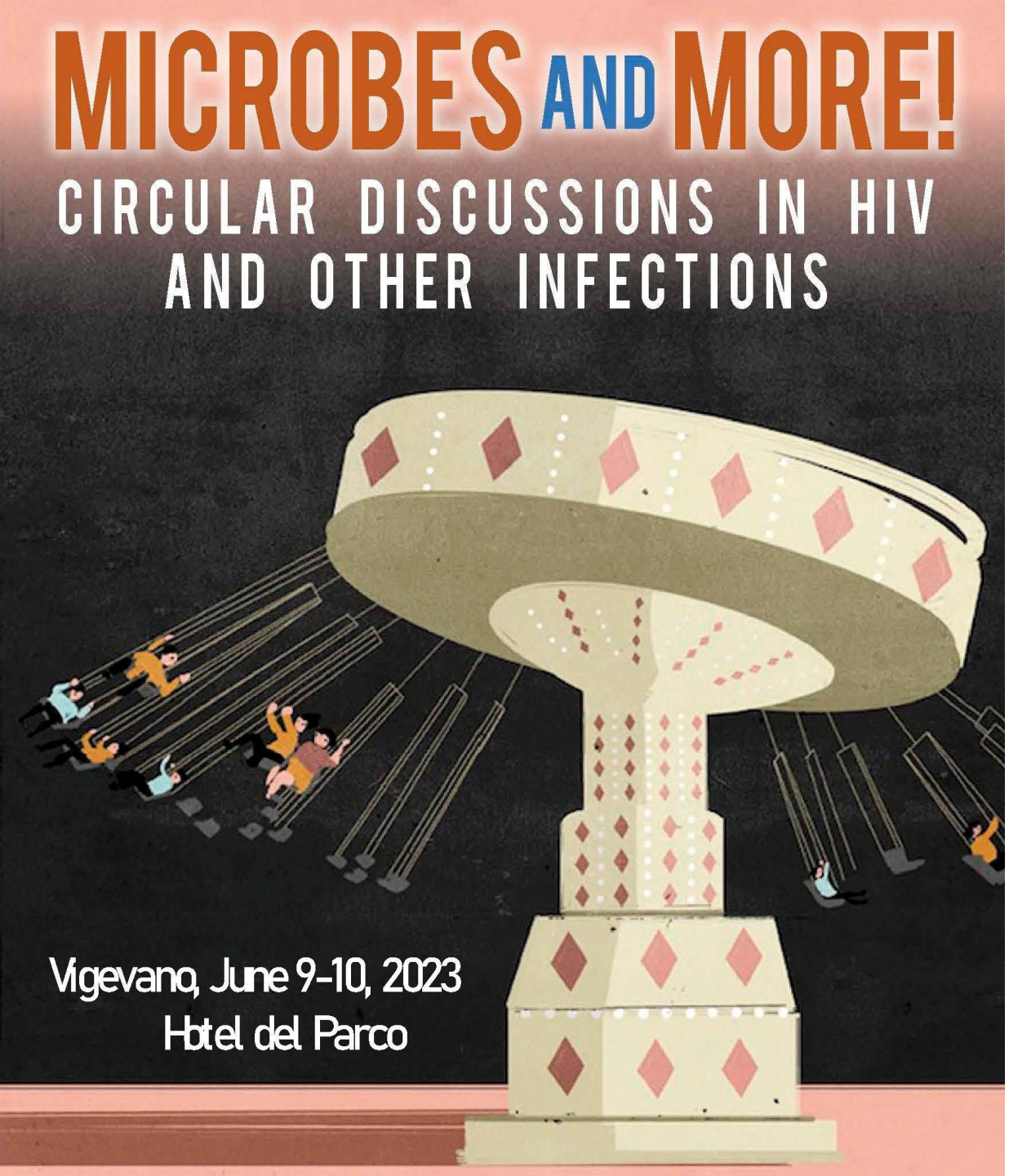


Sistema Socio Sanitario
Regione Lombardia
ASST Santi Paolo e Carlo

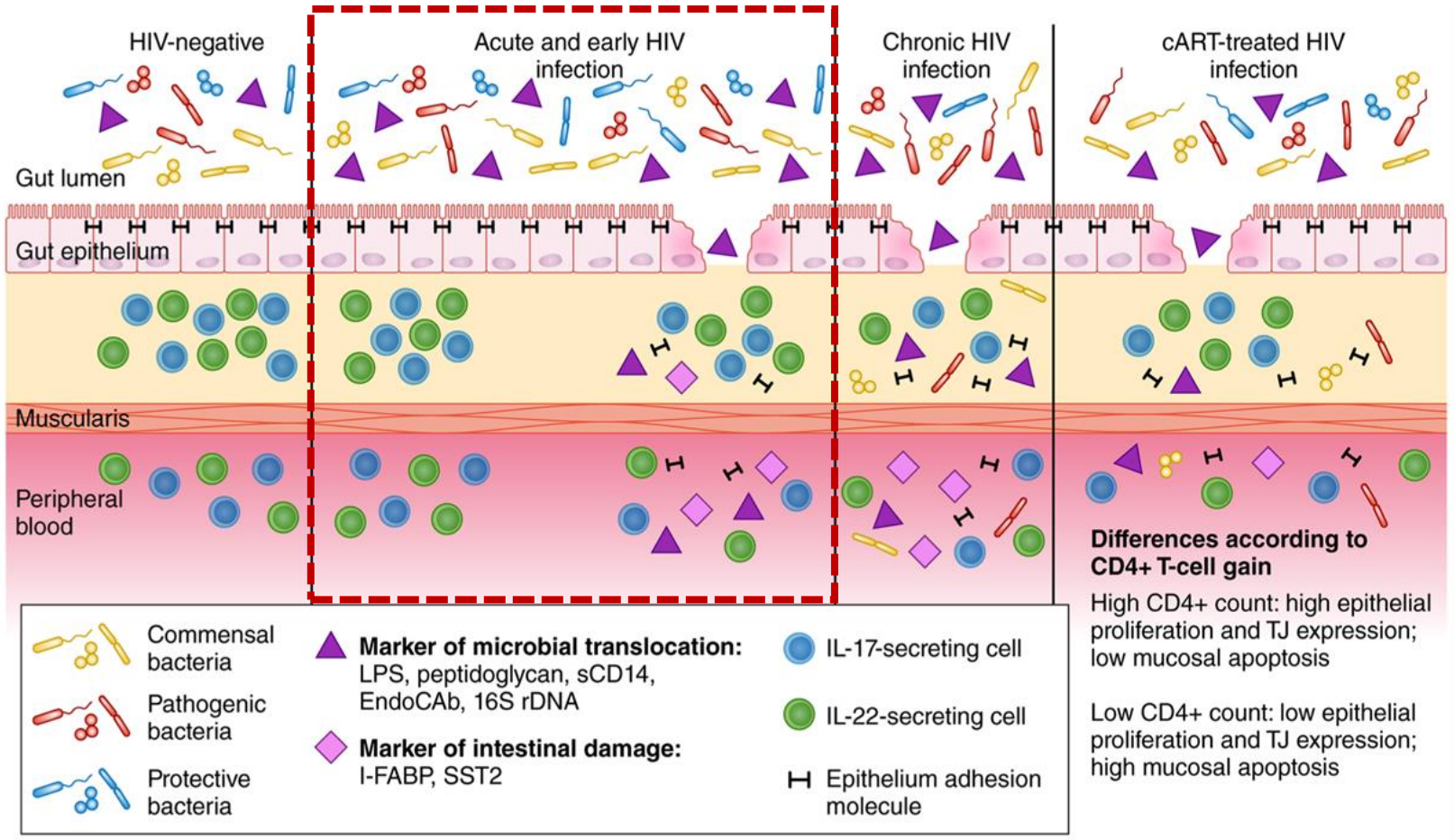
UNIVERSITÀ DEGLI STUDI DI MILANO

Study of the gastrointestinal tract in acute HIV infection

Camilla Falcinella

10/06/2023

Background



Tincati *et al. AIDS Res Ther (2016) 13:19* DOI 10.1186/s12981-016-0103-1

Aims

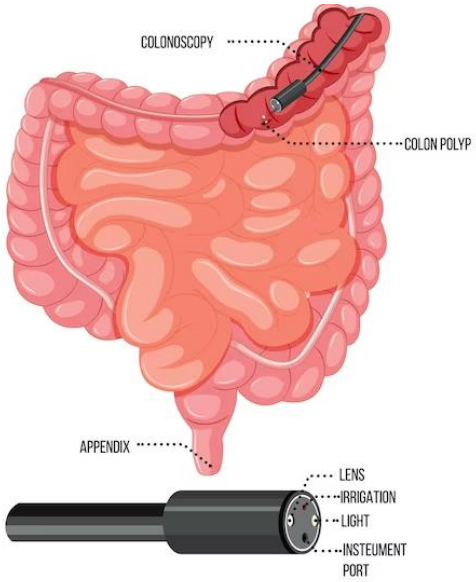
Investigating gut structural damage and fibrosis, mucosal immunity and microbiome alterations in:

- ✓primary (**PHI**) and chronic (**CHI**) HIV infection before cART introduction
- ✓primary HIV infection (**PHI**) before and after cART introduction (**T0 – T12**)

Materials & methods

- Enrollment between October 2019 – March 2021
- HIV-infected, treatment-naive patients:
  - PHI (Fiebig I-V) → INACTION P25 trial, GALT substudy
  - chronic HIV
- Colonoscopy at t0 (both PHI and chronic naive) and at t12 (PHI only) → <u>bioptic specimens:</u>
  - structure (E-cadherin/collagen) and monocyte/macrophage polarization (CD14, CD68, CD163) [immunohistochemistry]
  - T-cell phenotyping (Th17, Treg, γδ) [flow cytometry]
  - microbial analysis [16s rRNA sequencing, MiSeq Illumina]
- <u>Peripheral blood samples:</u>
  - PBMCs: T-cell phenotyping (Th17, Treg, γδ) [flow cytometry]
  - plasma: inflammation (IL-6), microbial translocation (sCD14) and gut integrity (E-cadherin) [ELISA]
  - HIV-1 DNA normalized to RPP30 reference gene [Droplet Digital PCR, Biorad QX100]

4 ileum samples:
1 OCT-preserved
2 lysate
1 fresh frozen
(+ standard biopsies)



4 colon samples:
1 OCT-preserved
2 lysate
1 fresh frozen
(+ standard biopsies)

Sample processing	**Analyses**
OCT	Immunohistochemical staining → gut barrier integrity and microbial translocation
Lysate	Flow cytometry → immunophenotyping of lamina propria mononuclear cells
Fresh frozen	Microbiome characterization
[Formalin-fixed (standard)	Histopathology]

Results

- **Demographic and clinical data**
- Gut barrier integrity, structural damage and peripheral inflammation
- Mucosal and peripheral immunity
- Microbiome and its interplay with viral reservoir

Study population

	PHI (N = 11)	Chronic naive (N = 10)	p value
Age at diagnosis [years], median (IQR)	41 (29-45)	42 (34-51)	0.3408
Male sex, n (%)	11 (100)	8 (80)	0.2143
Caucasian ethnicity, n (%)	10 (91)	7 (70)	0.3108
MSM, n (%)	10 (91)	8 (80)	0.5865
Nadir CD4 [cell/mmc], median (IQR)	538 (493-609)	147 (12-279)	**0.0006**
Fiebig stage, n (%)			
I-III	2 (18)	/	/
IV-VI	9 (82)	/	/
CDC stage C1-C3 (AIDS-defining conditions)**,** n (%)	1 (9)	4 (40)	0.1486
ART regimen, n (%):			
TAF/FTC/DRV/C	3 (27)	/	/
TAF/FTC/DTG	6 (55)	/	/
TAF/FTC/DTG/DRV/C	2 (18)	/	/

Viro-immunologic parameters at colonoscopy

	PHI (N = 11)	Chronic naive (N = 10)	p value
CD4 count [cell/mmc], median (IQR)	538 (446-615) **T12: 756 (590-940)**	176 (94-279)	**0.0008** **0.004**
%CD4, median (IQR)	25 (14-28) **T12: 37.6 (33-43-7)**	17 (10-18)	0.1295 **0.001**
CD8 count [cell/mmc], median (IQR)	1332 (826-1752) **T12: 816 (554-900)**	688 (248-925)	**0.0183** **0.001**
%CD8, median (IQR)	56 (49-72) **T12: 35 (28.4-40.7)**	58 (49-66)	0.5256 **0.001**
CD4/CD8 ratio, median (IQR)	0.50 (0.19-0.57) **T12: 1.23 (0.76-1.44)**	0.26 (0.15-0.32)	0.1693 **0.001**
HIV-RNA [log_{10} cp/mL], median (IQR)	5.58 (5.16-5.92) **T12: 1.6 (1.55-1.91)**	5.13 (4.80-5.42)	0.0528 **0.001**

Results

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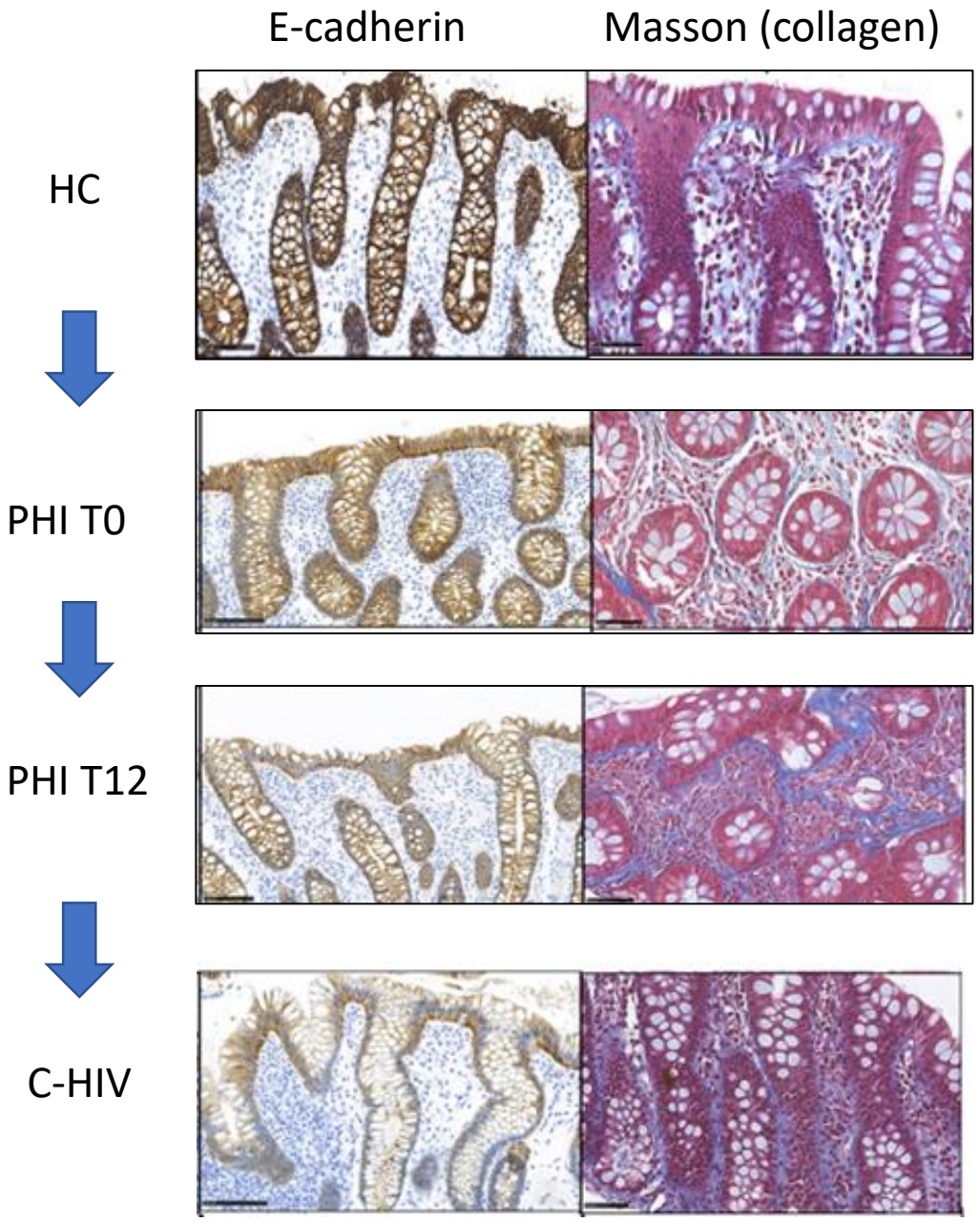
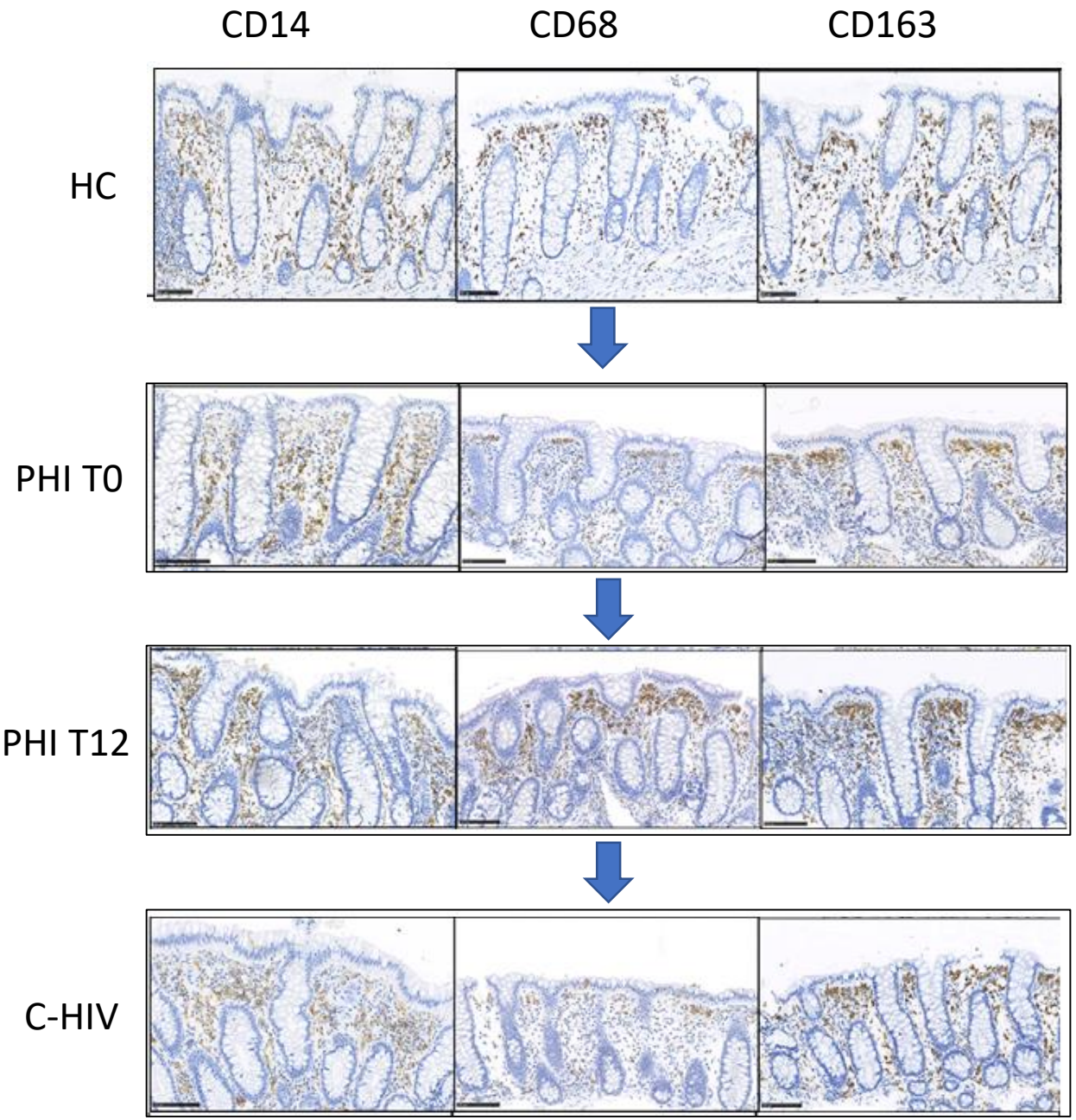


Loss of barrier integrity and fibrosis

While PHI patients present a preserved intestinal structure (very similar to healthy controls), chronic HIV counterparts display a high degree of fibrosis and a markedly reduced E-cadherin expression.

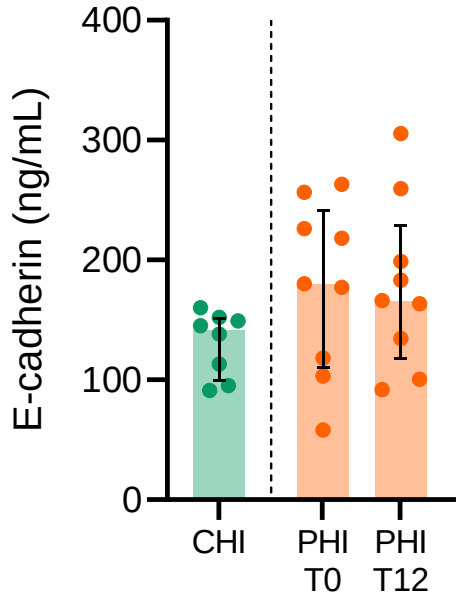
At 12 weeks after ART initiation, PHI patients present progressive collagen deposition and loss of E-cadherin within colonic mucosa.

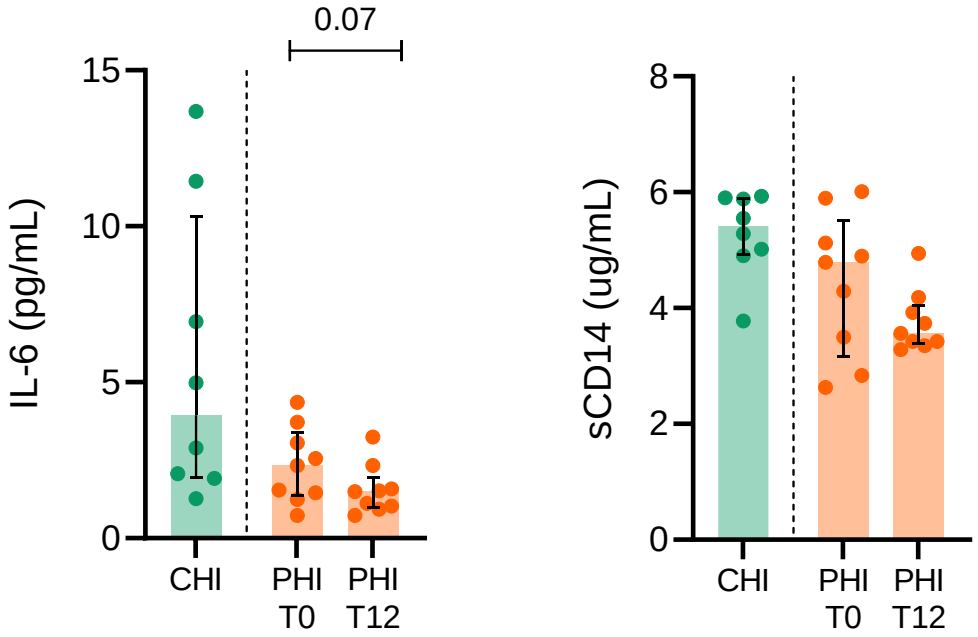


Monocyte/macrophage distribution and polarization along intestinal villi

As HIV-related damage progresses, the physiological distribution of monocytes and macrophages along the villus and their differentiation/maturation is progressively altered.

In particular, concentration of CD14+ cells towards lymphatic vessels reflects damage-induced macrophagic differentiation, while CD163+ cells (M2 macrophages) progressively move towards the intestinal lumen driven by barrier injury (tissue repair function).





Peripheral inflammation and intestinal barrier integrity markers

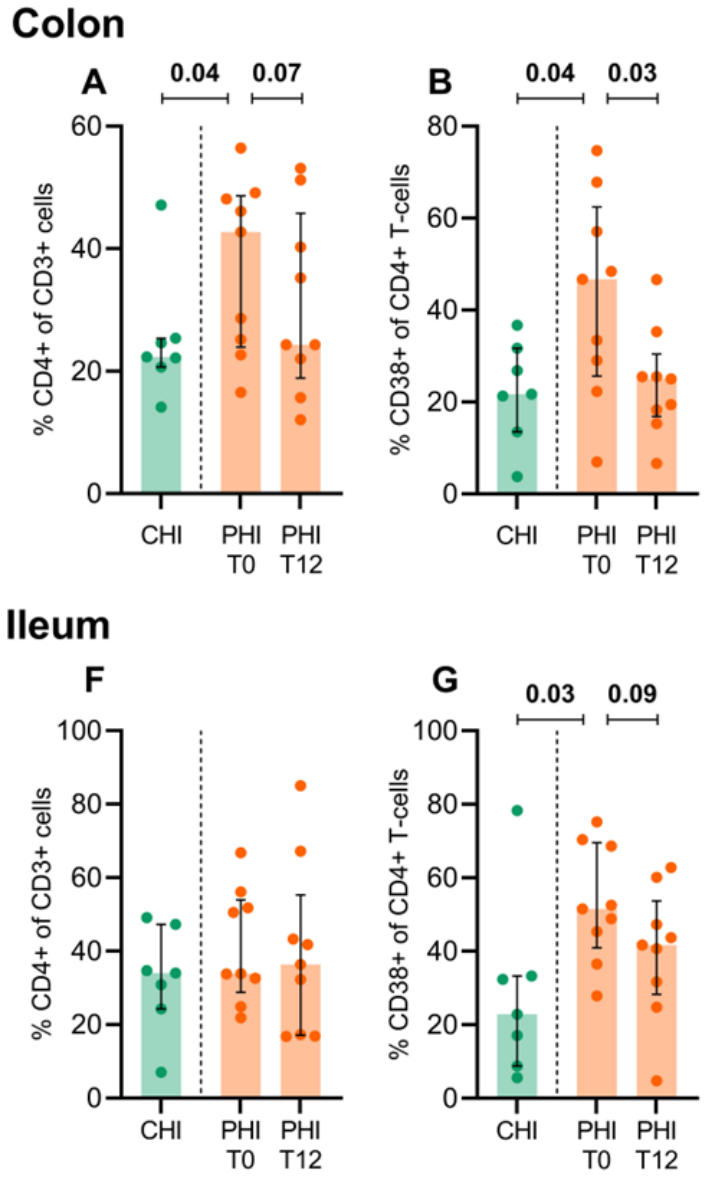
While we didn't find a major effect of short-term cART on plasma markers in PHI, we noticed a trend to a significant reduction of peripheral inflammation (T0-T12).

This suggests a potential role of **<u>early</u>** ART initiation in reducing HIV-related inflammatory state.

Tincati et al., HIV Glagsgow, 2022

Results

- Demographic and clinical data
- Gut barrier integrity, structural damage and peripheral inflammation
- **Mucosal and peripheral immunity**
- Microbiome and its interplay with viral reservoir



CD4+ T cells phenotype

PHI at T0: preserved gut CD4+ T cells with higher degree of activation compared to CHI;

PHI at T12: frequency and activation of gut CD4+ T cells decreases to CHI levels after short-term ART.

Results

- Demographic and clinical data
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- Mucosal and peripheral immunity
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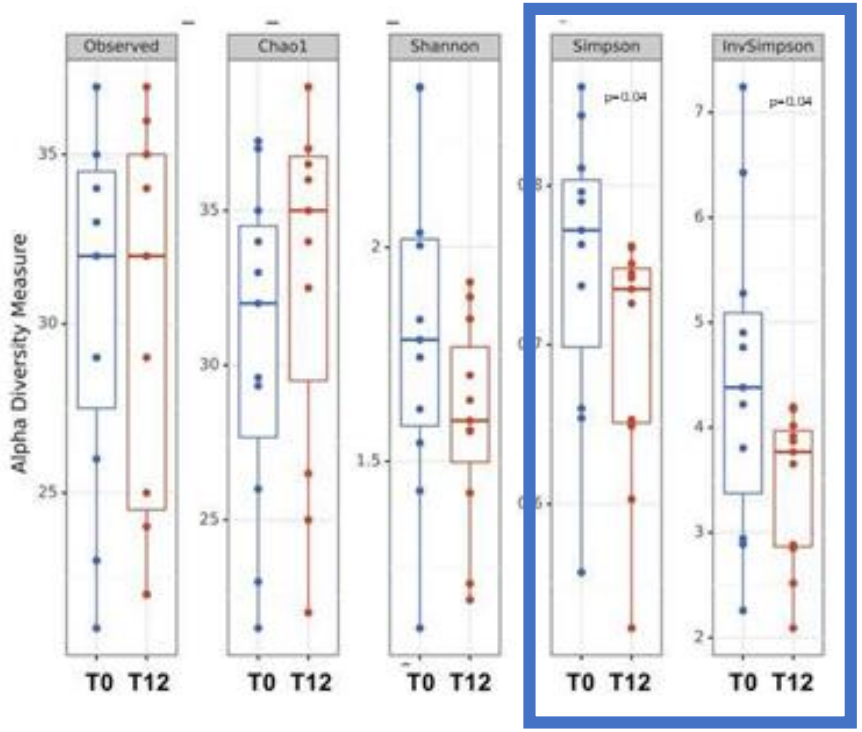
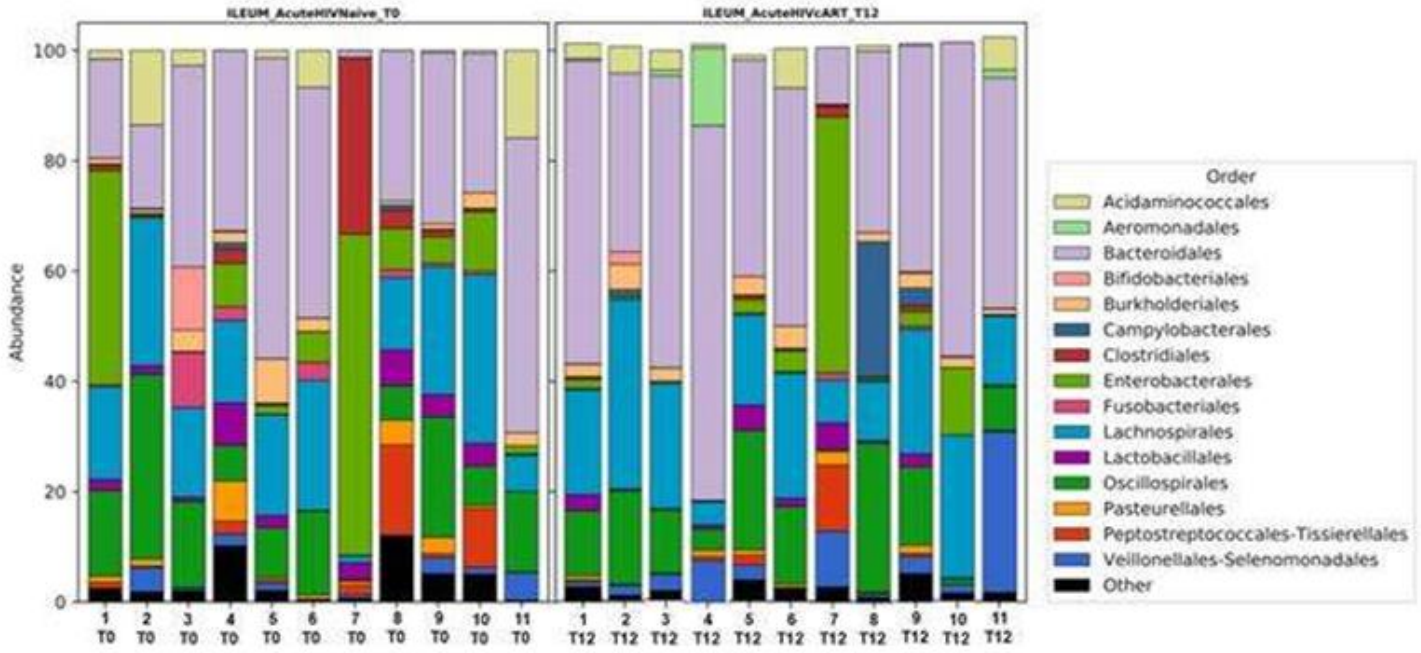




In ileum, significant differences were observed in α-diversity within bacterial order in PHI subjects before and after ART introduction (T0-T12); no differences were observed in β-diversity.

Also, no differences were found in colon tissue in alpha and beta-diversity parameters at all taxonomy levels.

Bono et al., ICAR 2023

Borgognone *et al. Microbiome* (2022) 10:59
https://doi.org/10.1186/s40168-022-01247-6

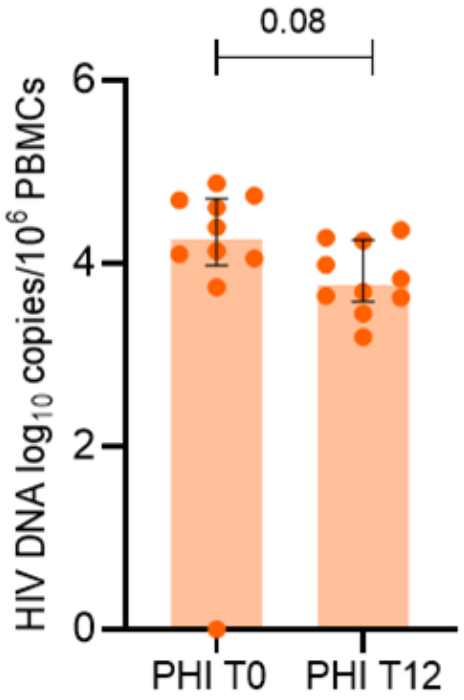
Microbiome

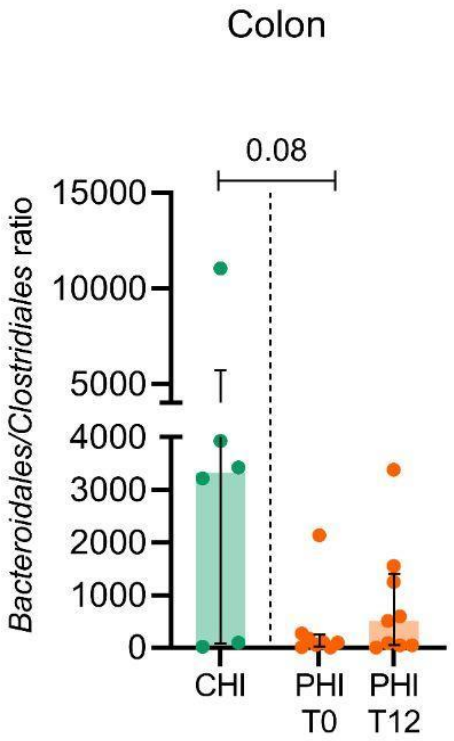
RESEARCH Open Access

Gut microbiome signatures linked to HIV-1 reservoir size and viremia control

Alessandra Borgognone[1*], Marc Noguera-Julian[1,2,3], Bruna Oriol[1,4], Laura Noël-Romas[5,6], Marta Ruiz-Riol[1,2], Yolanda Guillén[7], Mariona Parera[1], Maria Casadellà[1], Clara Duran[1,4], Maria C. Puertas[1,2], Francesc Català-Moll[1], Marlon De Leon[5], Samantha Knodel[5,6], Kenzie Birse[5,6], Christian Manzardo[8], José M. Miró[2,8], Bonaventura Clotet[1,2,3,4,9,10], Javier Martinez-Picado[1,2,3,11], José Moltó[2,9,10], Beatriz Mothe[1,2,3,9,10], Adam Burgener[5,6,12], Christian Brander[1,2,3,11], Roger Paredes[1,2,3,4,5,9,10*] and the BCN02 Study Group

"Viremic controllers during the MAP (controllers) exhibited higher Bacteroidales/Clostridiales ratio and lower microbial gene richness before vaccination and throughout the study intervention when compared to non-controllers. […] The Bacteroidales/Clostridiales ratio as well as host immune-activation signatures inversely correlated with HIV-1 reservoir size.
The present proof-of-concept study suggests the Bacteroidales/Clostridiales ratio as a novel gut microbiome signature associated with HIV-1 reservoir size and immune-mediated viral control after ART interruption."

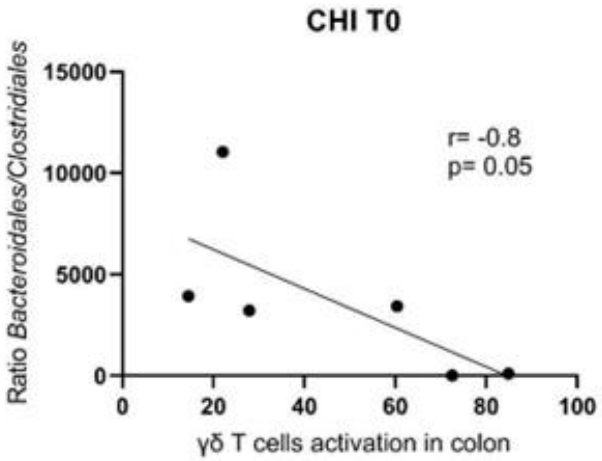


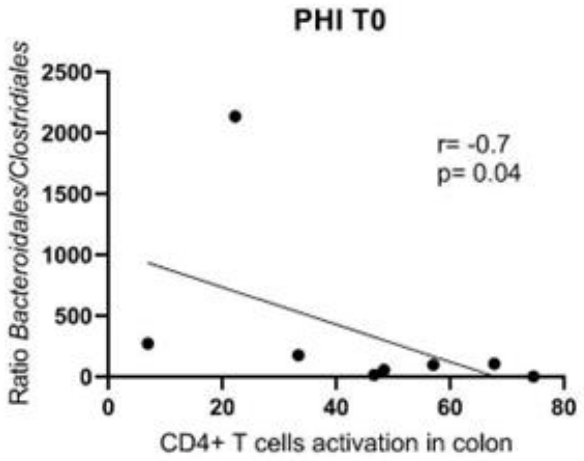


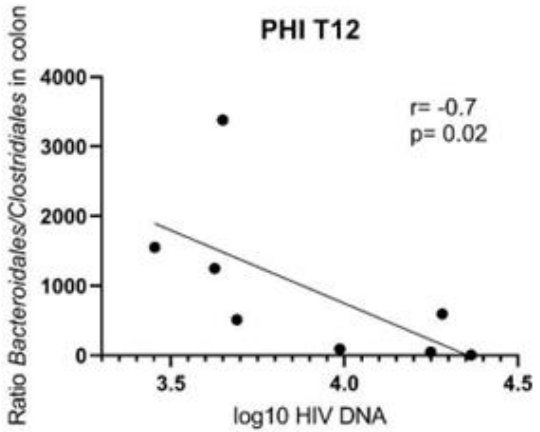
A non-significant decrease in HIV-DNA was measured in PHI after 12 weeks of ART.

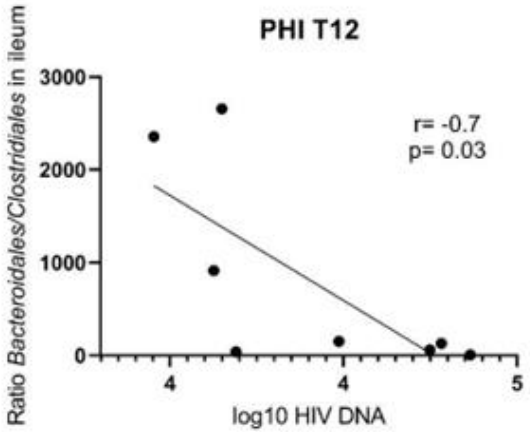
No major differences were observed in the percentage of *Bacteroidales* and *Clostridiales* in both colon and ileum between PHI and CHI individuals. Conversely, when the *Bacteroidales/Clostridiales* ratio was calculated, a non-significant lower value was found in PHI compared to CHI in colon tissue.

Bacteroidales/Clostridiales ratio negatively correlates with immune activation and HIV reservoir size









Bacteroidales/Clostridiales ratio negatively correlated with γδ T cells activation in CHI individuals in colon.

In PHI individuals, *Bacteroidales/Clostridiales* ratio negatively correlated with both CD4+ T cells activation in colon at T0 and HIV DNA at T12 in both tissues.

Conclusions

- Early involvement of the gut in the course of HIV infection
- Progression of immune, structural and microbial alterations despite early ART introduction vs potential (?) benefit on peripheral inflammation
- Uncertain effect on mucosal immunity (reduced activation vs progressive depletion?)
- Potential effect of microbiome signature on viral reservoir size and immune activation

Limitations: short follow-up, small sample size

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