor 568-labeled polyclonal goat antimouse IgG (Molecular Probes). The numbers of positive cells were counted by a single observer and presented as cells/mm$^2$ of lamina propria or intraepithelial regions (above the basement membrane) of duodenal mucosa.
- **Cell associated- (CA) HIV DNA and CA-HIV RNA:** levels of CA-HIV DNA were determined by real-time PCT and normalized to $10^6$ cells. CA-HIV RNA levels were determined by reverse transcriptase real time PCR (qRT-PCR) and results are expressed in copies/mL.
- **Assays in cryopreserved plasma:** IL-6 (R&D Systems), sCD14 (R&D Systems), hs-CRP (CardioPhase hs-CRP assay, Siemens), lipoteichoic acid (LTA, R&D Systems) and zonulin-1 (ALPCO).
- **Statistical analysis:** Longitudinal associations between CD4/CD8 ratio changes and variations of continuous variables were analyzed using linear mixed models with random intercepts, considering the CD4/CD8 ratio as fixed effect. Continuous variables were log-transformed when necessary to satisfy model assumptions.

## RESULTS

•Data were available for 26 HIV+ subjects who completed the follow-up period.
•Eight subjects received EFV, 10 MRV and 8 MVC+RAL, each in combination with FTC/TDF.
•Median age was 37 years [25-42] vs. 34 [28-43] in the control group
•Median CD4 T-cell count was 435 cell/ml [283-572].
•All subjects in the study achieved viral suppression.

### The CD4/CD8 ratio improved in all compartments, but remained more impaired in duodenal mucosa




- The CD4/CD8 ratio significantly improved in all compartments (**A**: blood; **B**: duodenum; **C**: rectum), but the extent of the recovery was smaller in duodenum.
- No differences in the extent of CD4/CD8 ratio recovery was observed between the regimens (P=0.102) (**D**).

### Higher CD4/CD8 ratio increase was associated with greater decay of markers of viral persistence in blood and gut

| | Compartment | Coef. | p value |
|---|---|---|---|
| HIV DNA | Blood | -0.39 | 0.014 |
| | Duodenum | -0.64 | 0.014 |
| | Colon | -0.64 | 0.032 |
| HIV RNA | Blood | -1.20 | <0.001 |
| | Duodenum | -0.91 | 0.095 |
| | Colon | -2.85 | <0.001 |

*A positive coefficient denotes a positive correlation between CD4/CD8 ratio changes in blood and the outcome variable (HIV DNA or HIV RNA). A negative coefficient denotes a negative correlation.*

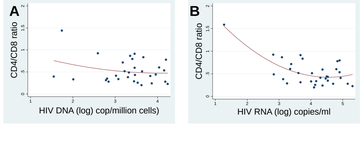
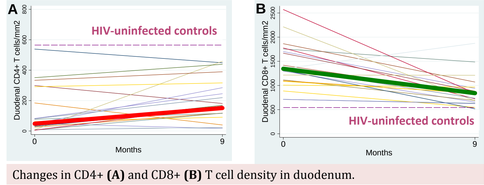


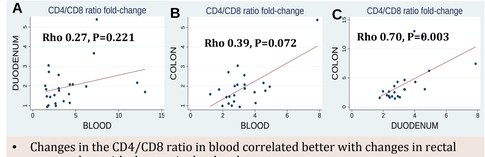

**Figure A:** correlation between the CD4/CD8 ratio and CA-HIV DNA at baseline (Rho -0.52, P=0.007).
**Figure B:** correlation between the CD4/CD8 ratio and CA-HIV RNA at baseline (Rho -0.36, P=0.065).

### Poor recovery of CD4+ T cell density in duodenum




Changes in CD4+ **(A)** and CD8+ **(B)** T cell density in duodenum.

### Correlations between CD4/CD8 ratio changes in blood and gut mucosa




- Changes in the CD4/CD8 ratio in blood correlated better with changes in rectal mucosa than with changes in duodenal mucosa.
- Changes in the CD4/CD8 ratio at both gastrointestinal sites correlated well between them.
- Correlations calculated using linear mixed models yielded similar results (all P<0.001).

### An increased CD4/CD8 ratio recovery in blood was associated to amelioration of CD4+ and CD8+ T cell density in duodenum

| | Coef. | p value |
|---|---|---|
| **Duodenal CD4+ T cells/mm$^2$** | 1.36 | <0.001 |
| **Duodenal CD8+ T cells/mm$^2$** | -0.41 | 0.002 |

*A positive coefficient denotes a positive correlation between CD4/CD8 ratio changes in blood and the outcome variable. A negative coefficient denotes a negative correlation. Higher CD4/CD8 ratio decreases reflected greater CD4+ T cell density increase and greater CD8+ T cell density decrease.*

### Improvement of the CD4/CD8 ratio in blood correlated with changes in markers of bacterial translocation and epithelial barrier disfunction

| | Coef. | p value |
|---|---|---|
| **Interleukin-6** | 0.068 | 0.113 |
| **Soluble CD14** | 0.0008 | 0.927 |
| **Lipoteichoic acid** | -0.023 | 0.016 |
| **Zonuline-1** | 0.003 | 0.076 |

*A positive coefficient denotes a positive correlation between CD4/CD8 ratio changes in blood and the outcome variable. A negative coefficient denotes a negative correlation.*

An increase in the CD4/CD8 ratio was related to an increase in zonulin levels (P=0.076) and a decline in lipoteichoic acid levels (P=0.016).

### Decreases of systemic and mucosal T cell activation and improvements of T cell maturation were associated with greater CD4/CD8 ratio recovery in blood

| | Compartment | Coef. | p value |
|---|---|---|---|
| %CD4+HLA-DR+CD38+ T cells | Blood | -0.77 | <0.001 |
| | Duodenum | -0.03 | 0.697 |
| | Colon | -0.28 | 0.004 |
| %CD8+HLA-DR+CD38+ T cells | Blood | -0.75 | <0.001 |
| | Duodenum | -0.43 | <0.001 |
| | Colon | -0.31 | 0.001 |
| CD4 Naive/Memory Ratio | Blood | 0.34 | 0.023 |
| | Duodenum | 0.19 | 0.377 |
| | Colon | 0.12 | 0.625 |
| CD8 Naive/Memory Ratio | Blood | 0.84 | <0.001 |
| | Duodenum | 0.35 | 0.080 |
| | Colon | 0.10 | 0.004 |

*A positive coefficient denotes a positive correlation between CD4/CD8 ratio changes in blood and the outcome variable. A negative coefficient denotes a negative correlation.*

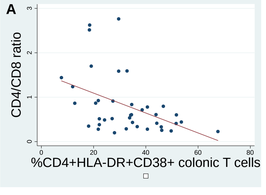



**Figure A.** Correlation between the CD4/CD8 ratio in blood and the numbers of activated CD4+ T cells in rectal mucosa.

## CONCLUSIONS

- CD4/CD8 recovery during ART correlates with improvements in markers of viral persistence, T cell activation and T cell maturation in PB and colon.
- The duodenum may represent a unique compartment for impairment of CD4 maturation and depletion.
- A quadruple ART regimen did not add detectable benefit to triple ART.

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