Sistema Socio Sanitario
Regione Lombardia
ASST Santi Paolo e Carlo

Il Microbioma in HIV in Corso di Storia Naturale e Risposta alla cART

Camilla Tincati M.D., Ph.D
Department of Health Sciences
Clinic of Infectious Diseases
San Paolo Hospital
University of Milan

Outline

1. Introduction

- HIV as a chronic disease
- the gut as a site of HIV pathogenesis: microbial translocation, GI epithelial damage, microbial dysbiosis

2. Does cART revert dysbiosis?

3. Is dysbiosis linked to non-infectious comorbidities?

Outline

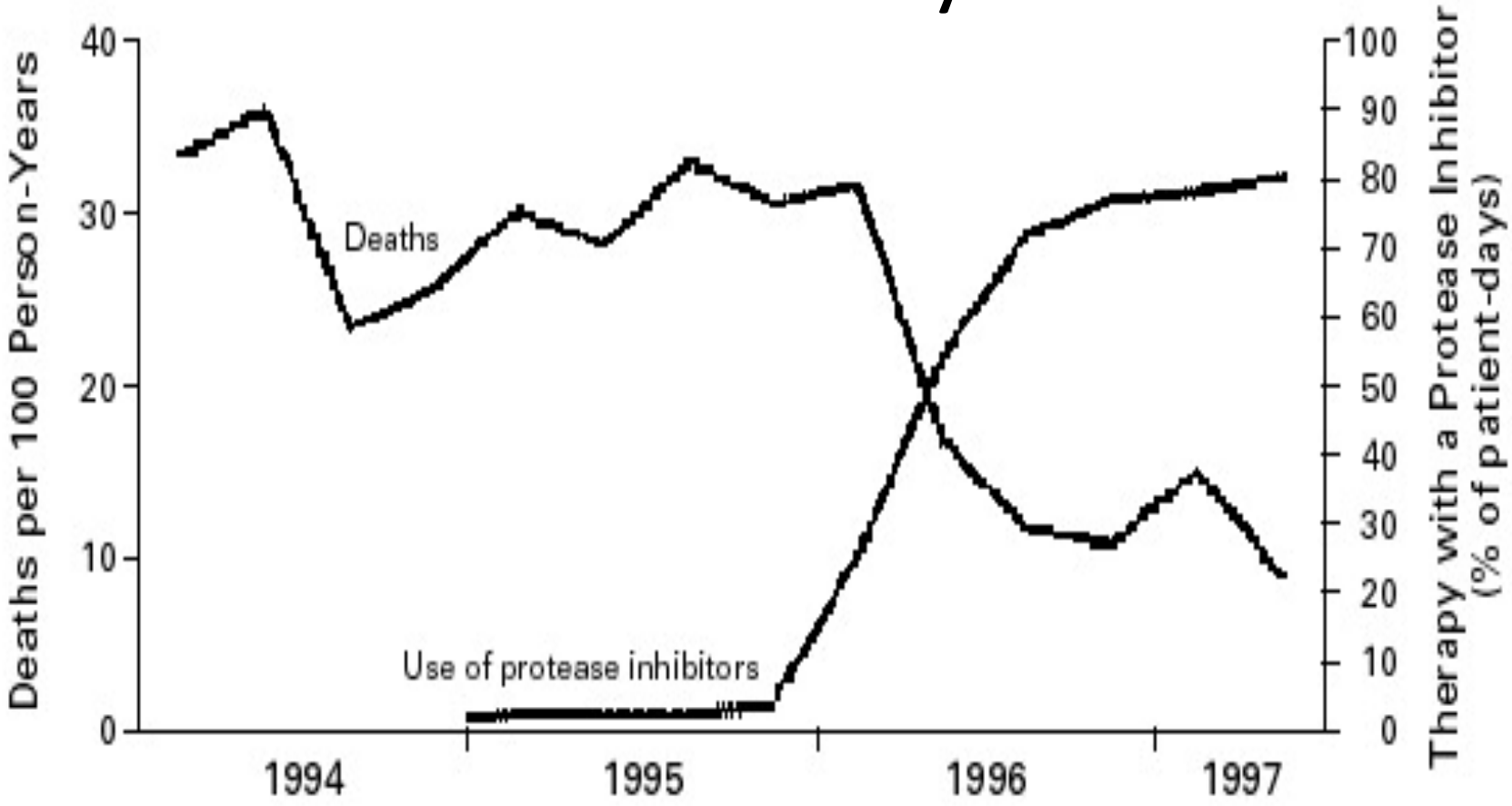
1. Introduction

- HIV as a chronic disease
- the gut as a site of HIV pathogenesis: microbial translocation, GI epithelial damage, microbial dysbiosis

2. Does cART revert dysbiosis?

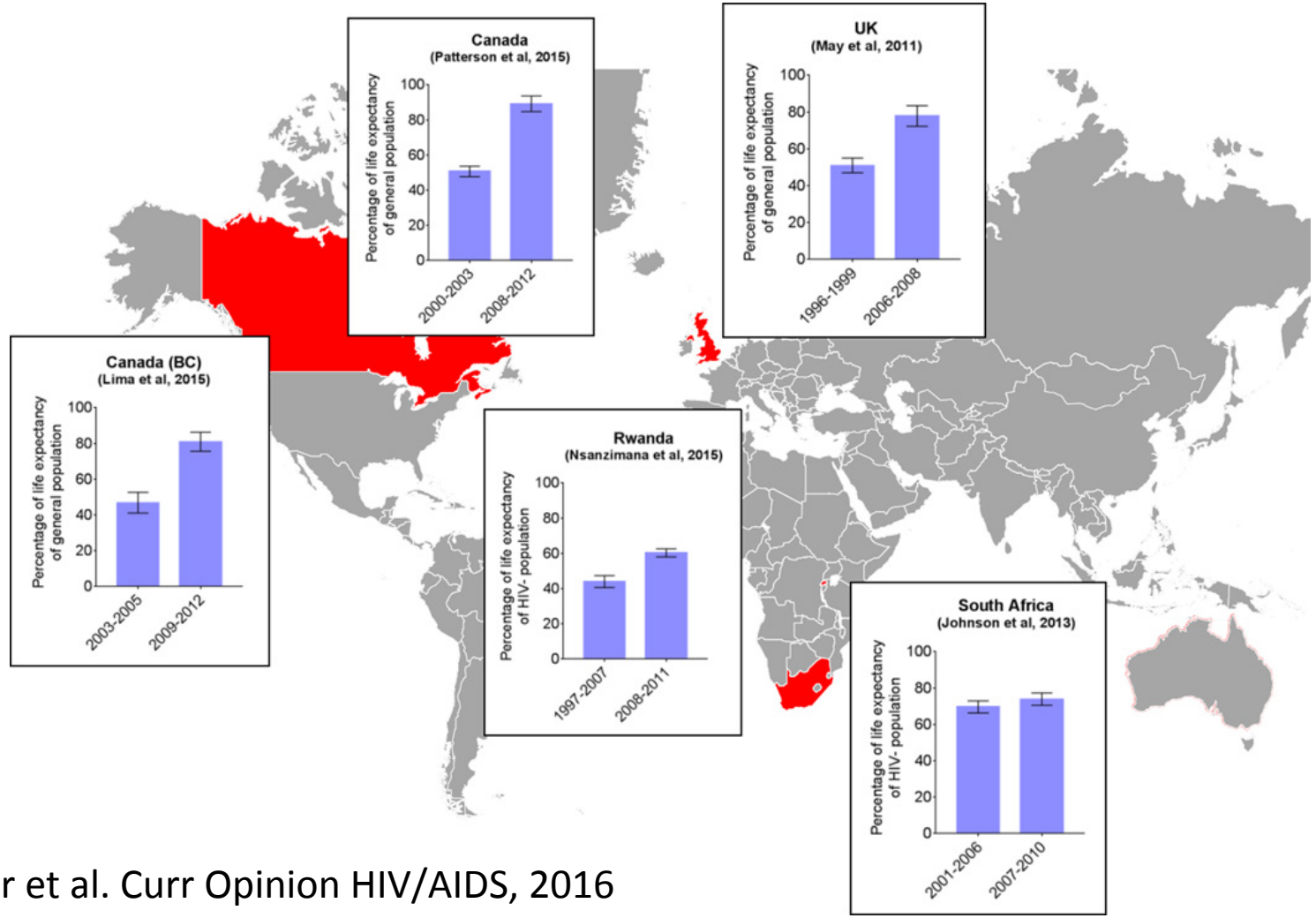
3. Is dysbiosis linked to non-infectious comorbidities?

The **Revolution** of cART: decreased HIV-related morbidity and mortality



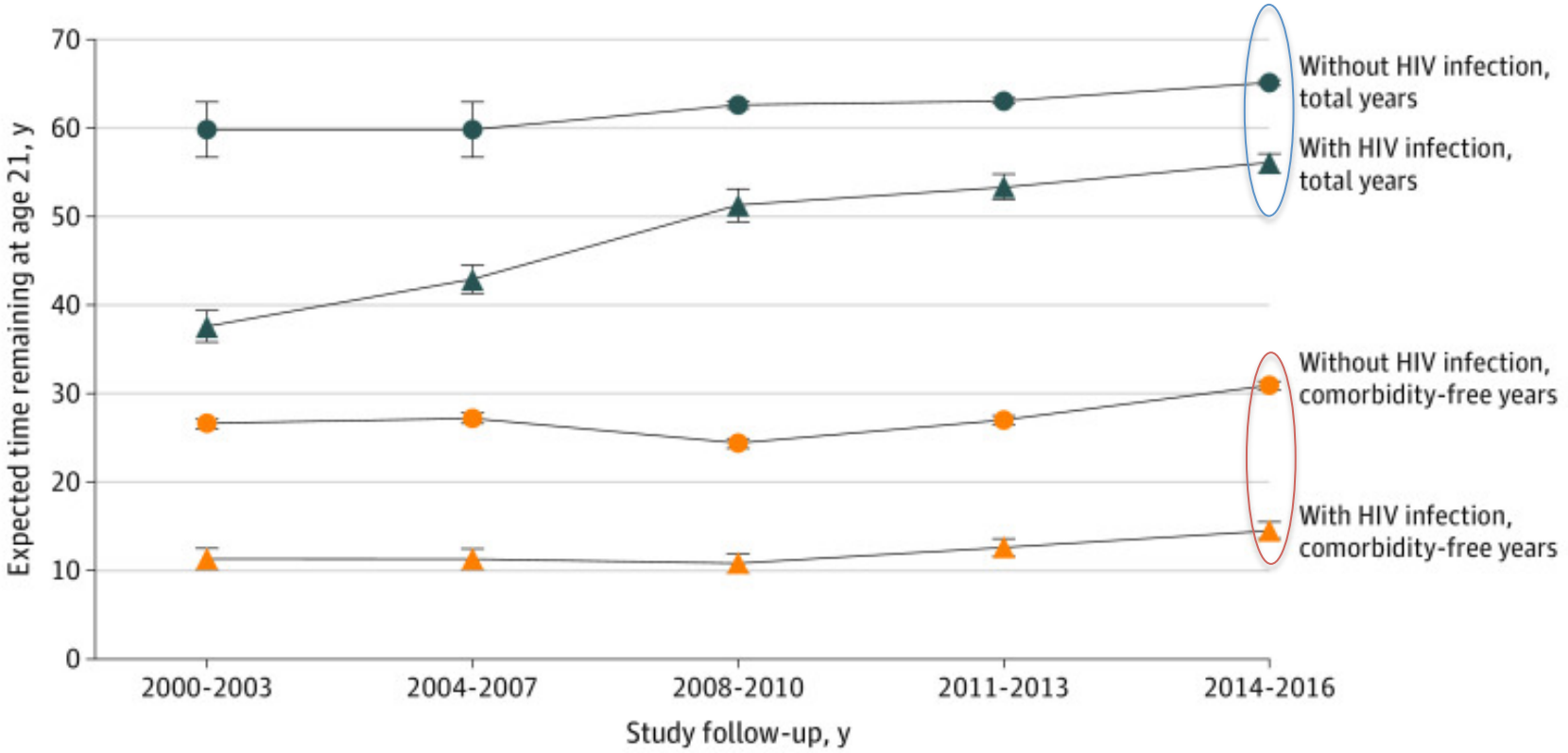
Palella et al. N Engl J Med 1998

Increased life expectancy in treated HIV, yet lower than HIV-uninfected counterparts



Wandeler et al. Curr Opinion HIV/AIDS, 2016

Lower comorbidity-free life expectancy in PLWH



Overall and Comorbidity-Free Life Expectancy at Age 21 Years for Individuals With and Without HIV Infection, Kaiser Permanente, 2000-2016

Comorbidity-free years were those lived before incident diagnosis of any of 6 common comorbidities: **chronic liver disease, chronic kidney disease, chronic lung disease, diabetes, cancer, or cardiovascular disease**. Error bars indicate 95% CIs.

Marcus et al. JAMA Netw Open, 2020

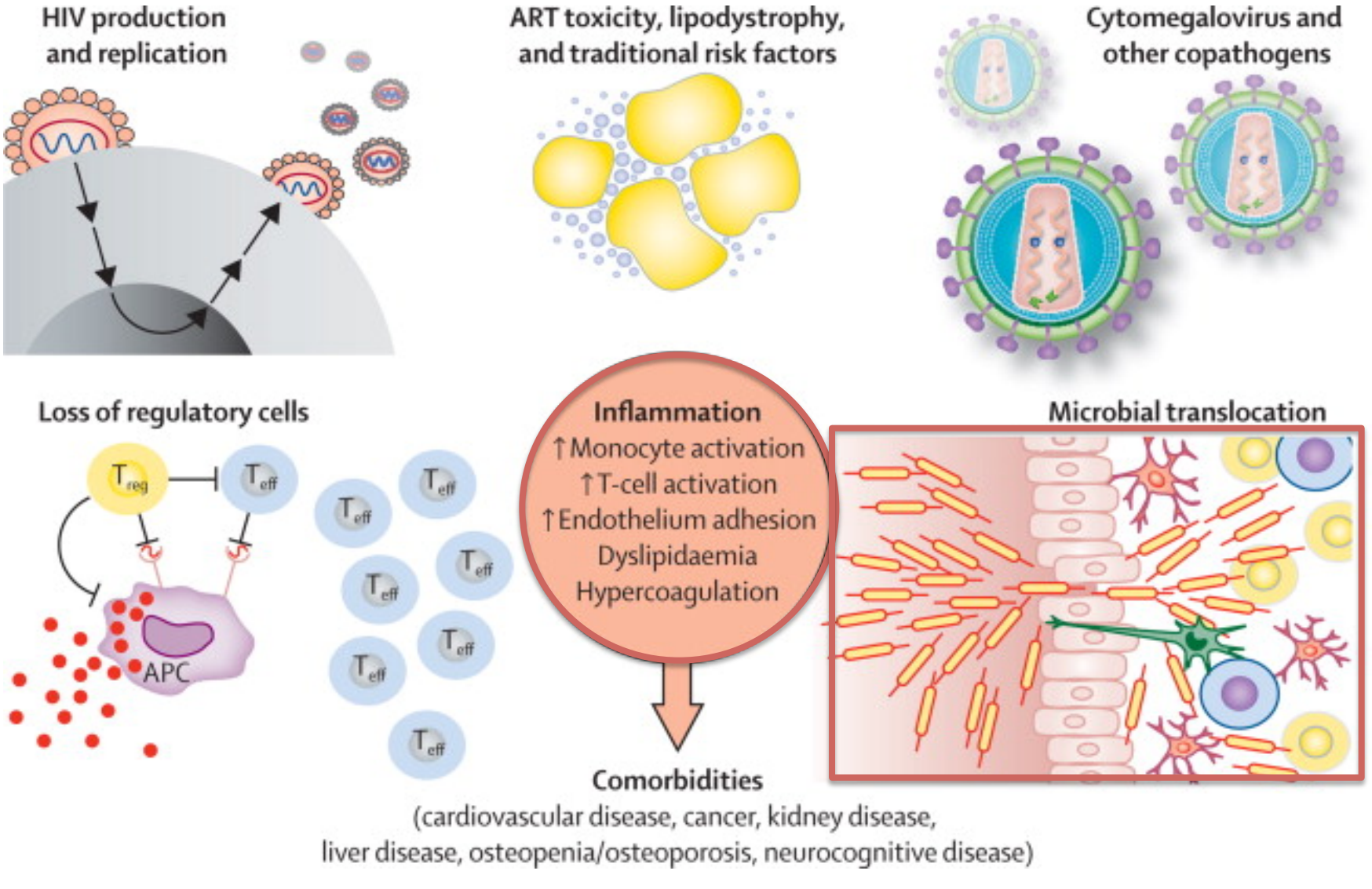
JAMA Network | Open™

Invited Commentary | Infectious Diseases

What It Means to Age With HIV Infection

Years Gained Are Not Comorbidity Free

Lauren F. Collins, MD; Wendy S. Armstrong, MD



Deeks et al., Lancet, 2013

Biomarkers linked to non-infectious comorbidities, serious non-AIDS events and death in cART-treated, HIV-infected subjects

Clinical event	Study design	Patient population	Aim	Markers	Main findings	References
CVD	Cross-sectional	61 cART-treated	Investigate whether microbial translocation and monocyte activation associate with endovascular dysfunction, inflammation and altered coagulation	LPS, sCD14, hs-CRP, d-dimer	sCD14 associates with endovascular dysfunction in HIV	[14]
CVD	Cross-sectional	147 cART-treated	Explore the relationship between inflammation, T-cell/monocyte activation and coronary calcification/subclinical vascular disease	sCD14, sCD163, hs-CRP, IL-6, sTNFR-II, sVCAM-1, OPG, RANKL	sCD14 independently associates with coronary artery calcification	[19]
CVD	Cross-sectional	252 cART-treated	Explore the association of soluble biomarkers with patient demographics, HIV progression, components of the metabolic syndrome, viral co-infections, Framingham risk score and the VACS Index	IL-6, cystatin C, hs-CRP, TNF-α, d-dimer	Elevated inflammation and coagulation associate with higher scores on risk stratification indices	[15]
CVD	Cross-sectional	242 cART-treated and 348 uninfected controls	Investigate the association of inflammatory markers with subclinical coronary artery disease	IL-6, sICAM-1, fibrinogen, d-dimer, hs-CRP, sTNFR I and II	Higher inflammatory marker levels associated with greater prevalence of coronary stenosis	[16]
CVD		149 cART-treated	Determine the associations of markers of immune activation with atherosclerosis and mortality	IL-6, hs-CRP, sCD14, sCD163, d-dimer, T cell activation (HLA-DR/CD38) and senescence (CD57/CD28), monocytes subsets (CCR2/CX3CR1/CCR5)	Monocyte CCR5 expression and plasma IL-6 associate with atherosclerosis. IL-6 and carotid artery IMT associate with all-cause mortality	[17]

Tincati et al. European Geriatric Medicine 2016

Biomarkers linked to non-infectious comorbidities, serious non-AIDS events and death in cART-treated, HIV-infected subjects

Clinical event	Study design	Patient population	Aim	Markers	Main findings	References
CVD/Bone disorders	Cross-sectional	94 cART-treated and 41 uninfected controls	Investigate the role of traditional factors, T-cell phenotype and osteoprotegerin in bone and cardiovascular disease	OPG, T cell activation (HLA-DR/CD38) and senescence (CD57/CD28)	OPG and T-cell activation/senescence linked to bone and cardiovascular disease	[28]
Bone disorders	Cross-sectional	142 cART-treated	Determine the association between bone health and inflammation, T-cell activation and monocyte activation	RANKL, OPG, sVCAM-1, sICAM-1, IL-6, TNF-α, sTNFR I and II, hs-CRP, d-dimer, sCD14, sCD163	Weak correlation between BMD and markers of inflammation/immune activation. Bone resorption associated with ICAM-1	[29]
Neurogognitive impairment	Longitudinal	99 cART-treated	Examine the relationship between mild HAND and CSF NFL/neopterin	Neopterin, NFL	Mild HAND was associates with increased intrathecal immune activation	[35]
Neurogognitive impairment	Cross-sectional	41 cART-treated	Investigate the role of cell-free mitochondrial DNA in the CSF	mitochondrialDNA, sCD14, TNF-α, IL-6, IL-8, MCP-1, IP-10, NFL	Higher cell-free mitochondrial DNA is associates with inflammation	[39]
Neurogognitive Impairment	Longitudinal	51 acutely-infected, cART-treated and 18 uninfected controls	Explore whether early cART impacts CD163 shedding, with possible implications on the CNS	sCD163	Early treatment reduces sCD163 which is linked to neuropsychological perfomances	[32]
Neurogognitive Impairment	Cross-sectional	90 cART-treated, 94 uninfected controls	Assess the impact of HIV on complex motor performances	d-dimer, IL-6, MCP-1, sCD14, TNF-α	Inflammation accounts for worse complex motor skills	[36]

Tincati et al. European Geriatric Medicine 2016

Biomarkers linked to non-infectious comorbidities, serious non-AIDS events and death in cART-treated, HIV-infected subjects

Clinical event	Study design	Patient population	Aim	Markers	Main findings	References
Death	Case–control	64 cases—128 controls (LCOSA cohort) 27 cases—54 controls (SCOPE cohort)	Assess immunologic predictors of mortality	IL-6, I-FABP, sCD14, hs-CRP, zonulin-1, sTNFR-1, d-dimer, KT ratio, T cell activation (CD38/HLA-DR), exhaustion (PD1), senescence (CD57/CD28)	Gut epithelial barrier dysfunction, innate immune activation, inflammation, independently predict mortality	[52]
SNAEs/death	Case–control; longitudinal	cART-treated: 143 cases (non-AIDS events) 315 controls	Assess the role of biomarkers on disease outcomes	IL-6, IFN- γ, sCD14, IP10, sTNFR-1, sTNFR-II, d-dimer T cell activation (CD38/HLA-DR), exhaustion (PD1) senescence (CD57/CD28)	IL-6, sTNFR-I and II, sCD14,, plasma KT ratio, and d-dimer level associate with a higher risk of non–AIDS-related morbidity and mortality	[72]
SNAEs	Case–control; longitudinal	cART-treated: 39 cases (non-AIDS events) 39 controls	Investigate whether unchanged or smaller decreases in systemic inflammation predict SNAEs	sCD14, sCD163, IL-6	Unchanged or slow rates of decrease of sCD14 and IL-6, levels predict SNAEs	[83]
SNAEs	Longitudinal	249 cART-treated	Examine the longitudinal changes in biomarkers following cART their association with future non-AIDS events	IL-6, d-dimer	Persistent elevation of d-dimer levels associate with an increased risk of non-AIDS events	[43]
SNAEs/death	Cross-sectional	3756 cART-treated: 1748 SMART, 1446 ESPRIT, 572 SILCAAT cohorts	Examine the associations of biomarkers with SNAEs/death in 3 large cohorts	IL-6, d-dimer, hs-CRP	IL-6 and d-dimer are independently associatee with SNAEs	[42]

Tincati et al. European Geriatric Medicine 2016

Outline

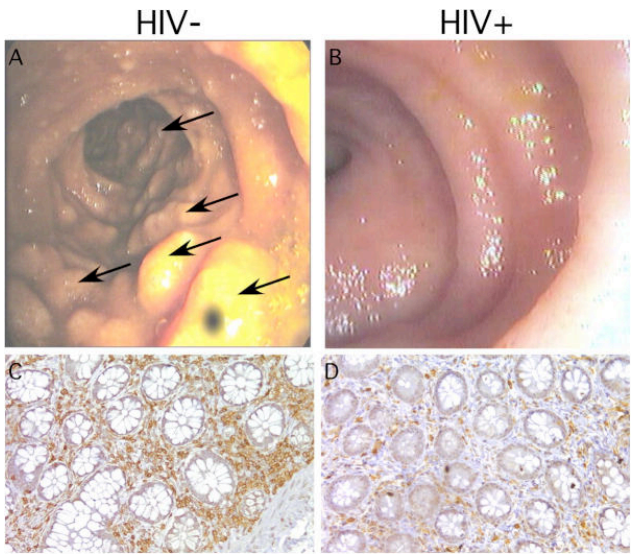
1. Introduction

- HIV as a chronic disease
- the gut as a site of HIV pathogenesis: microbial translocation, GI epithelial damage, microbial dysbiosis

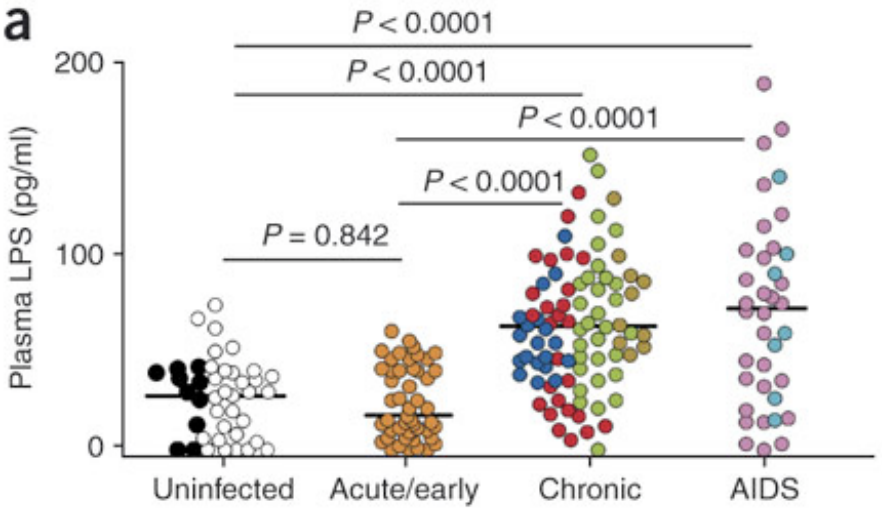
2. Does cART revert dysbiosis?

3. Is dysbiosis linked to non-infectious comorbidities?

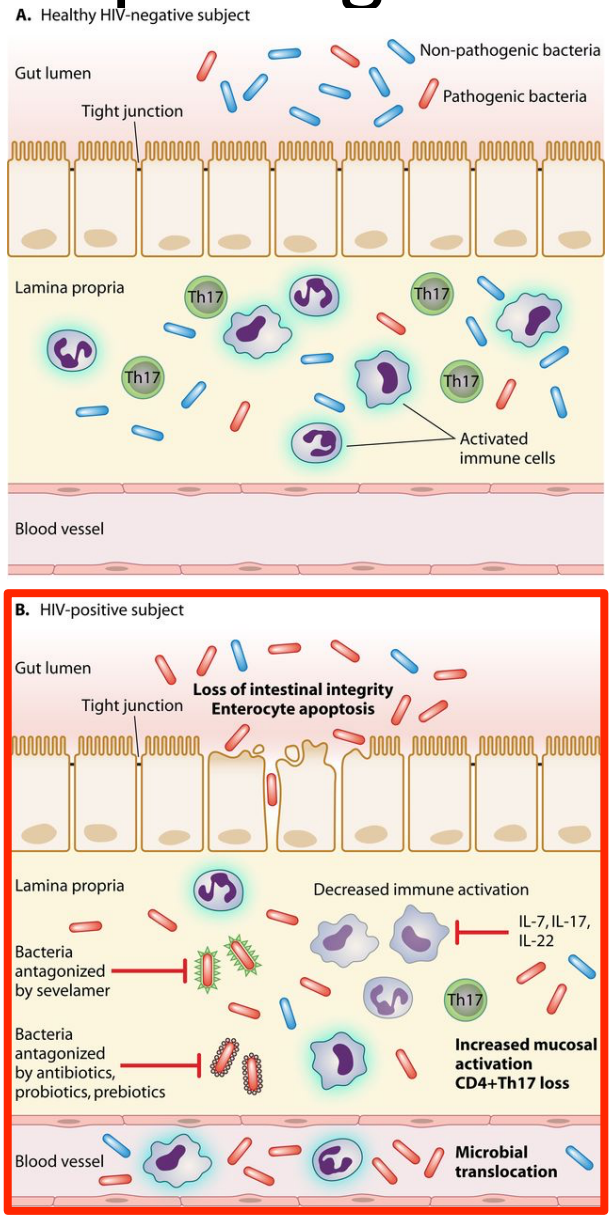
The GI tract as a site of HIV pathogenesis



Brenchley et al., J Exp Med 2004

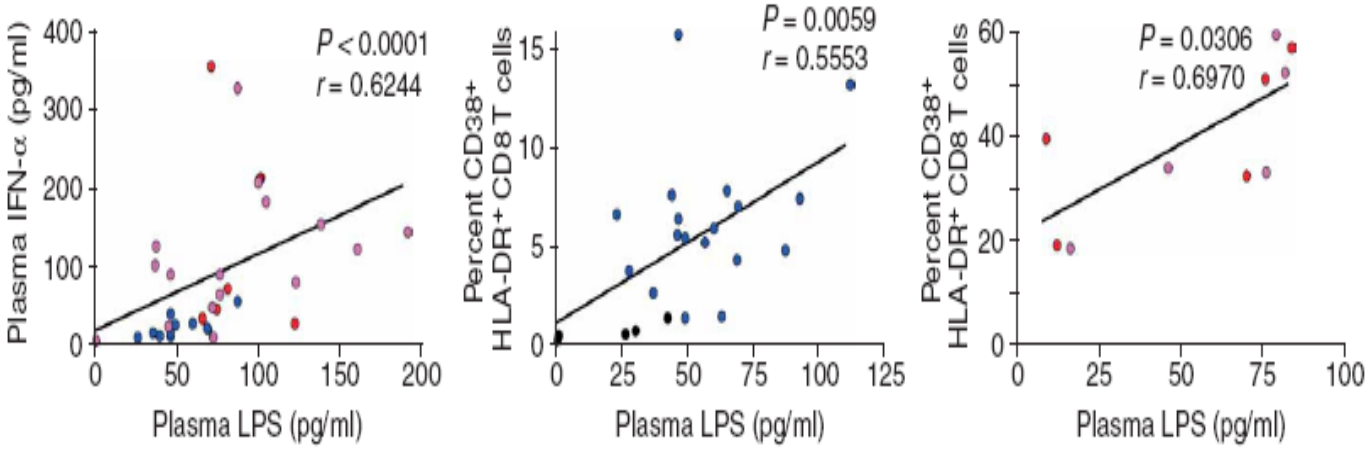


Brenchley et al., Nat Med 2006

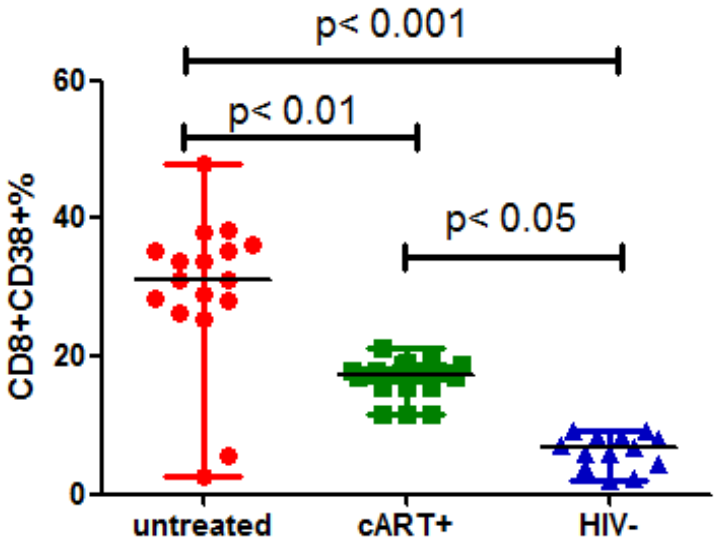


Marchetti et al. Clin Microb Rev, 2013

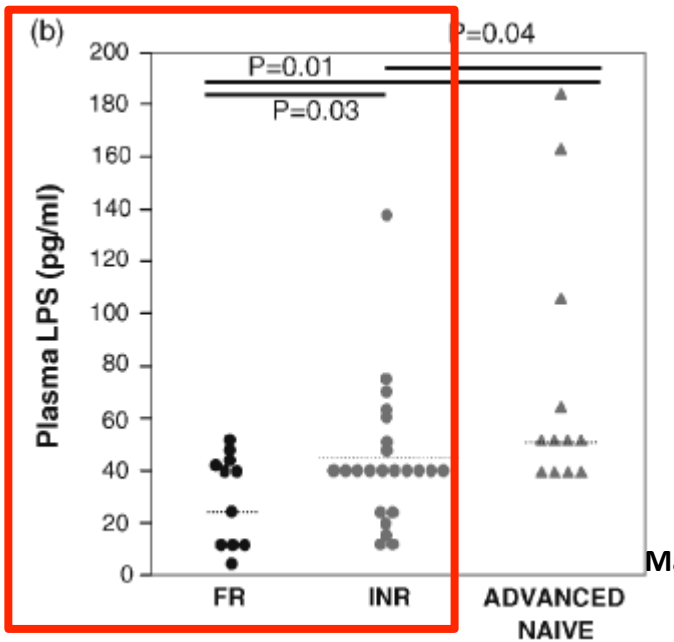
Microbial translocation is a cause of immune activation in HIV infection and persists despite effective cART



Brenchley et al., Nat Med 2006



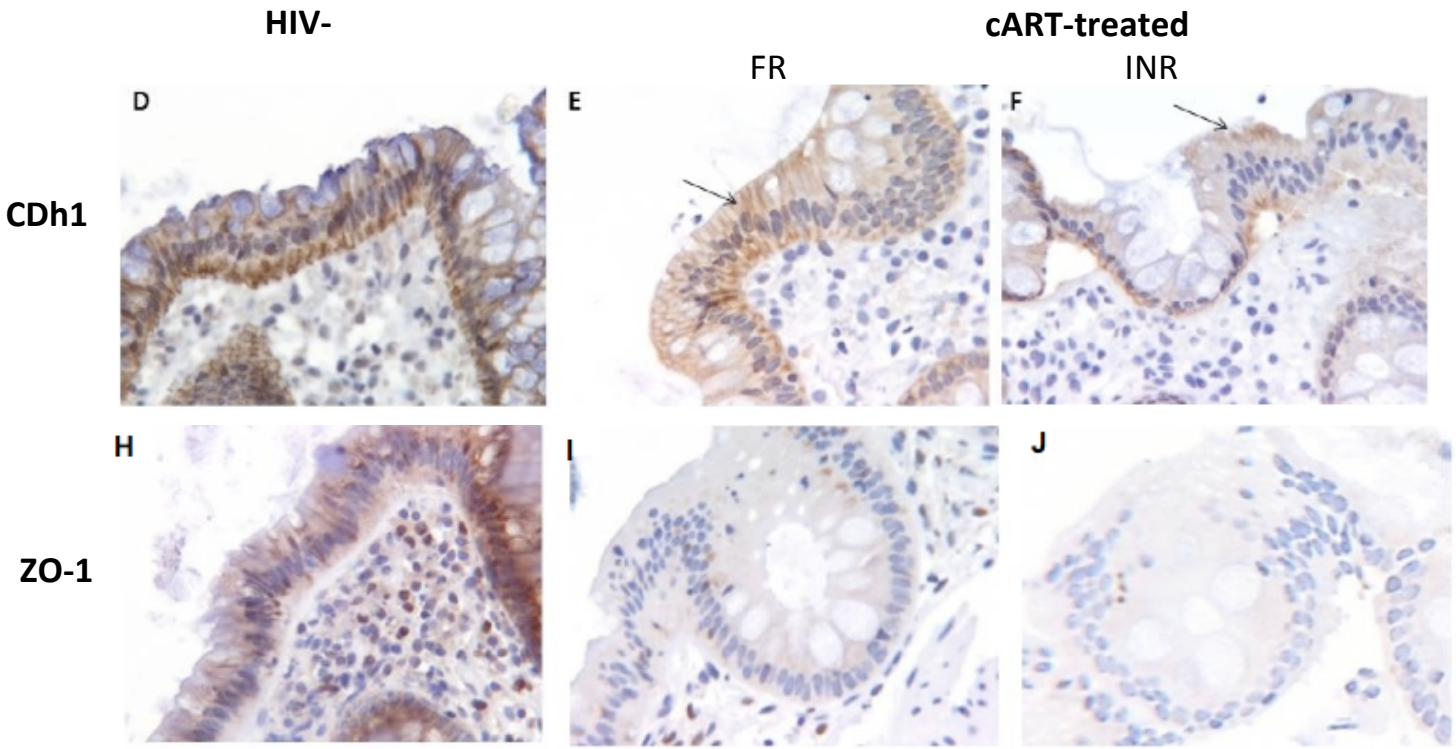
Cannizzo et al. JID 2015; see also Hunt et al. JID 2003



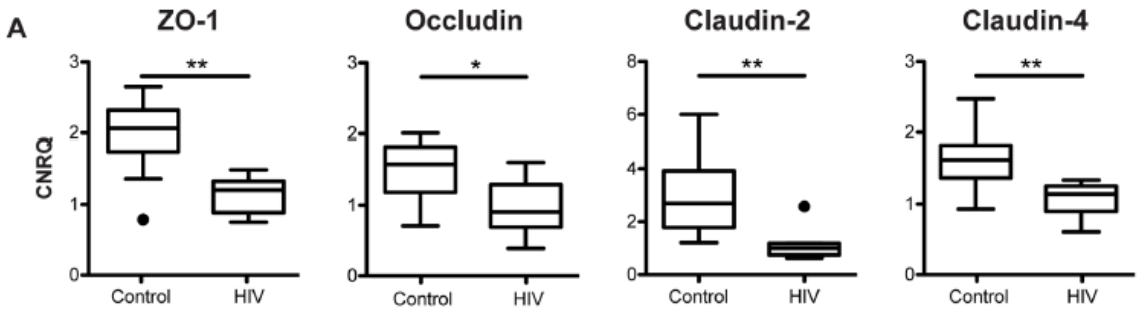
Marchetti et al., AIDS 2008

(see also Jiang JID 2009)

Gut damage in the course of cART

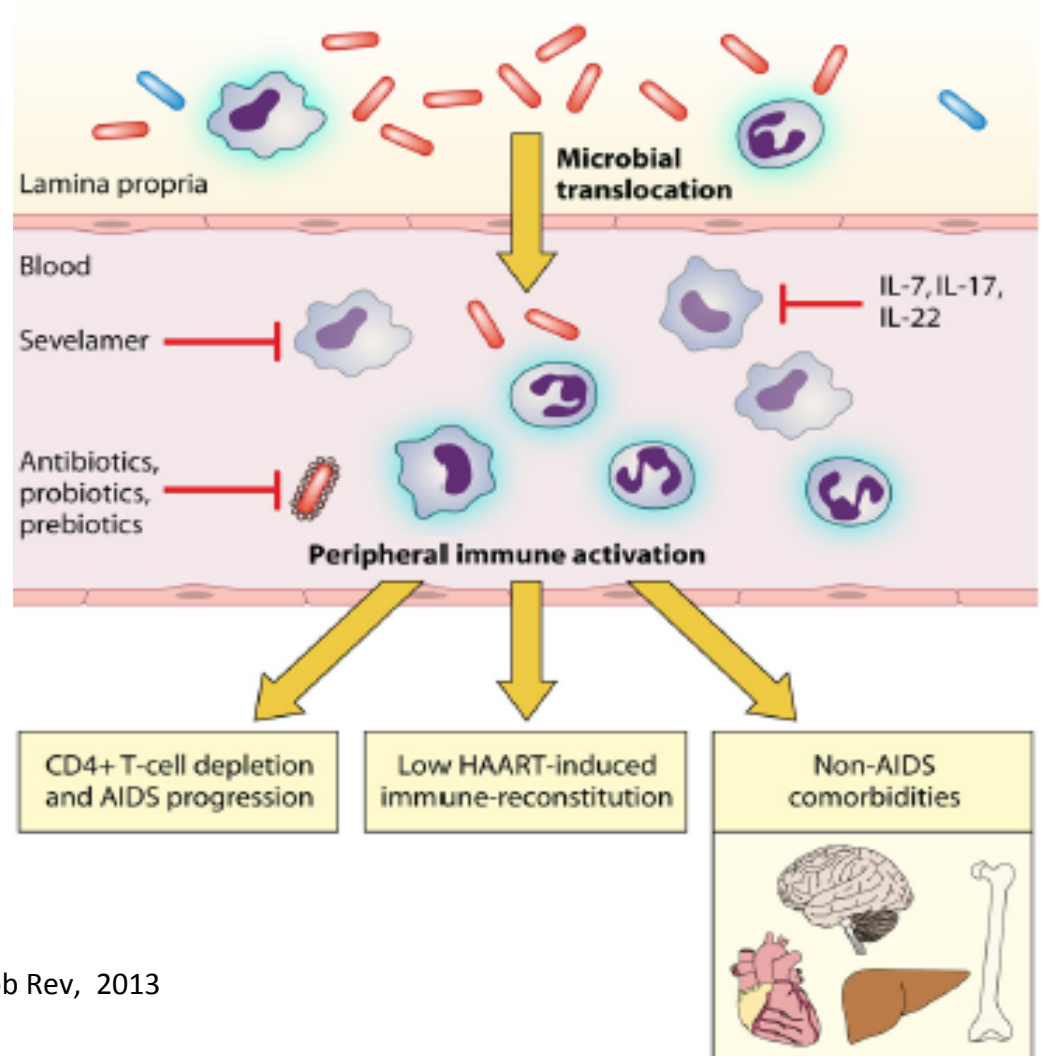


Tincati et al. AIDS 2016;
See also Somsouk et al, AIDS 2015



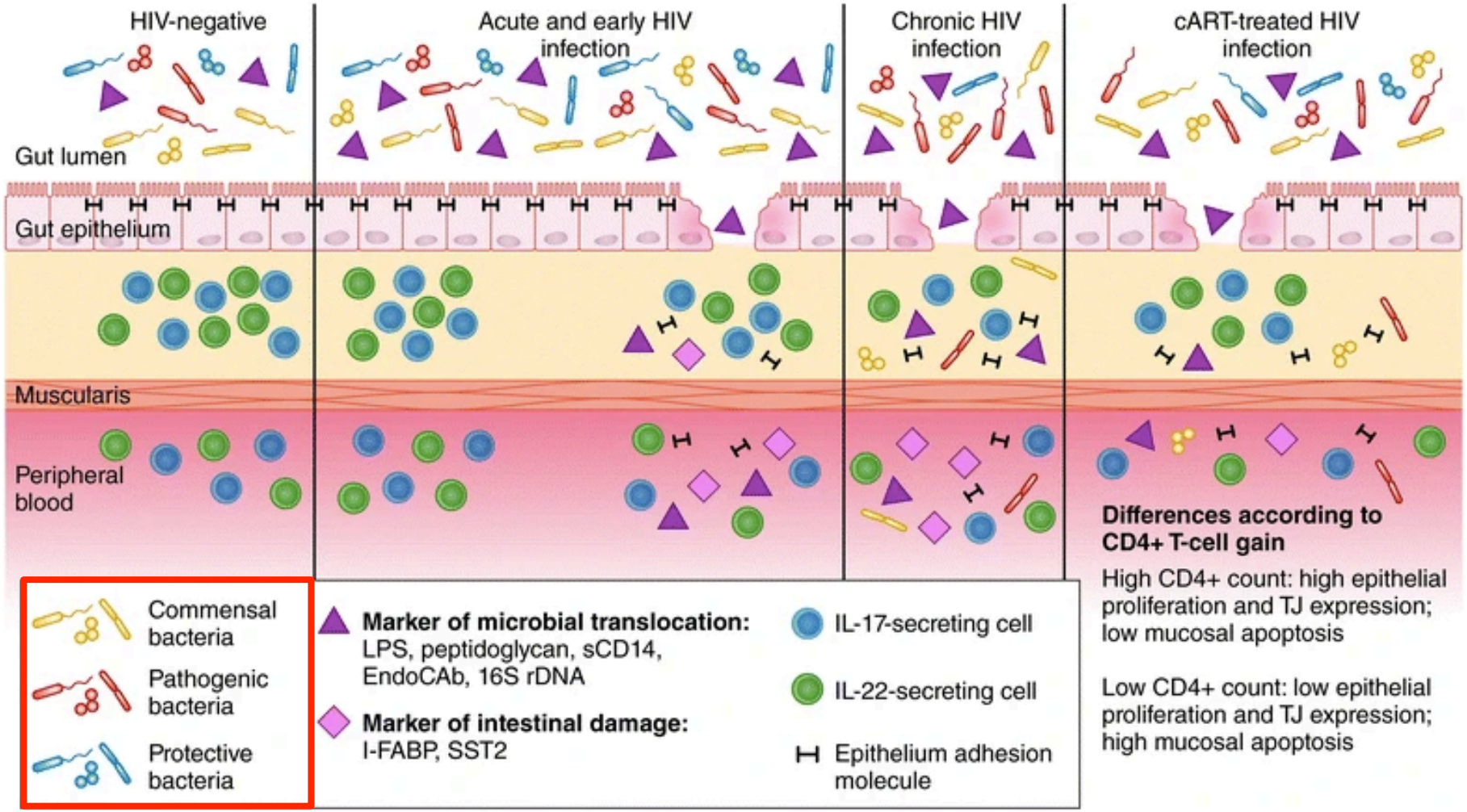
Chung et al., Plos Path , 2014

Possible adjuvant therapies to counteract MT-dependent immune activation in cART-treated individuals



Marchetti et al. Clin Microb Rev, 2013

HIV-related dysbiosis



Tincati et al., AIDS Res Ther, 2016

Disruption of gut microbiota early in the course of SIV/HIV disease

		Macaque #575			Macaque #588		
		Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Gram–	*Escherichia coli*	++++	–	–	++++	–	–
	Kluyvera sp.	++++	–	–	++	–	–
	Pseudomonas sp.	+++	–	–	++++	–	–
	Klebsiella pneumoniae	+++	–	–	++++	–	–
	Citrobacter freundii	+++	++++	++++	++++	++++	++++
	Klebsiella oxyloca	+++	–	–	–	–	–
	Enterobacter sp.	–	–	–	+++	–	+++
	S. maltophilia	–	–	+	–	–	–
	Campylobacter sp.	–	–	–	++++	–	–
	Salmonella sp.	–	–	–	–	–	–
	Yersinia sp.	–	–	–	–	–	–
	Shigella sp.	–	–	–	–	–	–
Gram+	*Staphylococcus* sp.	++	++	+++	++	–	–
	Bacillus sp.	+++	++++	–	+++	–	++++
	Lactobacillus sp.	+++	–	+++	++++	–	–
	Enterococcus sp.	–	++++	–	–	++++	–

'+' signs signify relative amounts of bacterial species cultured

Brenchley et al., Nat Med 2006

fluorescence in situ hybridization or quantitative real-time PCR (Q-PCR)

↑ *P. aeruginosa*
C. albicans

↓ *lattobacilli*

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2008, p. 757–758
0095-1137/08/$08.00+0 doi:10.1128/JCM.01729-07

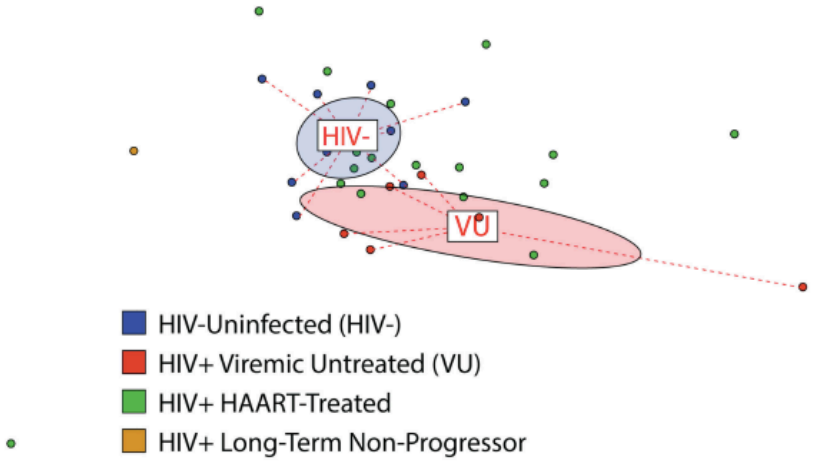
Vol. 46, No. 2

Early Impairment of Gut Function and Gut Flora Supporting a Role for Alteration of Gastrointestinal Mucosa in Human Immunodeficiency Virus Pathogenesis[▽]

Andrea Gori,[1,2*] Camilla Tincati,[2] Giuliano Rizzardini,[3] Carlo Torti,[4] Tiziana Quirino,[5] Monique Haarman,[6] Kaouther Ben Amor,[6] Jacqueline van Schaik,[6] Aldwin Vriesema,[6] Jan Knol,[6] Giulia Marchetti,[7] Gjalt Welling,[8] and Mario Clerici[9]

Gori et al., Journal of Clinical Microbiology,2008

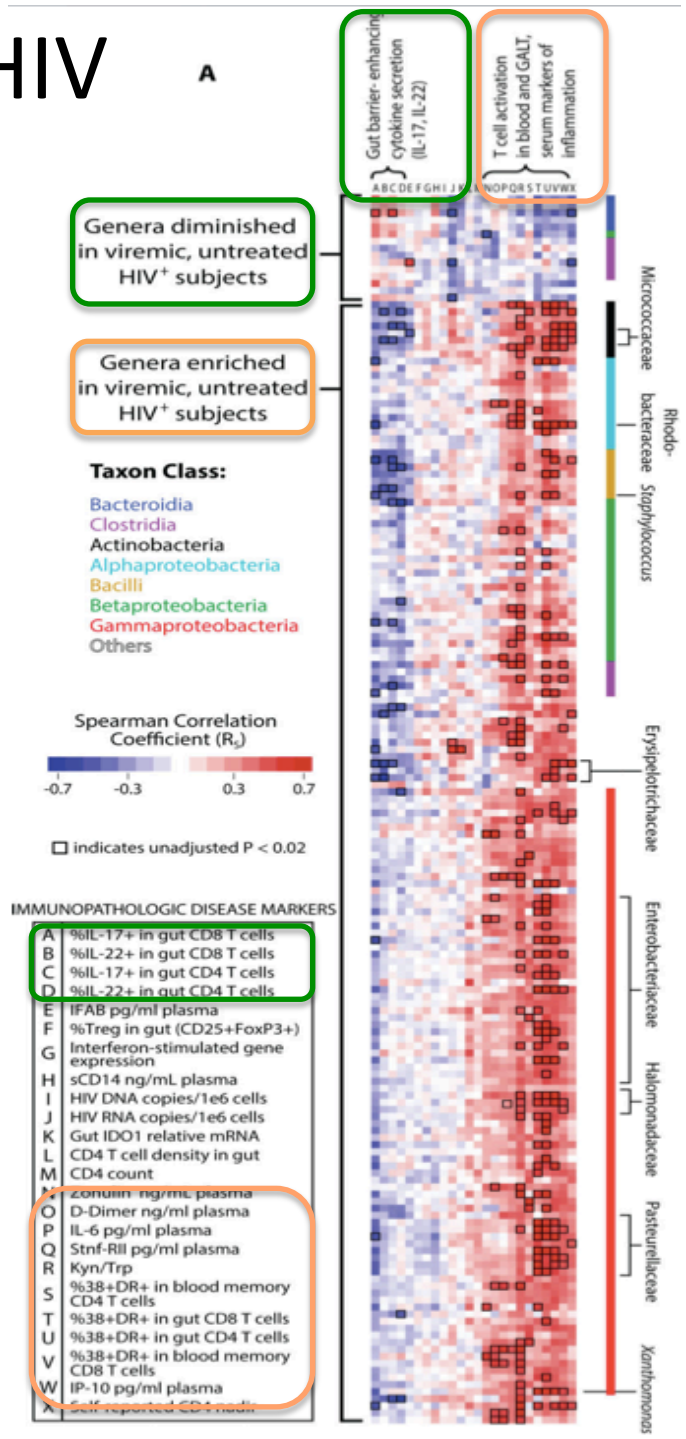
Gut dysbiosis is linked to HIV disease progression



- Bacteria **enriched** in VU subjects correlate with markers of **disease progression**

- Bacteria **depleted** in VU subjects correlate positively with **protective immune markers** (low T cell activation in the blood and gut, higher gut **IL-17 secretion**)

Vujkovic-Cvijin et al., Sci Transl Med, 2013



	Vujkovic-Cvijin *et al.*[51]	Lozupone *et al.*[40]	Perez-Santiago *et al.*[47]	McHardy *et al.*[42]
HIV-1-infected (M/F)	23 (23/0)	25 (22/3)	13 (13/0)	40 (40/0)
HIV-1 status				
Chronic	22	22	–	40
Early	–	3	13	–
AIDS	–	–	–	–
Other	–	LTNP $N=1$	–	–
ART	16 (+2)[a]	14 (8, 6)[b]	13[c]	20
Controls (M/F)	9 (9/0)	13 (8/5)	–	20 (20/0)
Geographical location	The United States	The United States	The United States	The United States
Sample site	Rectosigmoid biopsies	Fecal	Fecal	Rectal mucosa (sponge)

	Mutlu *et al.*[44]	Dillon *et al.*[37,38]	Lozupone *et al.*[41]	Vázquez-Castellanos *et al.*[49]
HIV-1-infected (M/F)	21 (16/5)	18 (13/5)	40 (M/F not reported)	15 (12/3)
HIV-1 status				
Chronic	21	18	40	15
Early	–	–	–	–
AIDS	–	–	–	–
Other	–	–	–	–
ART	19	–	28 (17/11)[e]	15
Controls (M/F)	22 (17/5)	14 (9/5)	15 (M/F not reported)	15 (8/7)
Geographical location	The United States	The United States	The United States	Spain
Sample site	Multiple biopsy sites[d], fecal	Colon biopsies, fecal	Fecal	Fecal

	Volpe *et al.*[50]	Dinh *et al.*[39]	Nowak *et al.*[46]	Yang *et al.*[52]
HIV-1-infected (M/F)	15 (13/2)	21 (17/4)	31 (16/15)	8 (5/3)
HIV-1 status				
Chronic	15	21	31	8[g]
Early	–	–	–	–
AIDS	–	–	–	–
Other	–	–	Elite controller $N=3$	–
ART	15	21	19[f]	–
Controls (M/F)	17 (13/4)	16 (12/4)	9 (5/4)	8 (4/4)
Geographical location	The United States	The United States	Sweden	The United States
Sample site	Fecal	Fecal	Fecal	Duodenum (mucosal brushes)[h]

	Noguera-Julian *et al.*[i] [45]	Monaco *et al.*[43]	Sun *et al.*[48]
HIV-1-infected (M/F)	129 (101/28)	82 (31/51)	13 (9/4)
HIV-1 status			
Chronic	129	82[k]	13
Early	–	–	–
AIDS	11	25	–
Other	Elite controller $N=8$	–	–
ART	84	40	11
Controls (M/F)	27 (23/3/1[j])	40 (20/20)	7 (5/2)
Geographical location	Spain	Uganda	China
Sample site	Fecal	Fecal	Fecal

ART, antiretroviral therapy; LTNP, long-term nonprogressor.
[a]Two viremic untreated study participants returned after 9 months of effective ART.
[b]Of the 14 chronically HIV-1-infected study participants on ART, eight study participants were on long-term (≥ 12 months) ART and six study participants on short-term (≤12 months) ART with three of the short-term ART sampled before and after ART initiation.
[c]All HIV-1-infected study participants started ART within 1 week of enrollment and followed for longitudinally for 48 weeks.
[d]Samples collected from terminal ileum, right colon, left colon.
[e]Of the 28 chronically HIV-1-infected study participants on ART, 17 were on long-term (≥ 12 months) ART and 11 study participants were on short-term (≤12 months) ART.
[f]Of the 31 HIV-1-infected study participants s, 19 were followed for a median 10 months after initiating ART.
[g]Years since first HIV-seropositive test reported as median 2.1 years with a range of 0.16–7 years.
[h]Samples were also collected from esophagus and stomach.
[i]Study included an external validation cohort; data reported for test cohort.
[j]Of uninfected study participants, one study participant reported as transgender.
[k]Time since diagnosis not reported.

AIDS

	Mucosal tissue	References	Fecal samples	References
Phylum Proteobacteria	↑	[37,51,52]	↑	[39,48,50]
Family				
Enterobacteriaceae	↑	[44,51]	↑	[39]
Brucellaceae	↑	[37]		
Xanthomonadaceae	↑	[37]		
Rhodspirillaceae	↓	[37]		
Genus				
Escherichia	↑	[44,51]		
Serratia	↑	[51]		
Shigella	↑	[51]		
Klebsiella	↑	[51]		
Ralstonia	↑	[44]		
Actinobacter	↑	[37]		
Burkholderia	↑	[52]		
Desulfovibrio			↑	[40]
Thalossospira	↓	[37]		
Phylum Bacteroidetes				
Family				
Rikenellaceae	↓	[42,44]	↓	[39,40]
Bacteroidaceae	↓	[37]	↓	[37,40,48]
Prevotellaceae	↑	[37]	↑	[40]
Genus				
Bacteroides	↓	[37,44,51]	↓	[37,40,48,49]
Alistipes	↓	[37,42,51]	↓	[37,39,40,50]
Barnesiella	↓	[37]	↓	[37]
			↑	[39]
Prevotella	↑	[37,44]	↑	[40,49]
Phylum Firmicutes	↓	[37,42,52]		
Family				
Lachnospiraceae	↓	[37,42,44]		
Ruminococcaceae	↓	[37,42,44]	↓	[37]
Christensenellaceae	↓	[37]		
Erysipelotrichaceae	↑	[51]	↑	[39,40]
Veillonellaceae			↑	[40]
Genus				
Coprococcus	↓	[37,42,44]		
Faecalibacterium	↓	[44]	↓	[46,48,49]
Blautia	↓	[37,44]		
Rumminococcus	↓	[42,44]		
Dorea	↓	[44]		
Oscillospira	↓	[44]		
Lachnospira	↓	[42,44]	↓	[48]
Roseburia	↓	[42,44]	↓	[48,49]
Dialister	↓	[44]	↑	[40]
Eubacterium	↓	[42,44]		
Lactobacillus	↓	[52]	↑	[46]
Mitsuokella			↑	[40]
Catenibacterium	↑	[44]	↑	[40]
Mogibacterium	↑	[44]		
Bulleidia			↑	[40]

AIDS

Dillon et al. AIDS 2016

Outline

1.Introduction

- HIV as a chronic disease
- the gut as a site of HIV pathogenesis: microbial translocation, GI epithelial damage, microbial dysbiosis

2. Does cART revert dysbiosis?

3. Is dysbiosis linked to non-infectious comorbidities?

frontiers in Immunology

ORIGINAL RESEARCH
published: 12 March 2019
doi: 10.3389/fimmu.2019.00463

Altered Immunity and Microbial Dysbiosis in Aged Individuals With Long-Term Controlled HIV Infection

*Nicholas Rhoades[1], Norma Mendoza[1], Allen Jankeel[1], Suhas Sureshchandra[1], Alexander D. Alvarez[1], Brianna Doratt[1], Omeid Heidari[2], Rod Hagan[3], Brandon Brown[4], Steven Scheibel[3], Theodore Marbley[3], Jeff Taylor[5] and Ilhem Messaoudi[1]**

[1] Molecular Biology and Biochemistry, University of California Irvine, Irvine, CA, United States, [2] School of Nursing, John Hopkins University, Baltimore, MD, United States, [3] Stonewall Medical Center, Borrego Health, Cathedral City, CA, United States, [4] School of Medicine, University of California, Riverside, Riverside, CA, United States, [5] HIV+ Aging-Palm Springs, Palm Springs, CA, United States*

Study design and population

- Cross-sectional study on 105 men ≥55 years of age
- HIV- n = 47
- LTC HIV+ n = 58

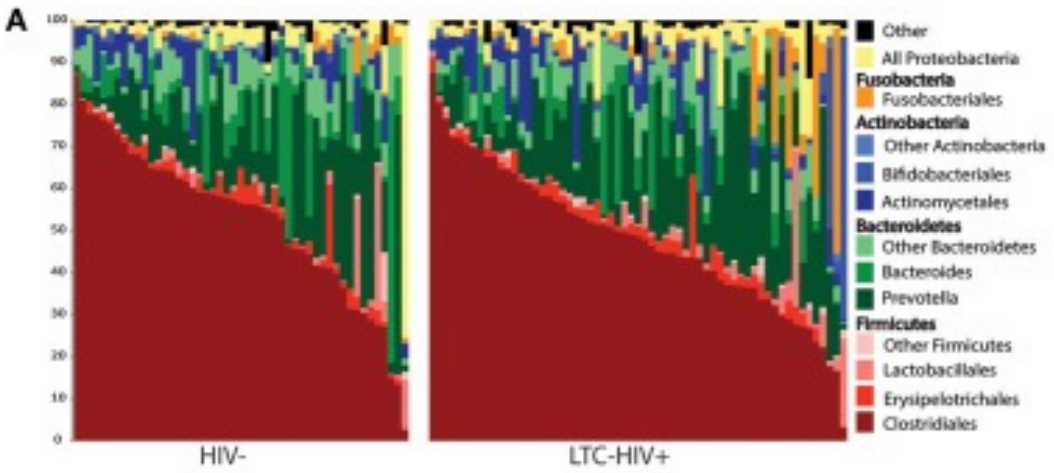
- LTC HIV+ on cART for >10 years
- undetectable viral loads
- median CD4+ cell counts 689 cells/mm3

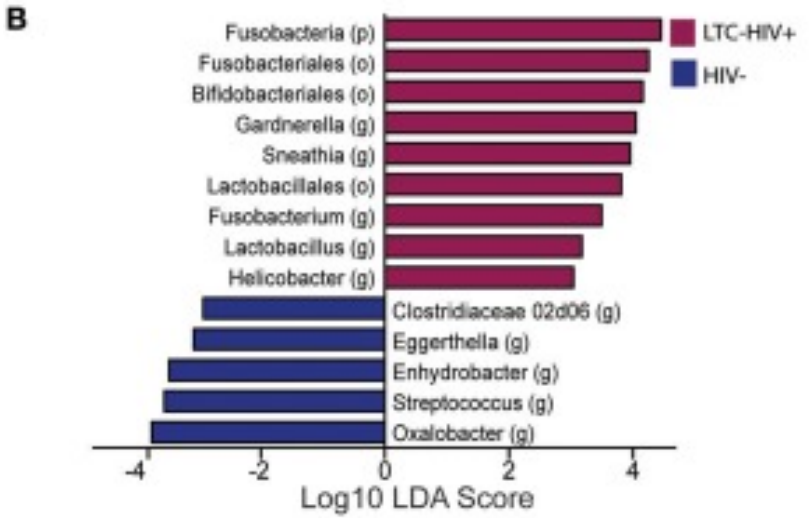
TABLE 1 | Demographics and co-morbidities.

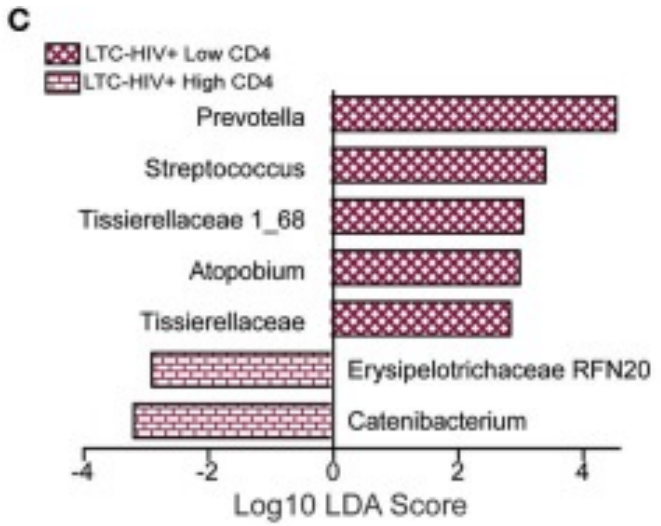
	Total *n* [*S.D.*](%)	LTC-HIV + *n* [*S.D.*](%)	HIV – *n* [*S.D.*](%)	*p*
DEMOGRAPHICS				
Age	64 [6.8]	65.7 [7.3]	62.7 [6.03]	0.02*
Race				0.82
Non-hispanic white	90 (84.6)	49 (84.5)	41 (87.2)	
Other	15 (15.5)	9 (15.5)	6 (12.8)	
Education				0.299
High school	8 (8.3)	7 (12.5)	1 (2.4)	
Some college	32 (33.3)	21 (37.5)	11 (26.8)	
Four-year university	25 (24.0)	13 (23.2)	12 (29.3)	
Graduate school	32 (33.3)	15 (26.8)	17 (41.4)	
Relationship status				0.10
Single	43 (41.7)	29 (49.2)	14 (31.8)	
In a relationship	18 (17.5)	10 (16.9)	8 (18.2)	
Engaged	2 (1.9)	1 (1.7)	1 (2.3)	
Married	34 (33.0)	14 (23.7)	20 (45.5)	
Widowed	6 (5.8)	5 (8.5)	1 (2.3)	
On disability	28 (28.28)	25 (43.86)	3 (7.14)	0.001***
COMORBIDITIES				
Depression	27 (25.96)	21 (35.59)	6 (13.33)	0.013*
Arthritis	30 (28.85)	17 (28.81)	13 (28.89)	1.000
Hepatitis	8 (7.69)	8 (13.56)	0 (0)	0.009***
Neuropathy	33 (31.73)	24 (40.68)	9 (20)	0.033*
Hypertension	38 (36.54)	18 (30.51)	20 (44.44)	0.156
Dermatitis	12 (11.54)	10 (16.95)	2 (4.44)	0.064
Herpes	13 (12.50)	8 (13.56)	5 (11.11)	0.773
Vision loss	17 (16.35)	9 (15.25)	8 (17.78)	0.792
Diabetes	13 (12.5)	8 (13.56)	5 (11.11)	0.773
Hearing loss	25 (24.04)	11 (18.64)	14 (31.11)	0.168
Respiratory problems	6 (5.78)	3 (5.08)	3 (6.67)	1.000
Heart condition	11 (10.58)	4 (6.78)	7 (15.56)	0.201
Broken bones	1 (0.96)	0 (0)	1 (2.22)	0.433

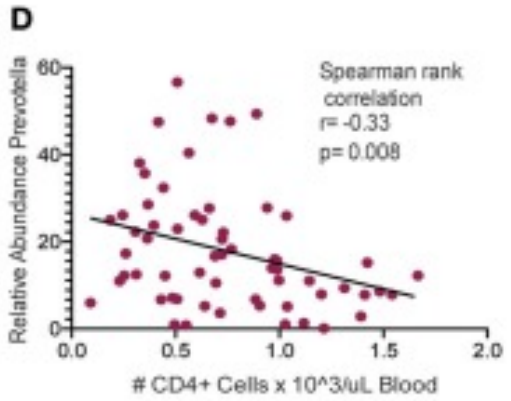
*Chi-squared or Fisher's exact tests were conducted to determine significant differences between the two *$p < 0.05$, ***$p < 0.001$.*

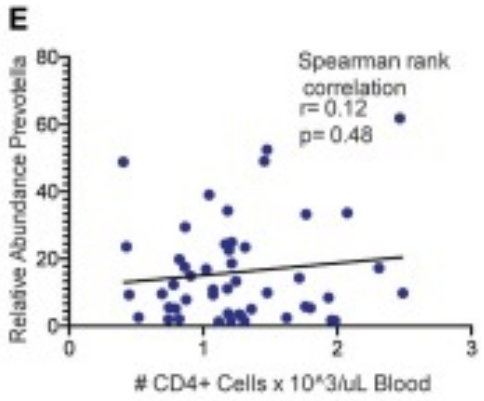
LTC-HIV+ are enriched in gut Gardnerella, Snethia, Fusobacterium, Lactobacillus, and Helicobacter; Prevotella is more abundant in individuals with a low CD4+ and negatively correlates with CD4+ T cell count











Rhoades et al. Front Immunol 2019

frontiers in Immunology

ORIGINAL RESEARCH
published: 26 February 2021
doi: 10.3389/fimmu.2021.639291

Long-Term Suppressive cART Is Not Sufficient to Restore Intestinal Permeability and Gut Microbiota Compositional Changes

Giuseppe Ancona[1†‡], *Esther Merlini*[1†‡], *Camilla Tincati*[1], *Alessandra Barassi*[2], *Andrea Calcagno*[3], *Matteo Augello*[1], *Valeria Bono*[1], *Francesca Bai*[1], *Elvira S. Cannizzo*[1], *Antonella d'Arminio Monforte*[1] *and Giulia Marchetti*[1*]

[1] *Clinic of Infectious Diseases, Department of Health Sciences, University of Milan, Azienda Socio Sanitaria Territoriale Santi Paolo e Carlo, Milan, Italy,* [2] *Biochemistry Laboratory, Department of Health Sciences, University of Milan, Azienda Socio Sanitaria Territoriale Santi Paolo e Carlo, Milan, Italy,* [3] *Unit of Infectious Diseases, Department of Medical Sciences, University of Turin, Turin, Italy*

OPEN ACCESS

Edited by:
Nicholas Funderburg, The Ohio State University, United States

Reviewed by:
Ivan Vujkovic-Cvijin, National Institutes of Health (NIH), United States
Sergio Serrano-Villar, Hospital Universitario Ramón y Cajal, Spain

Study design and population

Longitudinal study on naive HIV-infected individuals introducing cART (T12-T24)

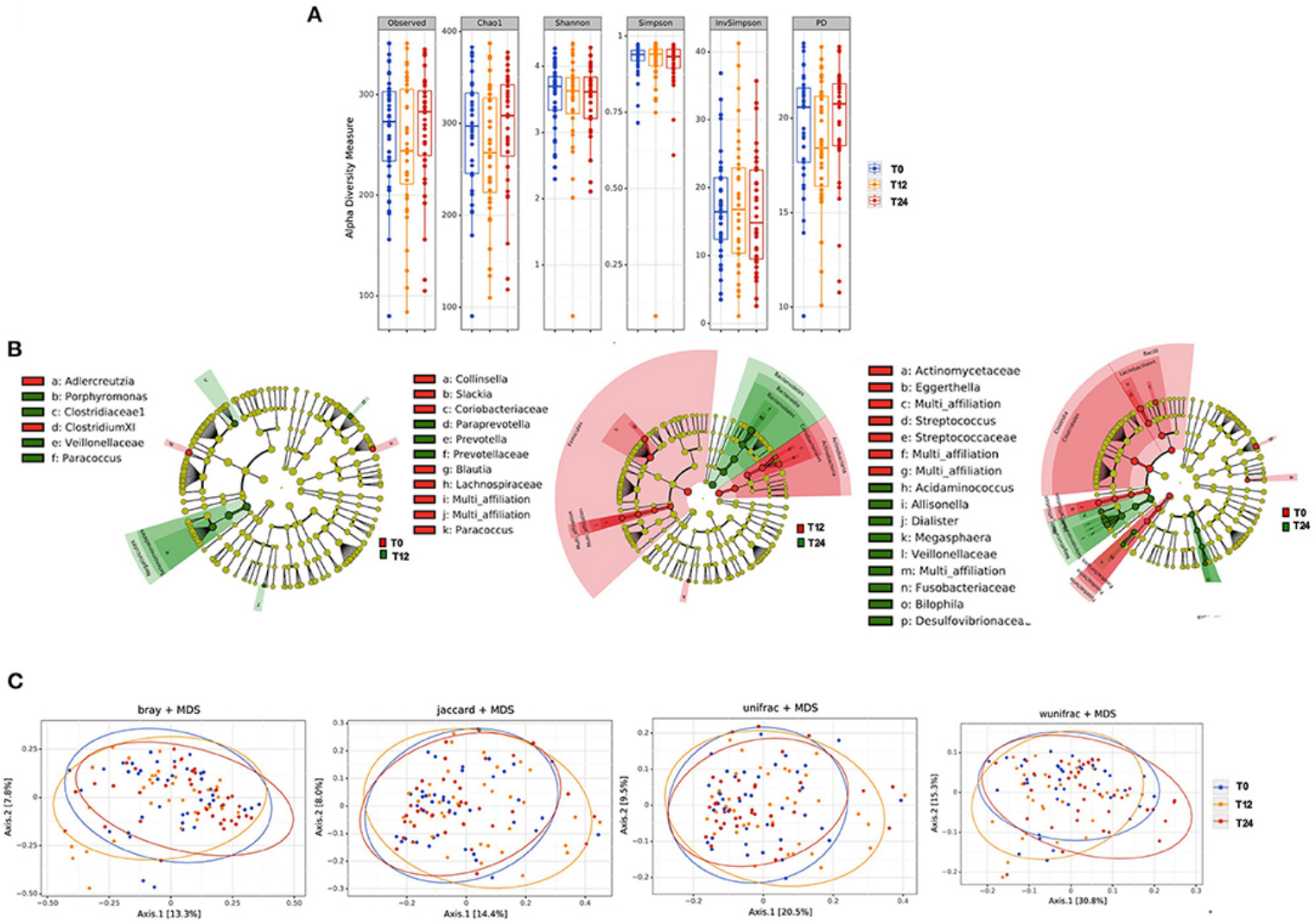
TABLE 1 | Epidemiological, Clinical, and HIV-related features of HIV-infected cohort.

	HIV -infected (*N* = 41)	HIV negative (*N* = 15)	*p*-value
Age, years (IQR)*	42 (31.5–50.5)	29 (24–33)	**0.0017**
Sex, male (n) (%)°	37 (90)	15 (100)	0.56
Sexual behaviors MSM, n (%)°	28 (68.2)	5 (33.3)	**0.039**
BMI, n (IQR)*	22.66 (22.02–25.18)	22.71 (20.98–24.91)	0.86
Vegetarian/vegan diet, n subjects (%)°	3 (7.3)	0	0.55
Ongoing antibiotic prophylaxis, n (%)°	8 (19.5)	0	0.09
Hepatitis coinfection, n (%)°	2 (4.8)	0	1
First cART regimen, n (%)		n/a	n/a
NNRTI-based	25 (61)		
PI-based	9 (22)		
INSTI-based	7 (17)		

**Data are median (IQR), statistical analysis Mann-Whitney test. °data are n (%), statistical analysis Fisher's Exact Test. IQR, Interquartile; cART, combination of antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; INI, integrase inhibitor.*

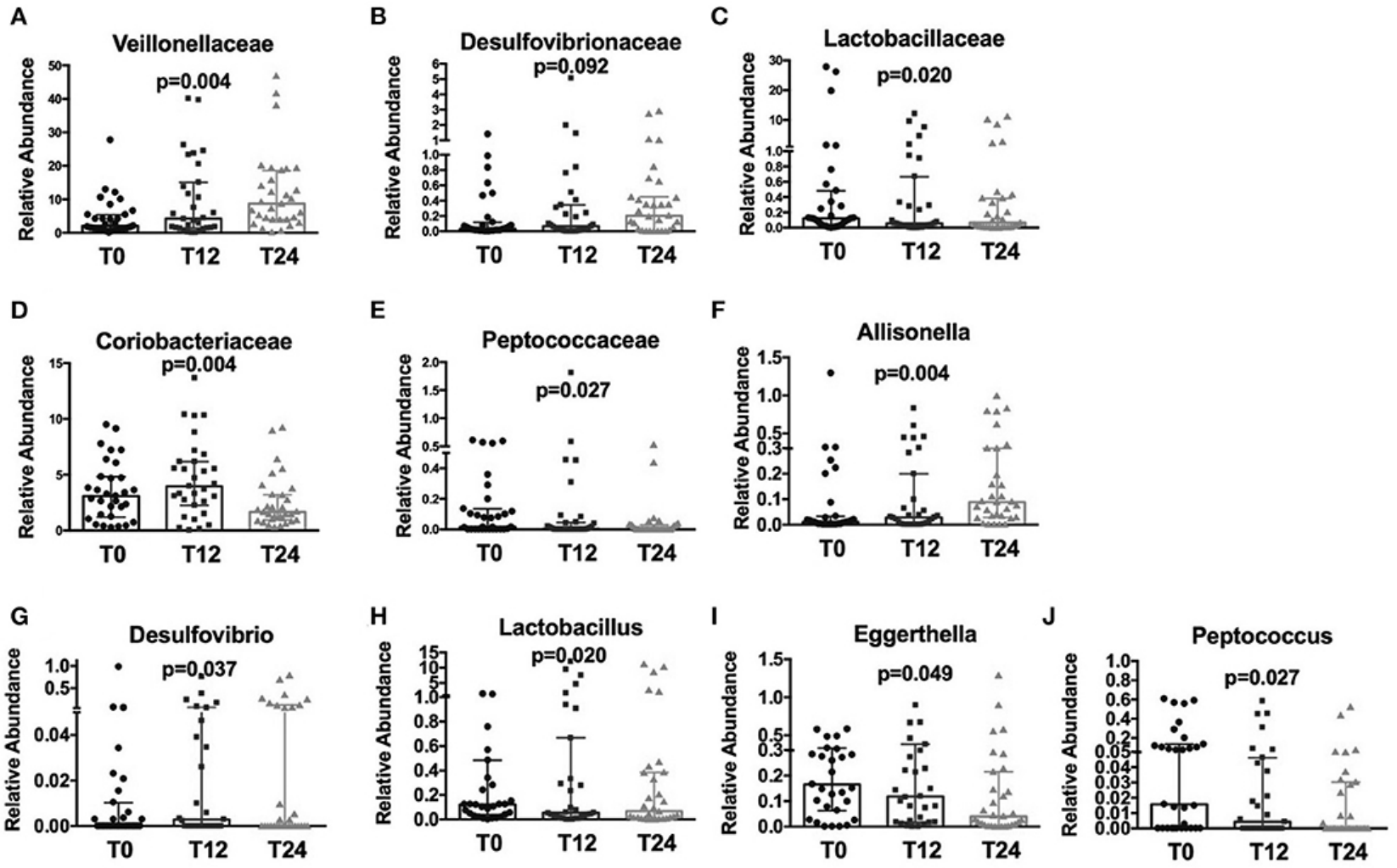
Ancona et al. Front Immunol 2021

No effect of cART alpha- (richness/ evenness) and beta-diversity (LEfSe; PcoA)



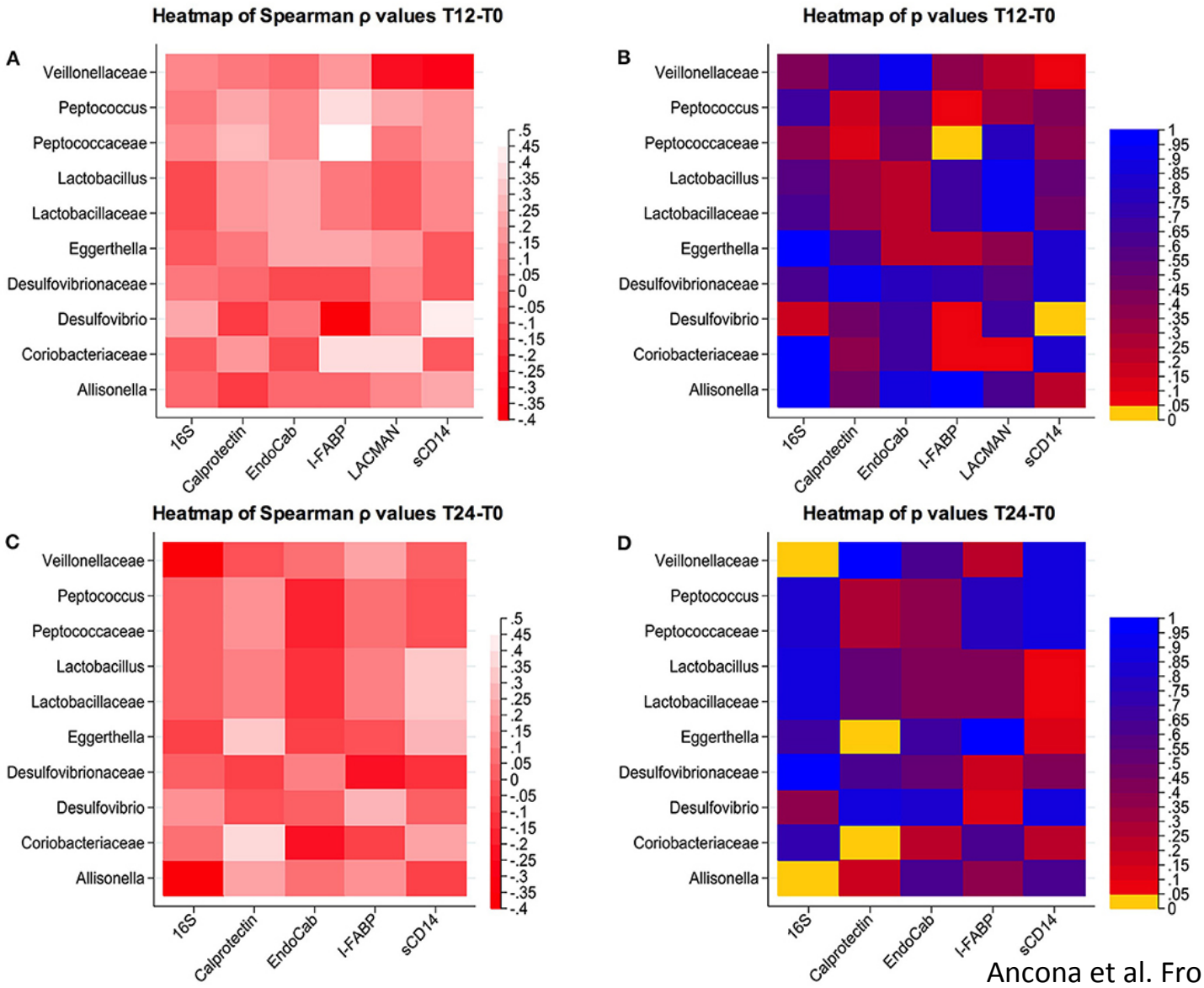
Ancona et al. Front Immunol 2021

Shifts in fecal abundance (family/ genus level) following cART



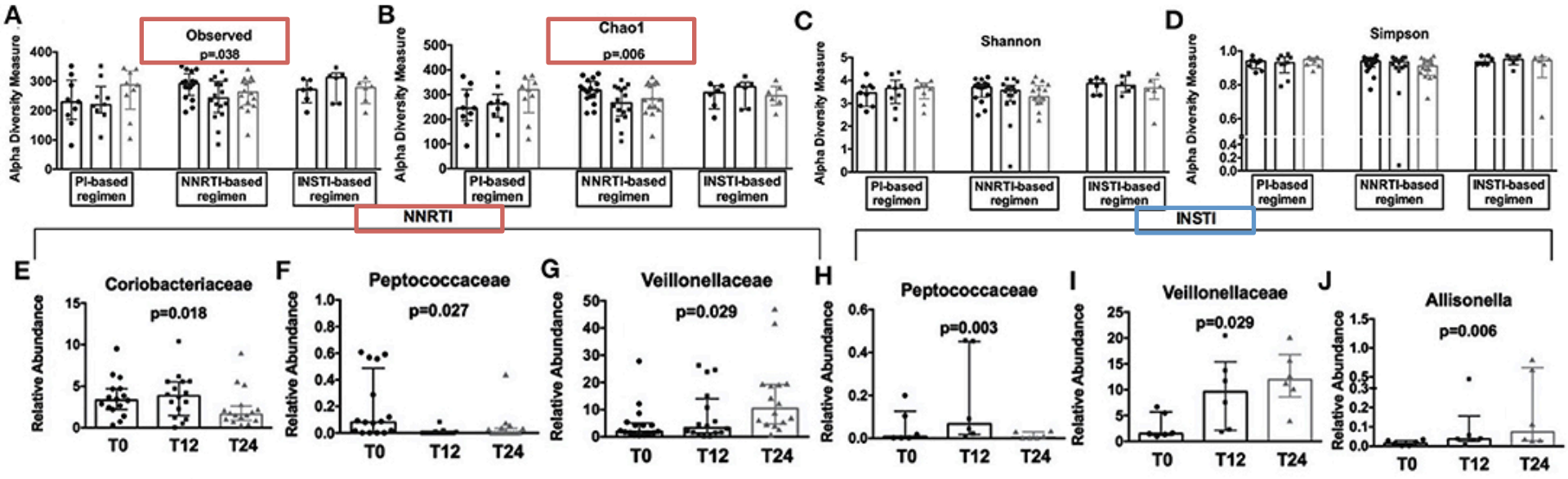
Ancona et al. Front Immunol 2021

Possible associations between bacterial composition and gut damage/microbial translocation



Ancona et al. Front Immunol 2021

Changes in Gut Microbiota Composition According to the cART Class



- **NNRTI-based therapy**
- reduced richness but not evenness indexes
- modified relative abundance: reduced Coriobacteriaceae, Peptococcaceae, increased Veillonellaceae

- **INSTI-based therapy**
- modified relative abundance: decreased Peptococcaceae, increased Veillonellaceae and Allisonella genus

Ancona et al. Front Immunol 2021

Does cART revert dysbiosis?

- Along with the well-known persisting structural defects of the GI tract, long-term cART apparently does not correct HIV-related dysbiosis

- Implications:

- elaboration of therapuetic strategies

- development of non-infectious comorbidities

Outline

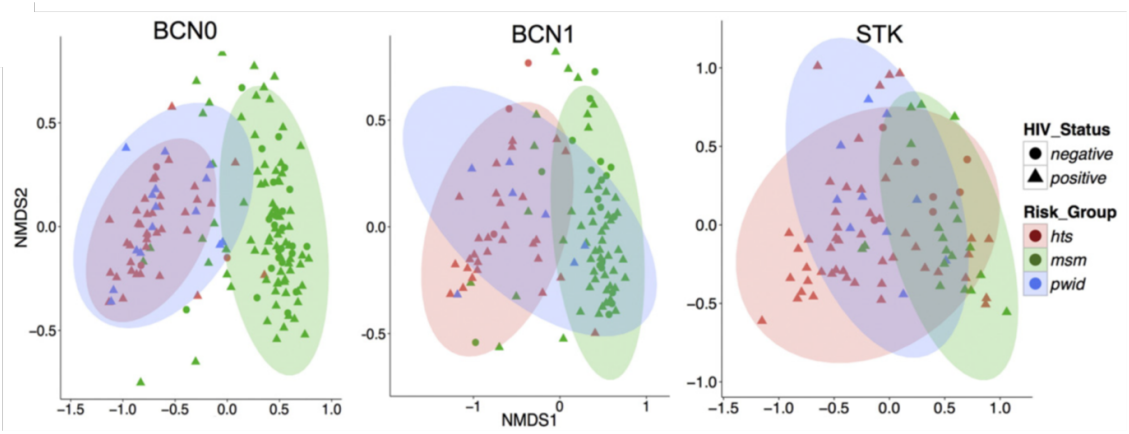
1. Introduction

- HIV as a chronic disease
- the gut as a site of HIV pathogenesis: microbial translocation, GI epithelial damage, microbial dysbiosis

2. Does cART revert dysbiosis?

3. Is dysbiosis linked to non-infectious comorbidities?

Dysbiotic microbiome drives immune activation in HIV-infected MSM

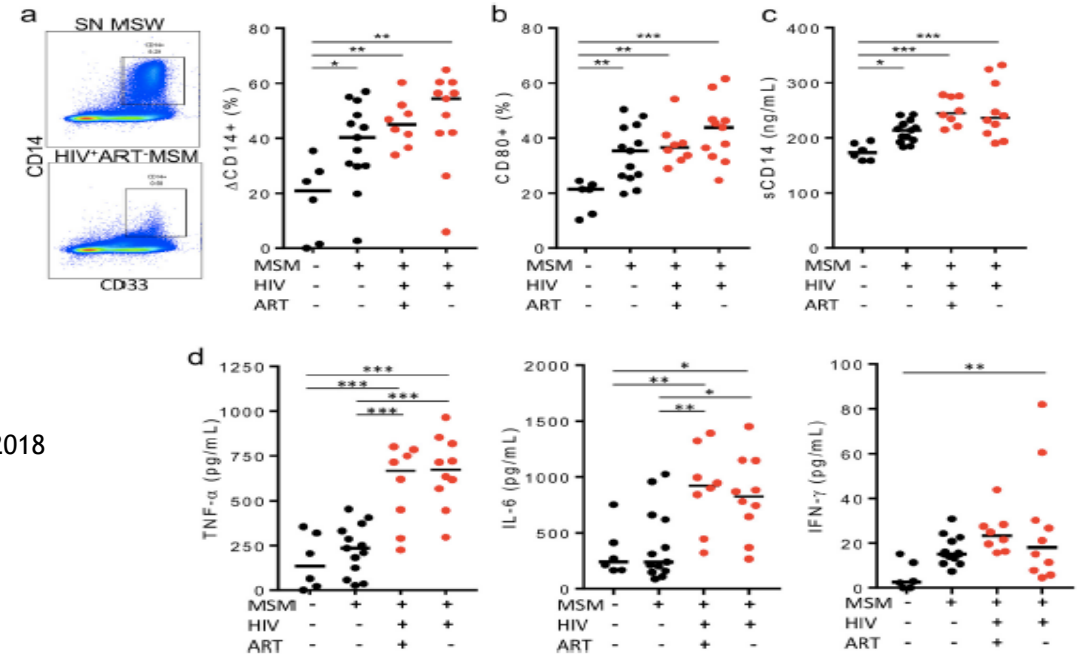


Bray-Curtis distances cluster by HIV transmission group (MSM, Men having Sex with Men, vs non-MSM) rather than by HIV serostatus

Noguera Julian et al 2016

Fecal Bacterial Communities (FBCs) from HIV-infected MSM induce monocyte activation and inflammatory cytokines

Neff et al. 2018



Hypotheses and Aims of the Study

- **Hypothesis:** does the fecal microbiome influence the cardio-vascular risk of HIV-infected, male subjects according to their sexual behaviour?

- **Aim:** evaluate the role of gut microbial dysbiosis in cardiovascular risk in a cross-sectional cohort of virologically-suppressive HIV patients stratified according to sexual behaviour

Study Design

Study design: cross-sectional study, single assessment

Study population: 44 HIV+ sex male patients treated with virologically suppressive cART stratified according to:

- **Sexual behaviour:**

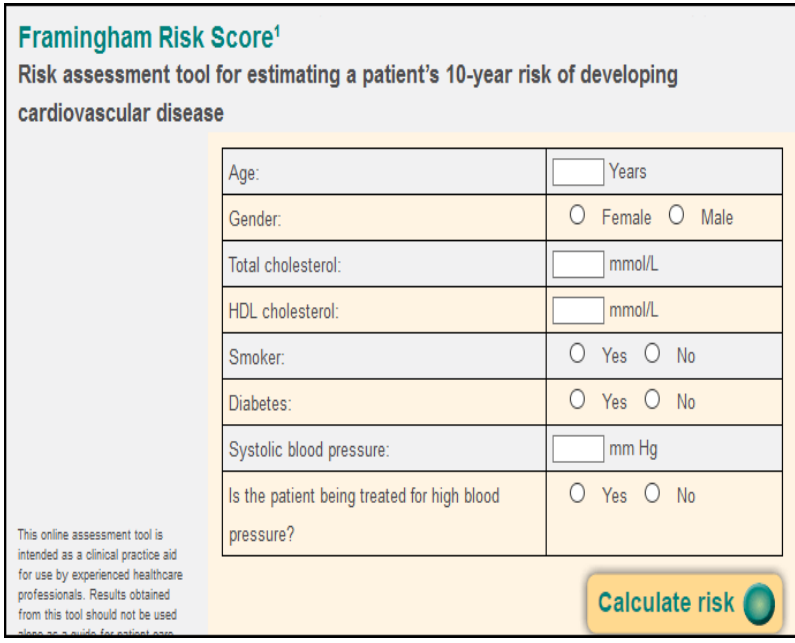
- Men having Sex with Men (MSM)
- Men having Sex with Women (MSW)

- **10-year Framingham Risk Score (FS)**: risk of developing a first CVD event (coronary heart disease, stroke, peripheral artery disease or heart failure) within 10 years

L; low (L) FS (< 10)**I/H: intermediate/high** FS (≥10)

Exclusion Criteria

- Female sex
- HBV or HCV
- Age > 60 years and <30 years
- Recent antibiotics (< 3 months)
- Recent probiotics
- Specific diets: vegan, vegetarians
- Diagnosis of DM
- Cardiac disease; GI disorders
- Alcohol intake > 14 drinks/week



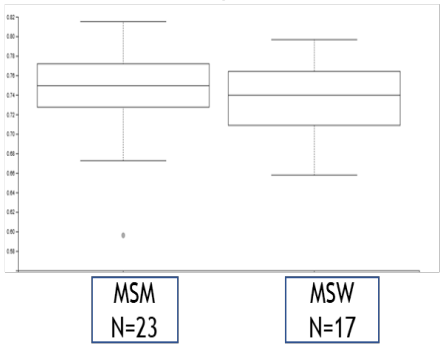
PATIENTS CHARACTERISTICHS	TOT (N=44)	MSW L (N=10)	MSW I_H (N=9)	MSM L (N=15)	MSM I_H (N=10)	p Value
Age, years (IQR) *	51 (45-55)	46 (42-53)	54 (51-55)	48 (42-55)	53 (49-55)	0,094
BMI, n (IQR) *	25,55 (23,5-27,7)	25,55 (23,4-27,8)	25,8 (24,2-27,1)	24 (22,2-26,5)	26,79 (24-31,1)	0,228
Previous ABT prophylaxis, n (%) °	14 (31,81)	4 (40)	5 (55,55)	3 (20)	2 (20)	0,27
AIDS diagnosis, n (%) °	7 (15,9)	1 (10)	4 (44,44)	1 (6,66)	1 (10)	0,073
Previous HCV coinfection, n (%)°	6 (13,63)	1 (10)	4 (44,44)	0 (0)	1 (10)	0,02
HIV infection months naïve, n (IQR)*	8 (1-50,5)	21 (0,75-71,25)	8 (0,5-57,5)	3 (1-46)	14 (2-91)	0,784
CD4 nadir, n/mmc (IQR) * Current CD4n, n/mmc (IQR) * Current CD4/CD8 ,n (IQR) *	348 (98-488) 659 (549-826) 0,82 (0,55-1)	200 (81-555) 648 (468-804) 0,78 (0,53-1,01)	127 (25-239) 632 (423-710) 0,69 (0,43-0,97)	310 (235-500) 778 (585-970) 0,92 (0,57-1,27)	248 (150-469) 674 (567-769) 0,84 (0,62-1,05)	0,133 0,494 0,595
VL zenith, log10 cp/ml (IQR) *	4,86 (4,2-5,4)	5,34 (4,1-6,2)	5,23 (4,2-5,8)	4,6 (4,1-5,1)	4,68 (4,2-5,6)	0,648
cART ongoing NNRTIs based,n (%) ° PI based,n (%) ° INIs based,n (%) °	 19 (43,18) 3 (6,81) 22 (50)	 6 (60) 1 (10) 3 (30)	 1 (11,11) 2 (22,22) 6 (66,66)	 8 (53,33) 0 (0) 7 (46,66)	 4 (40) 0 (0) 6 (60)	0,138
Current backbone with ABC, n (%)°	11 (25)	4 (40)	2 (22,22)	4 (26,66)	1 (10)	0,543
cART duration months, n (IQR) *	98,5 (36,2-186,3)	79,5 (28,5-153,5)	133 (47,5-273)	81 (36-192)	103 (53-214,5)	0,514
Smoke, n (%) °	17 (38,63)	4 (40)	3 (33,33)	4 (26,66)	6 (60)	0,4
AH, n (%) °	7 (15,9)	1 (10)	3 (33,33)	0 (0)	3 (30)	0,084
SBP, mmHg (IQR) *	130 (110-138)	130 (110-131)	140 (130-147)	120 (110-120)	132 (120-142)	0,0028
Tot-Col mg/dl (IQR) *	188 (171-215)	169 (161-190)	185 (174-201)	208 (177-230)	192 (173-222)	0,131
HDL-Col mg/dl (IQR) *	51 (42-56)	53 (41-65)	44 (37-51)	53 (51-74)	46 (34-49)	0,0031
Alcohol drinkers n, (%) °	19 (43,18)	7 (70)	4 (44,44)	3 (20)	5 (50)	0,093

Non-significant trend of a higher α-diversity in MSM vs MSW. Significant differences in β-diversity (all indexes) in MSM vs MSW

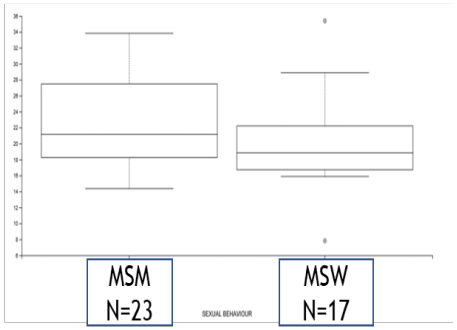
α-diversity in MSM vs MSW

β-diversity in MSM vs MSW

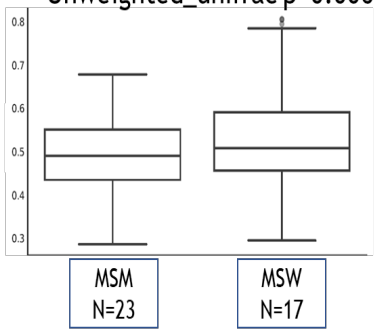
Evenness p=0.50



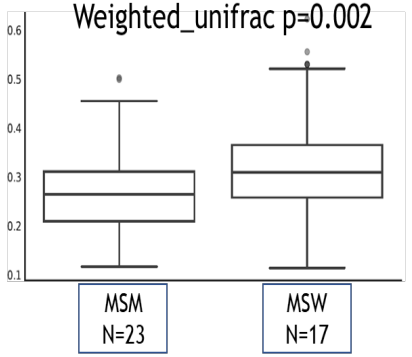
Faith p=0.20



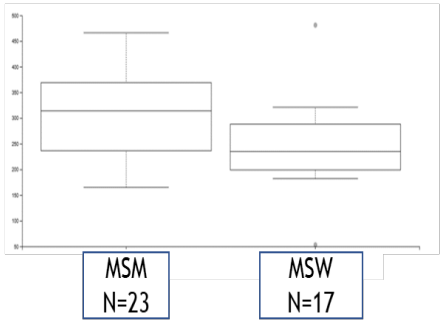
Unweighted_unifrac p=0.006



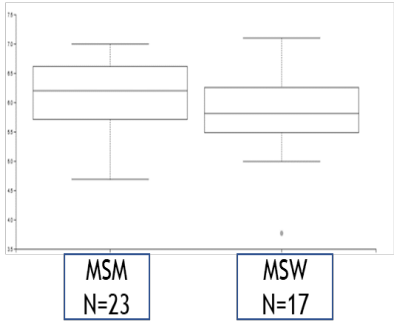
Weighted_unifrac p=0.002



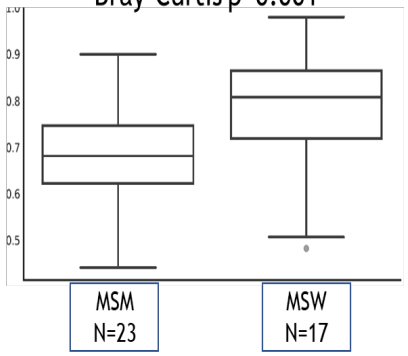
Observed p=0.09



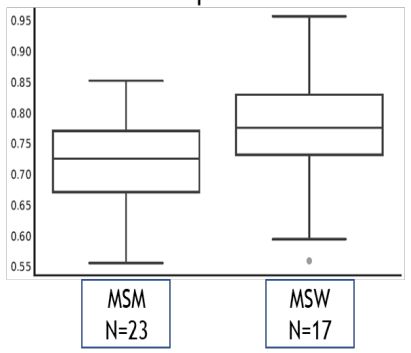
Shannon p=0.25



Bray-Curtis p=0.001



Jaccard p=0.001

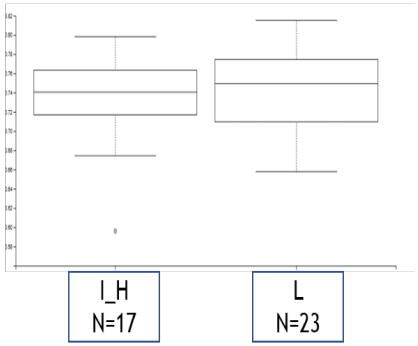


IAS 2019, ICAR 2020

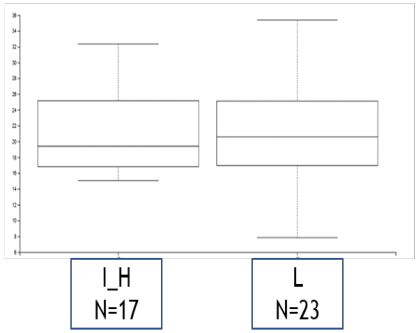
No differences in neither α-diversity nor β-diversity indexes according to Framingham Score (cut-off: 10)

α-diversity in I_H vs L

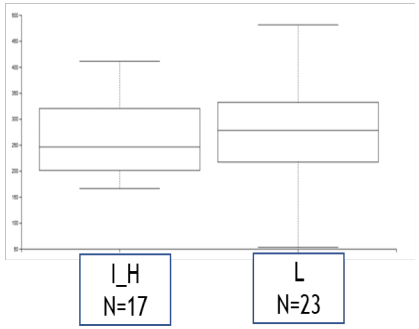
Evenness p=0.71



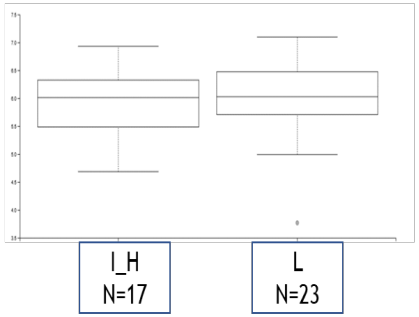
Faith p=0.98



Observed p=0.57

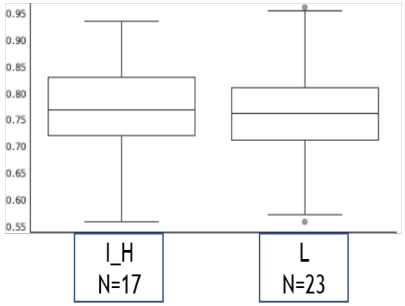


Shannon p=0.57

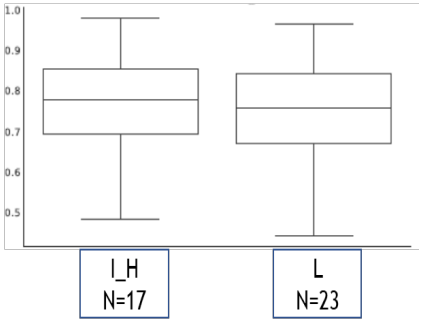


β-diversity in I_H vs L

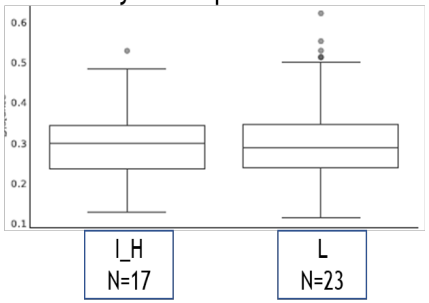
Unweighted_unifrac p=0.68



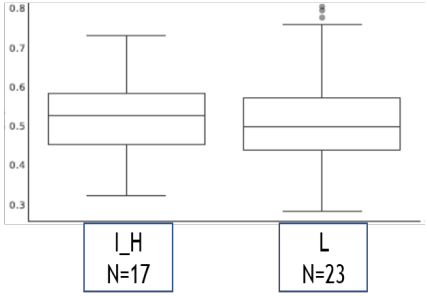
Weighted_unifrac p=0.53



Bray-Curtis p=0.75



Jaccard p=0.59

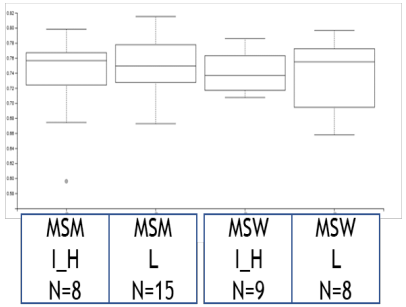


IAS 2019, ICAR 2020

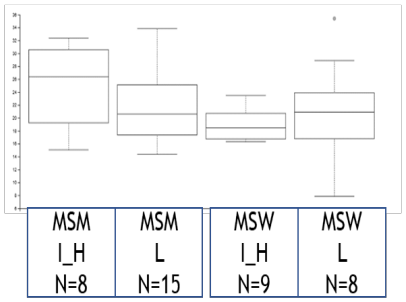
No differences in α-diversity indexes according to sexual behaviour **and** FS. Different in β-diversity indexes linked to sexual behaviour rather than FS

α-diversity in MSM vs MSW

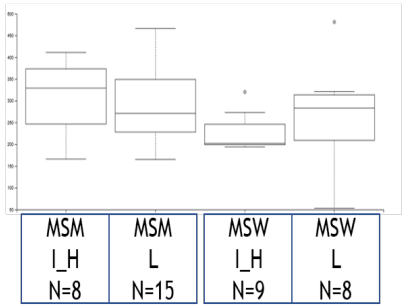
Evenness p=0.90



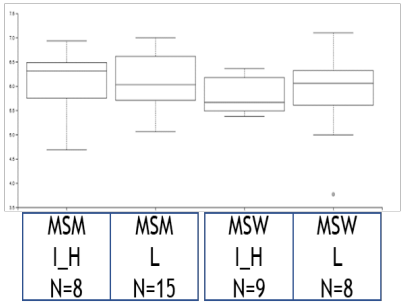
Faith p=0.32



Observed p=0.24

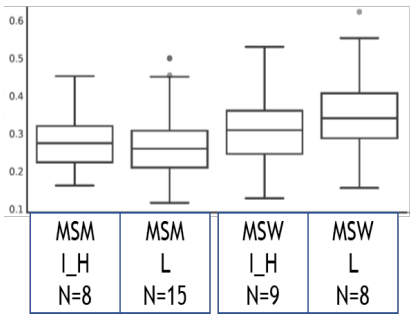


Shannon p=0.55

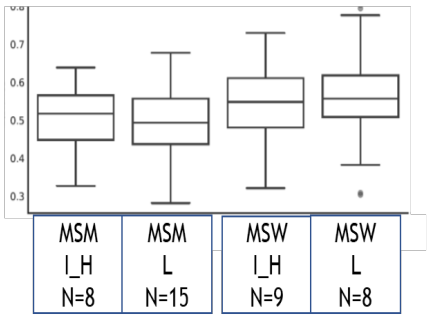


β-diversity in MSM vs MSW

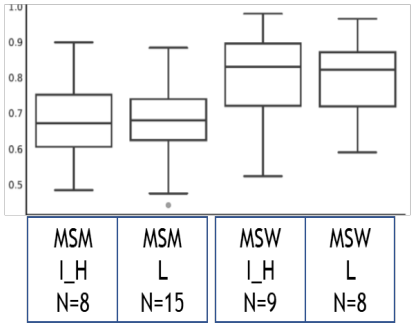
Unweighted_unifrac p=0.015



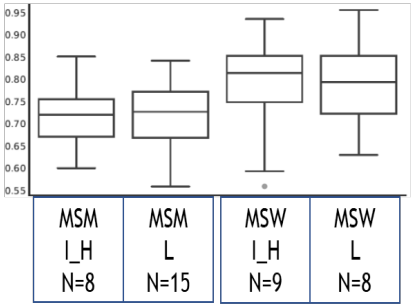
Weighted_unifrac p=0.022



Bray-Curtis p=0.002

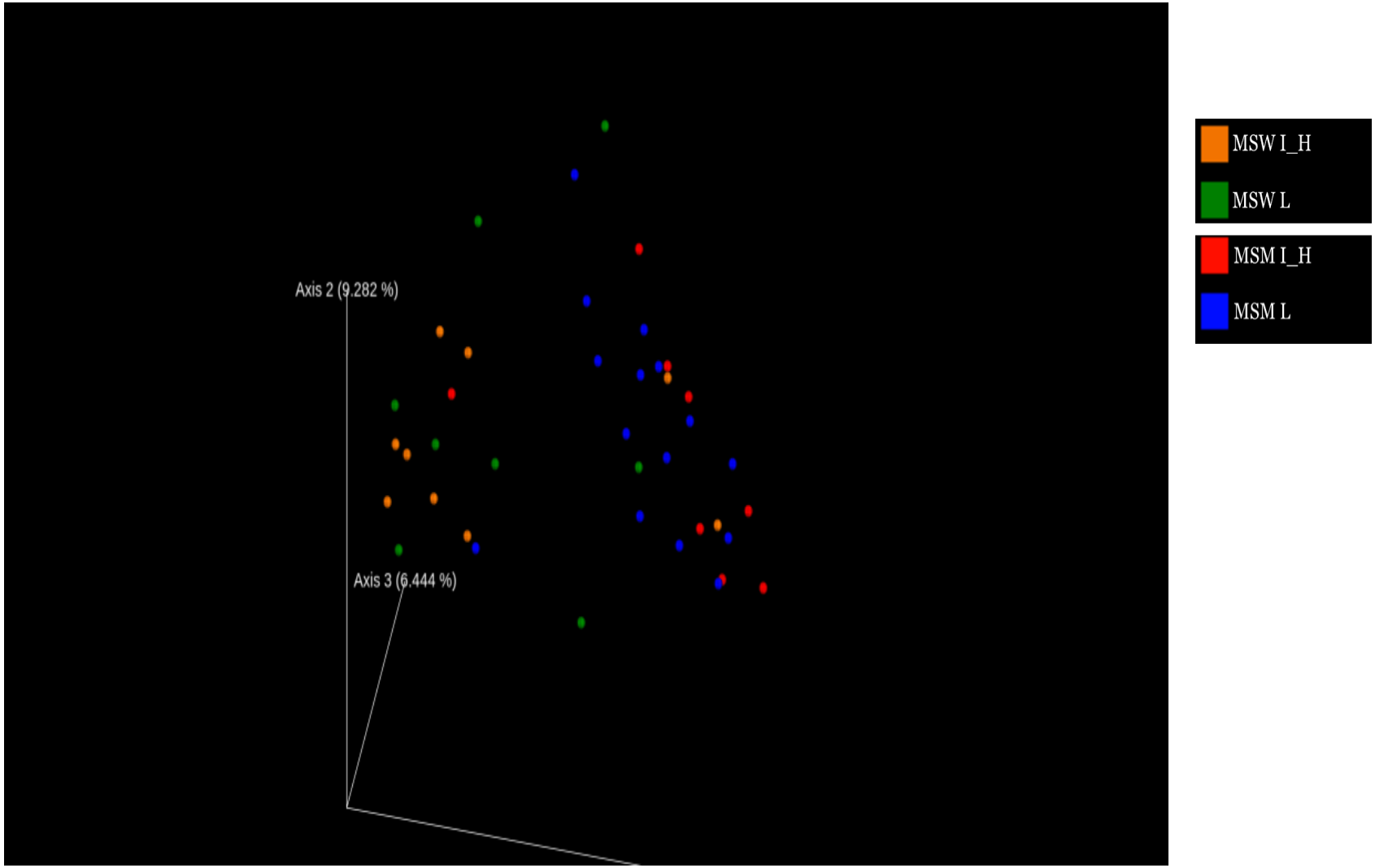


Jaccard p=0.004



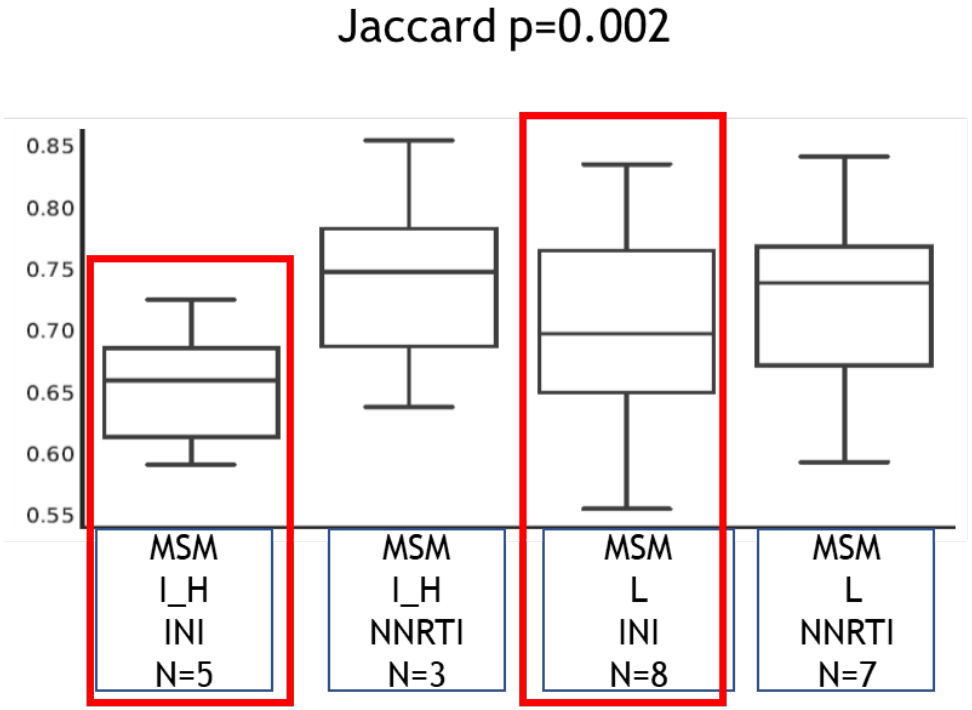
IAS 2019, ICAR 2020

Principal coordinate analysis (PCoA) in MSM I_H, MSM L, MSW I_H and MSW_L



IAS 2019, ICAR 2020

Significant difference in β-diversity (Jaccard) index in MSM according to FS and according to 3rd-drug



INI=Integrase Inhibitors

NNRTI=Non Nucleoside Reverse Transcriptase Inhibitor

IAS 2019, ICAR 2020

Conclusions

In a small, selected cohort of HIV-infected males on stable suppressive cART, our preliminary data show that:

- sexual behaviour is a major driver of microbiome alterations in HIV infection
- cardiovascular risk according to Framingham Risk score did not relate to differences in the fecal microbial composition
- cardiovascular risk may be modulated by the combined effect of microbes and antiretroviral drug exposure

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-020-16222-8 OPEN

HIV-associated gut dysbiosis is independent of sexual practice and correlates with noncommunicable diseases

I. Vujkovic-Cvijin[1,11], O. Sortino[2,3,11], E. Verheij[4], J. Sklar[1], F. W. Wit[4], N. A. Kootstra[5], B. Sellers[6], J. M. Brenchley[7], J. Ananworanich[4,8,9], M. Schim van der Loeff[10], Y. Belkaid[1,6], P. Reiss[4] & I. Sereti[3]✉

Study Design

- Cross-sectional, well-powered study on fecal microbiota
- HIV-infected people with suppressed viremia on cART (n=80)
- HIV-uninfected controls (n=80)

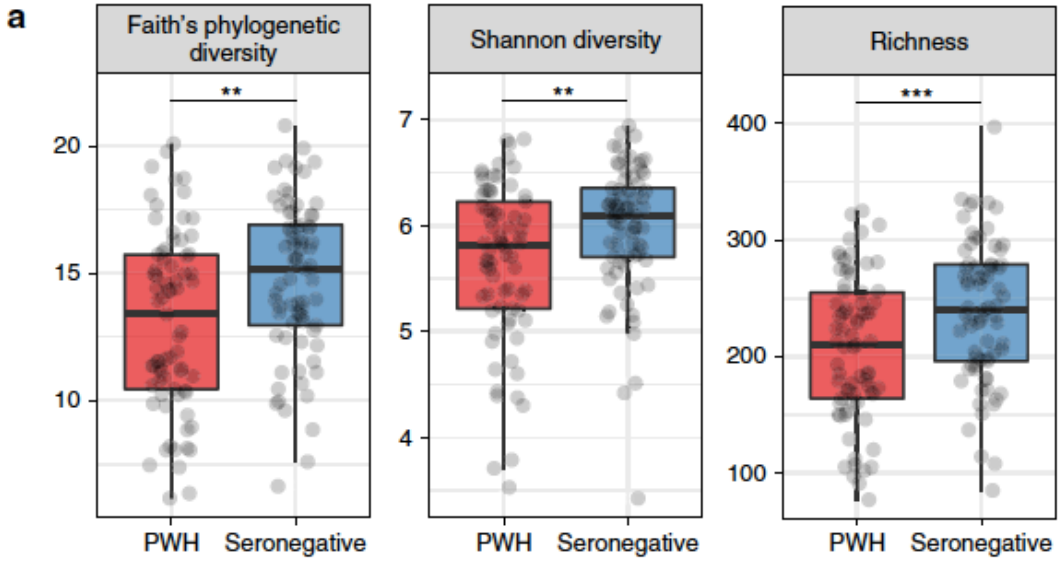
- stratified by: sex and sexual practice
- matched for age, sexual practice, BMI, and birth country

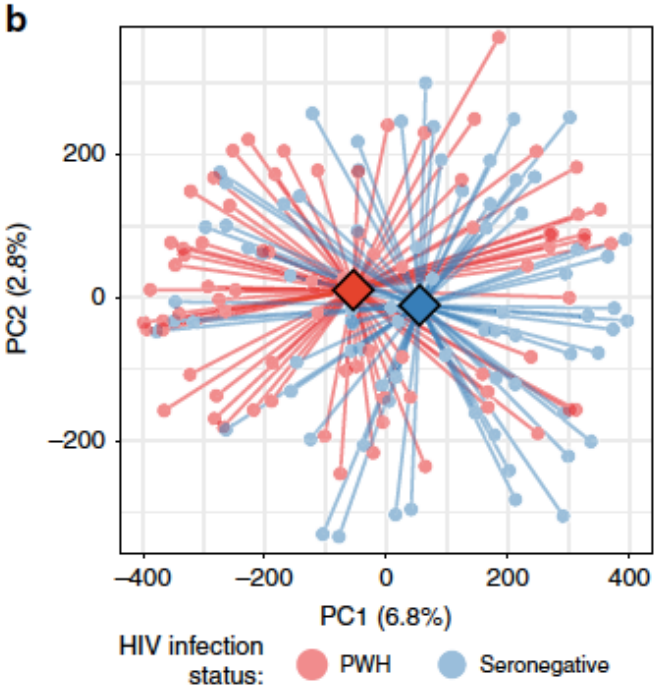
Comorbidities assessed:

- chronic obstructive pulmonary disease (COPD)
- advanced liver fibrosis (FIB-4 ≥ 3.25)
- decreased kidney function (eGFR < 60 mL/min)
- osteoporotic fractures
- diabetes mellitus
- heart failure
- non-AIDS defining cancers
- atherosclerotic disease (diagnosed by specialist; myocardial infarction, angina pectoris, peripheral arterial disease, ischemic stroke, or transient ischemic attack)

Vujkovic-Cvijin et al. Nat Comm. 2020

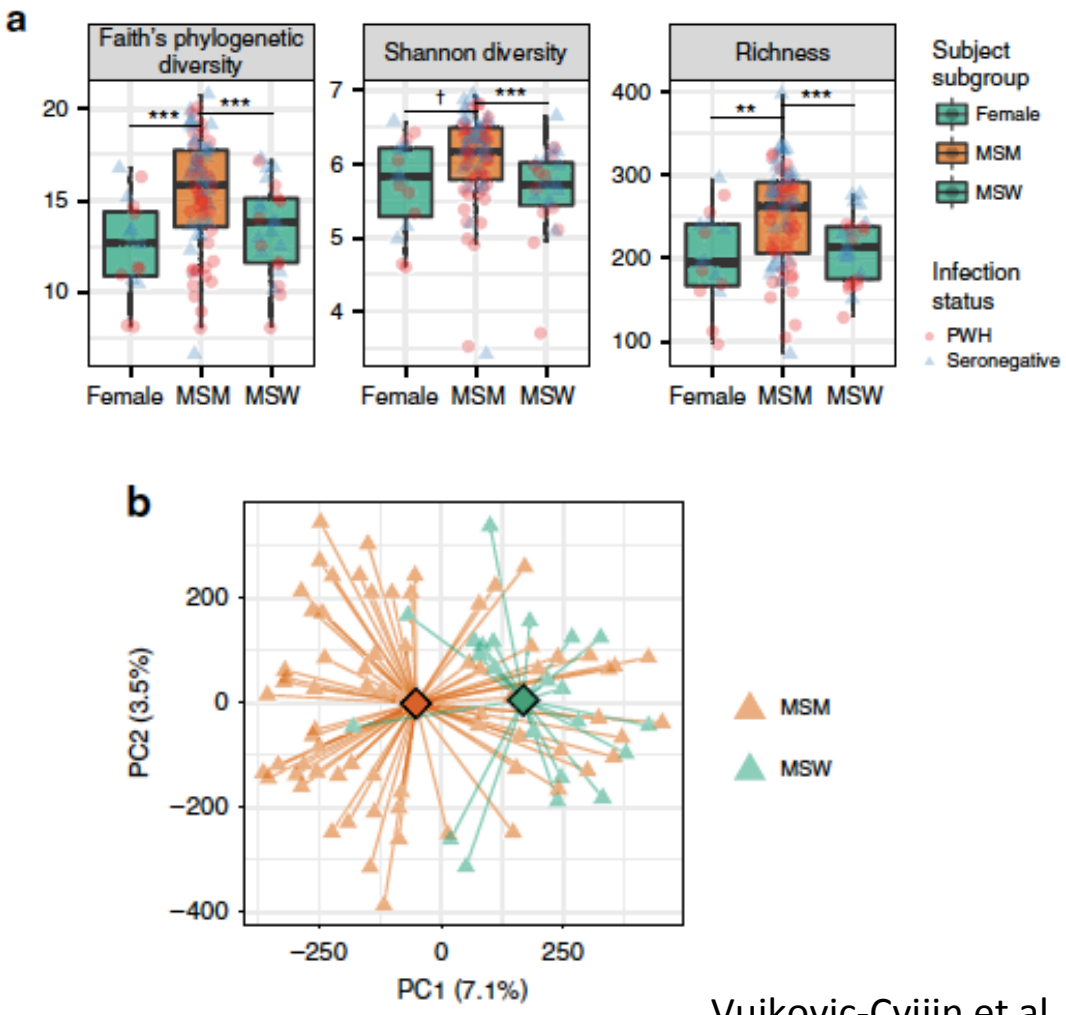
Gut microbiota differs between PWH and HIV-seronegative controls





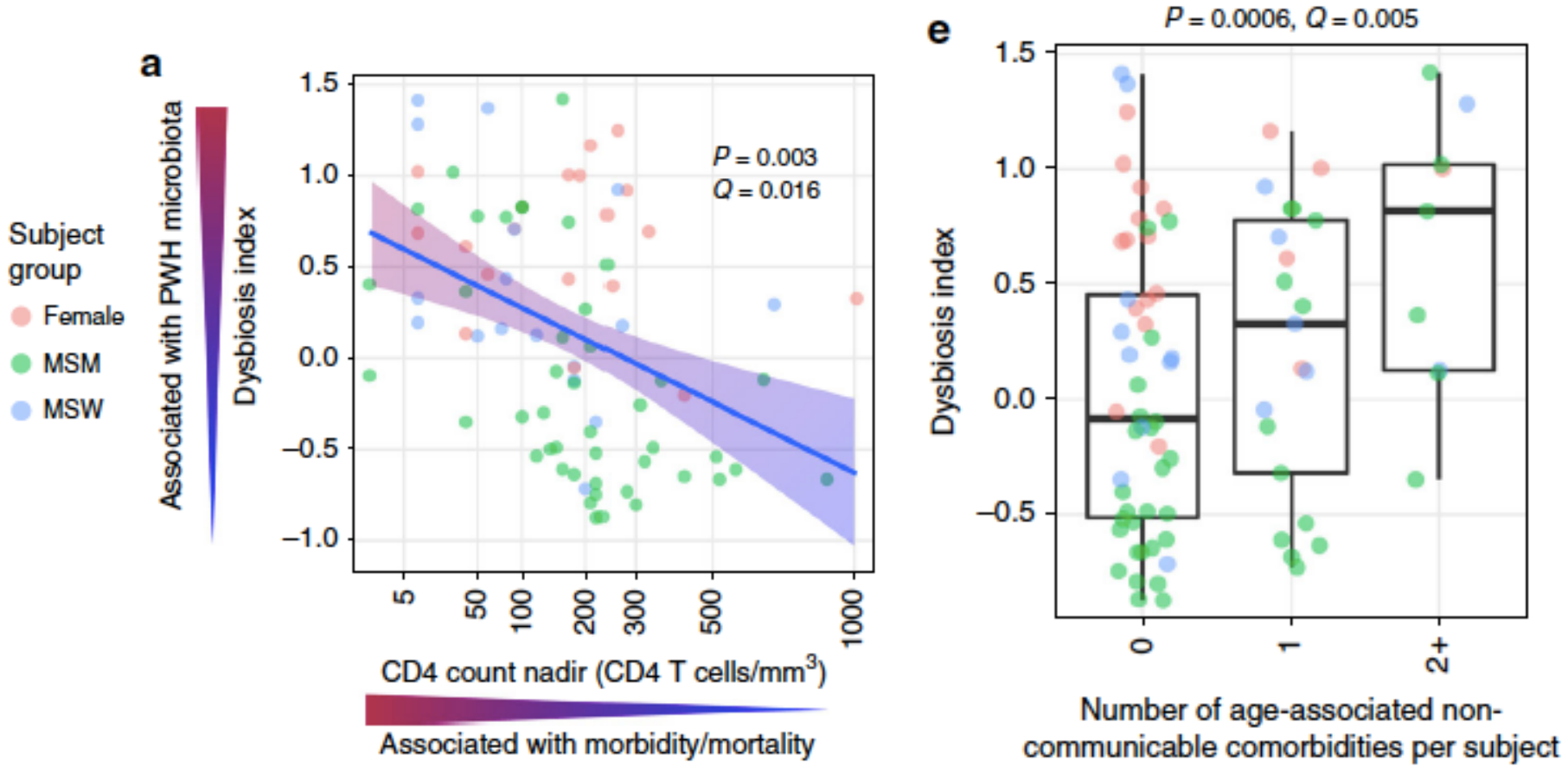
Vujkovic-Cvijin et al. Nat Comm. 2020

Sexual practice exerts major impact on gut microbiota composition



Vujkovic-Cvijin et al. Nat Comm. 2020

HIV-associated gut microbiota features correlate with markers of HIV disease progression and comorbidity prevalence



Vujkovic-Cvijin et al. Nat Comm. 2020

Conclusions

- Significant gut microbiota differences in PWH
- MSM exhibit a distinct microbiota signature characterized by Prevotella enrichment
- HIV-associated microbiota signature correlates nadir CD4 count, and prevalence of age-associated noncommunicable comorbidities

Vujkovic-Cvijin et al. Nat Comm. 2020

Future Directions

- ***Functional data?***

- ***When*** do GI alterations arise?
- PHI?
- Are they reverted by early cART? cART class?
- Different timing of GI abnormalities (anatomical, MT, dysbiosis, immunity)?

- ***Elaboration and timing*** of adjuvant therapies to prevent aging in PLWH
- anti-LPS (sevelamer)
- immuno-modulants
- Prebiotics/probiotics/antibiotics

Acknowledgments

Clinic and Laboratory of Infectious Diseases, Dept of Health Sciences, San Paolo Hospital

University of Milan

- Giuseppe Ancona
- Esther Merlini
- Stefania Cannizzo
- Matteo Augello
- Camilla Falcinella
- Valeria Bono
- Federica Gelpi
- Mohamad Hadla
- Roberta Rovito
- Francesca Bai
- Antonella d'Arminio Monforte
- Giulia Marchetti*
- Our patients

Endoscopy Unit, San Paolo Hospital, Milan

- Carmelo Luigiano

Microbiology Laboratory, Dept of Health Sciences, University of Milan

- Elisa Borghi

Servicio de Enfermedades Infecciosas Hospital Universitario Ramón y Cajal Madrid, Spain

- Sergio Serrano-Villar

University of Colorado Anschutz Medica Campus

- Catherine A. Lozupone
- Brent E. Palmer

Patholgy Unit, Dept of Health Sciences, San Paolo Hospital, University of Milan

- Federica Savi
- Delfina Tosi
- Gateano Bulfamante

***Funding**

Giovani Ricercatori- Regione Lombardia: GR-2009-1592029

Ministero della Salute-Progetto di Rete: NET-2013-02355333

Fondazione Regionale per la Ricerca Biomedica

Regione Lombardia