ROGER PAREDES, INFECTIOUS DISEASES DEPARTMENT & IRSICAIXA, HOSPITAL GERMANS TRIAS

THE MICROBIOME AND HIV VACCINES

Can bacteria teach immune cells how to fight HIV?

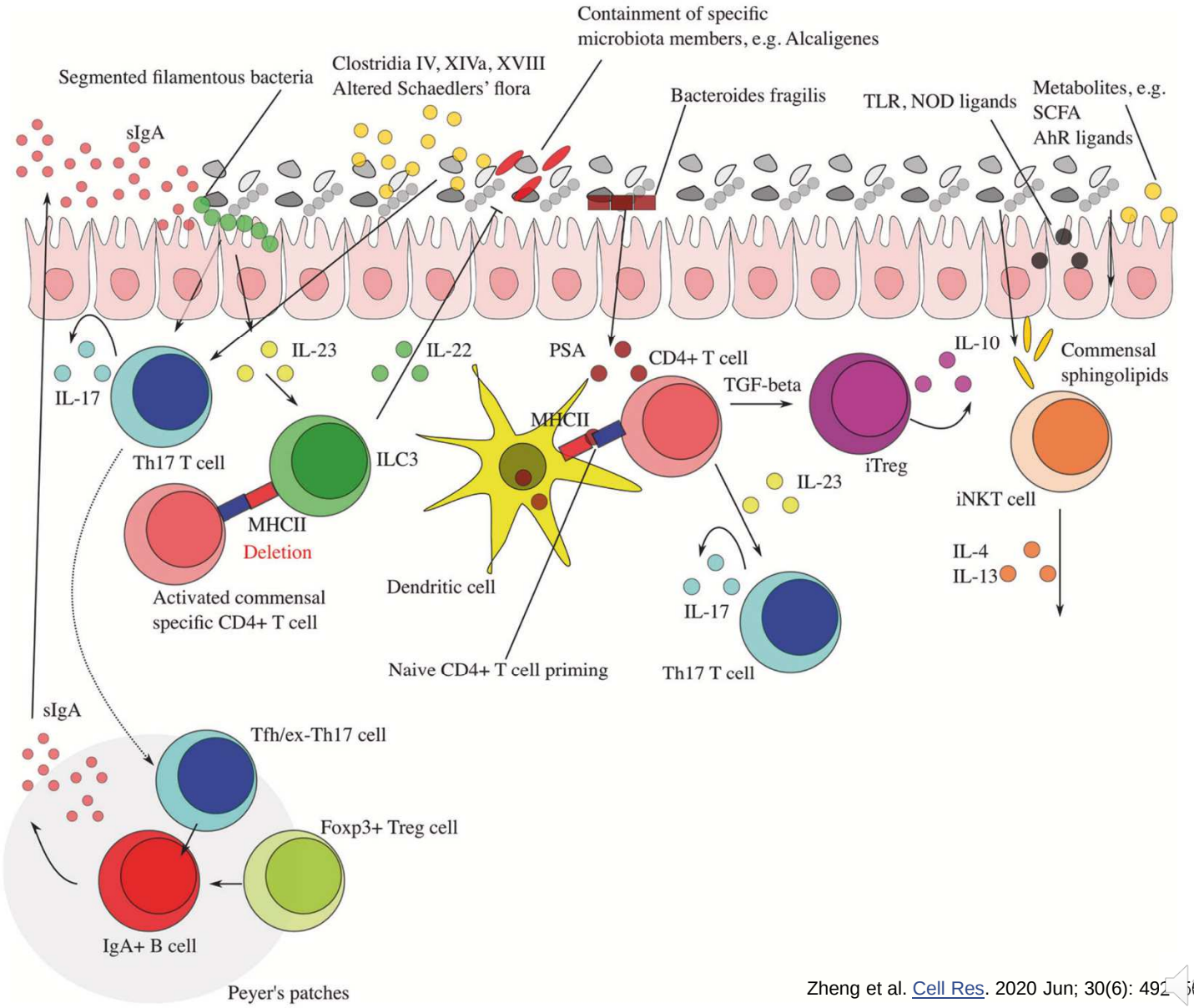
BUGS & IMMUNE CELLS

HOW BACTERIA MODULATE THE IMMUNE SYSTEM

THE MICROBIOTA MODULATES THE IMMUNE SYSTEM

Different bacteria selectively modulate different immune pathways, including:

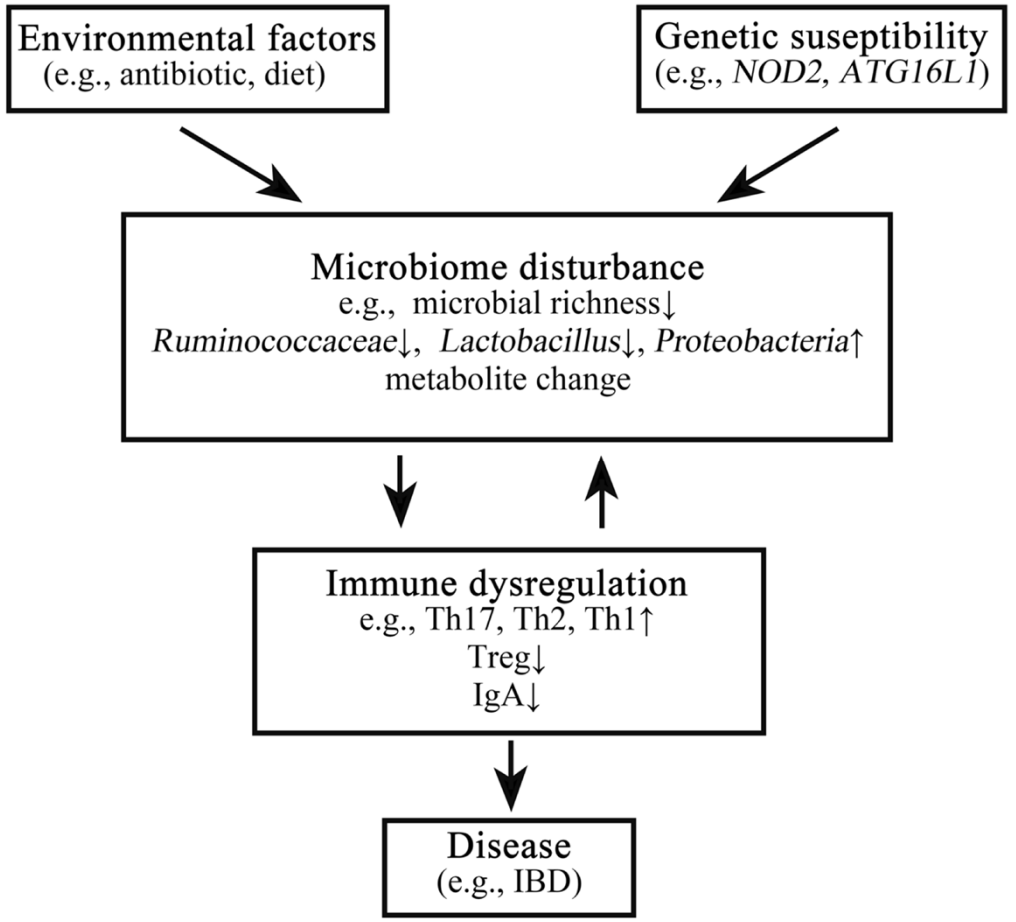
- Innate
- Acquired
  - Cellular
  - Humoral
  - Pro-inflammatory
  - Anti-inflammatory



Zheng et al. Cell Res. 2020 Jun; 30(6): 492–506

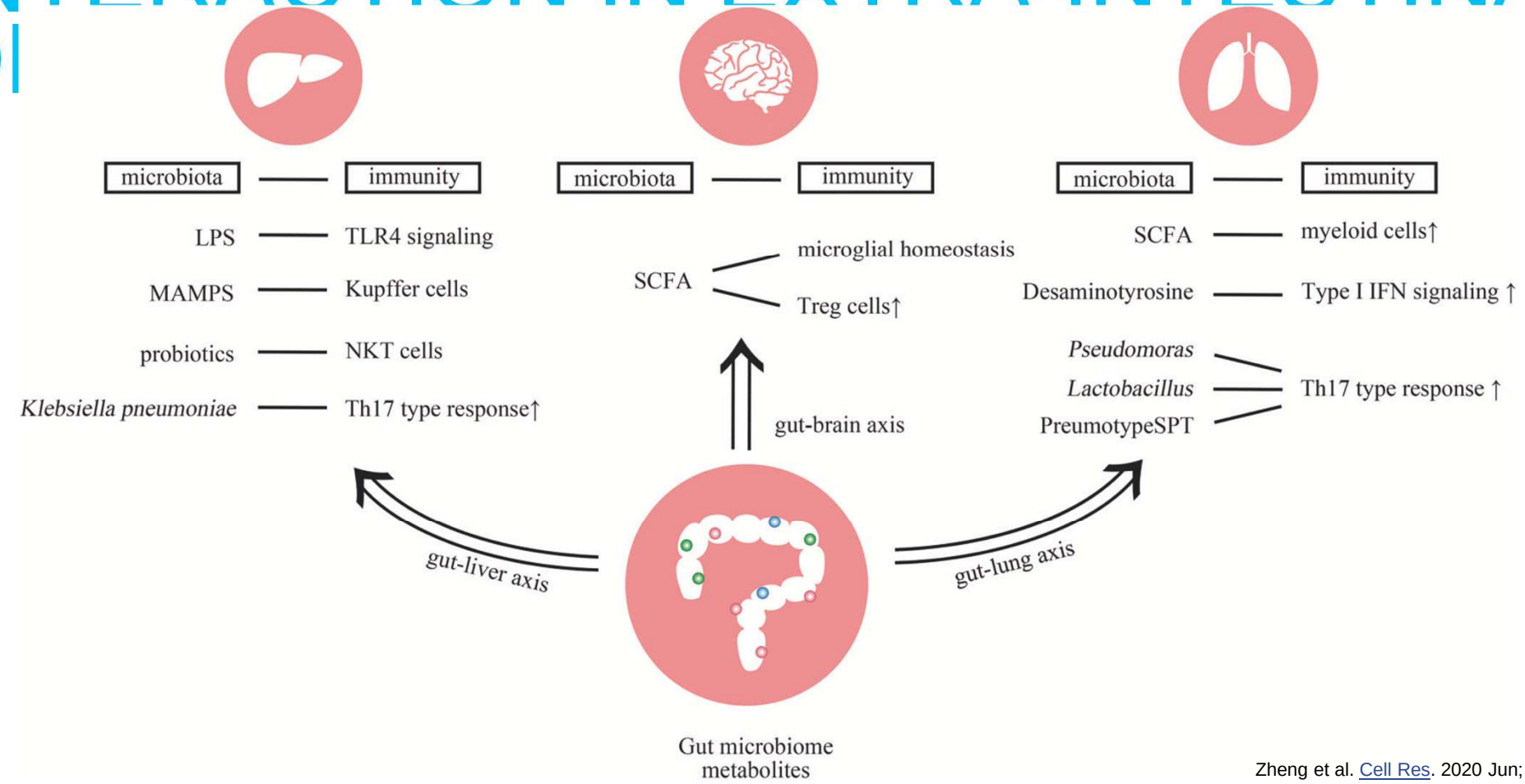
THE MICROBIOTA MODULATES THE IMMUNE SYSTEM

- Different bacteria selectively modulate different immune pathways, including:
  - Innate
  - Acquired
    - Cellular
    - Humoral
    - Pro-inflammatory
    - Anti-inflammatory



Zheng et al. Cell Res. 2020 Jun; 30(6): 492–506

MICROBIOME-IMMUNITY INTERACTION IN EXTRA-INTESTINAL O



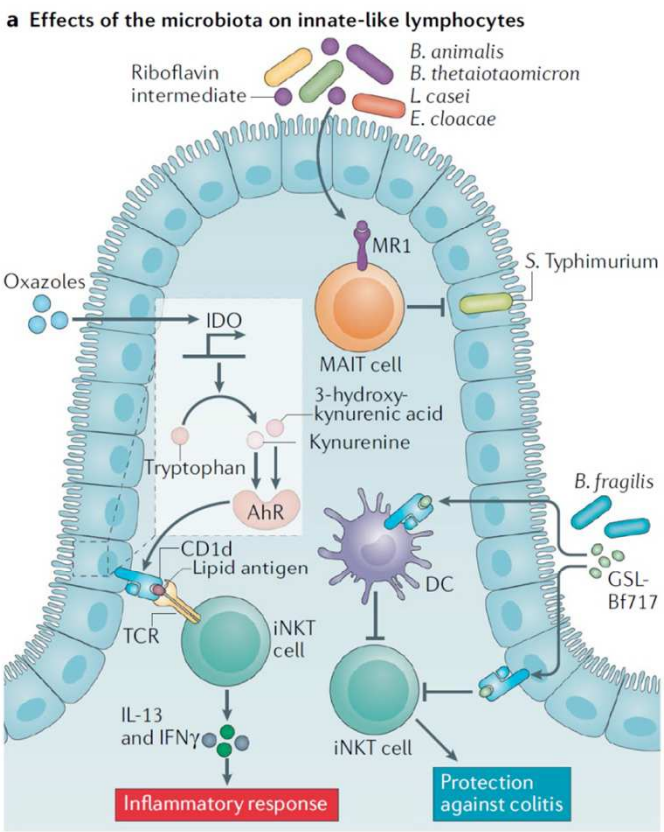
Zheng et al. Cell Res. 2020 Jun; 30(6): 492–506

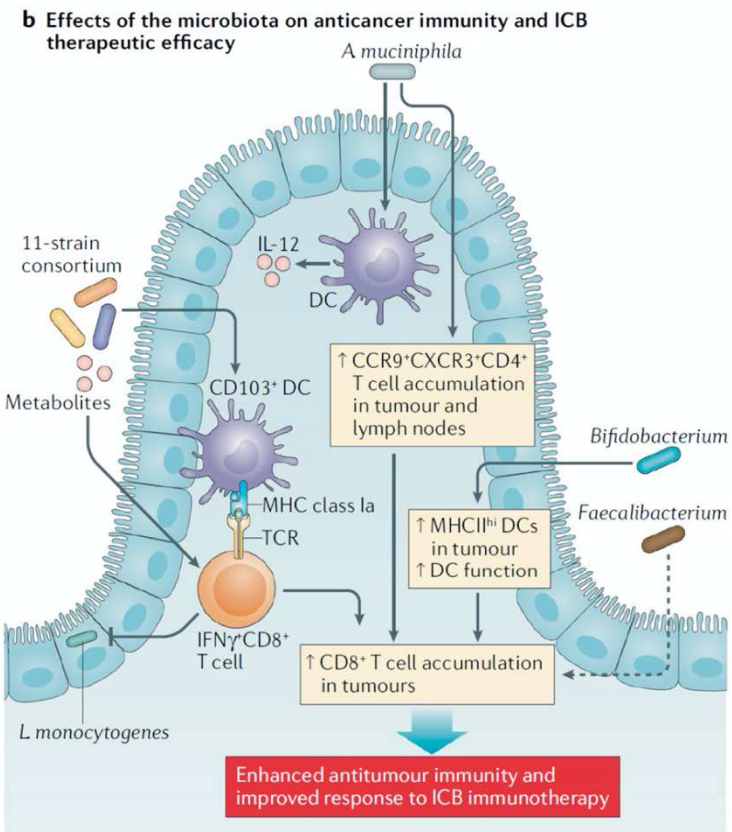
TARGETED IMMUNE MODULATION BY BACTERIA

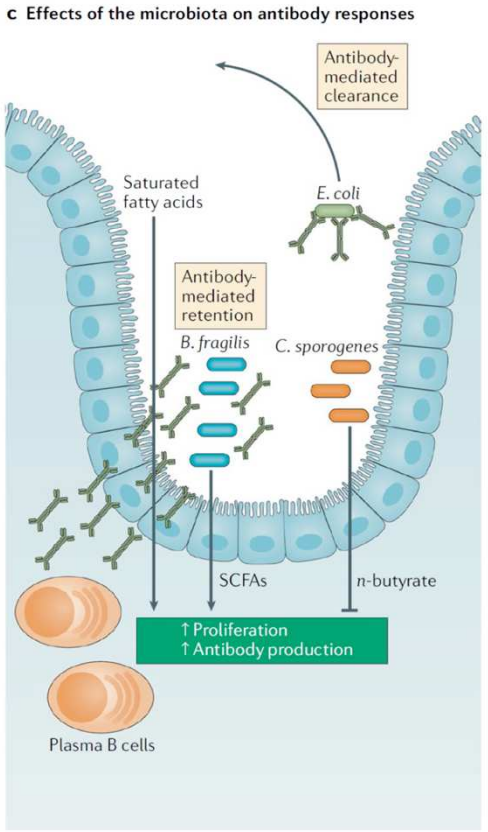
Innate immunity

Immune checkpoint blockade

Antibodies





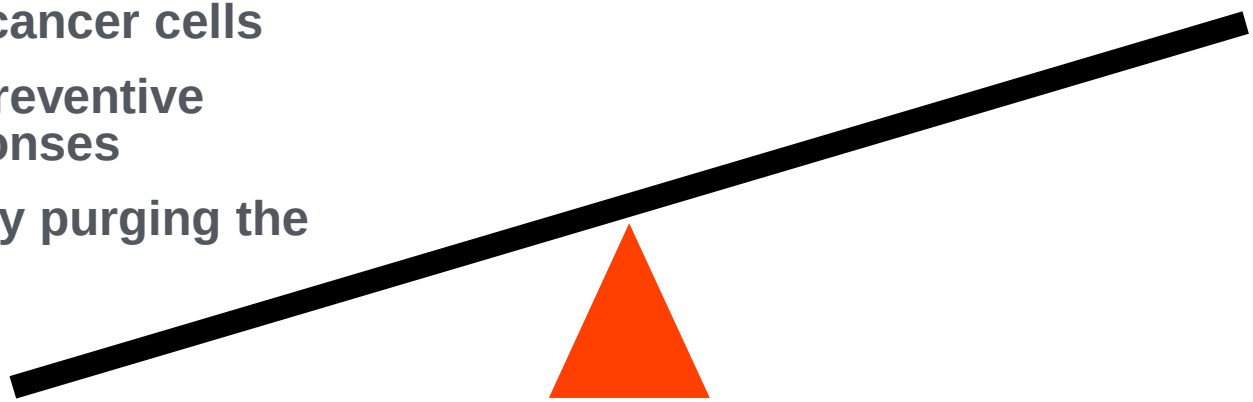


Skelly, et al. Nature Rev Microbiol 2019

TRADE-OFF OF IMMUNE ACTIVATION

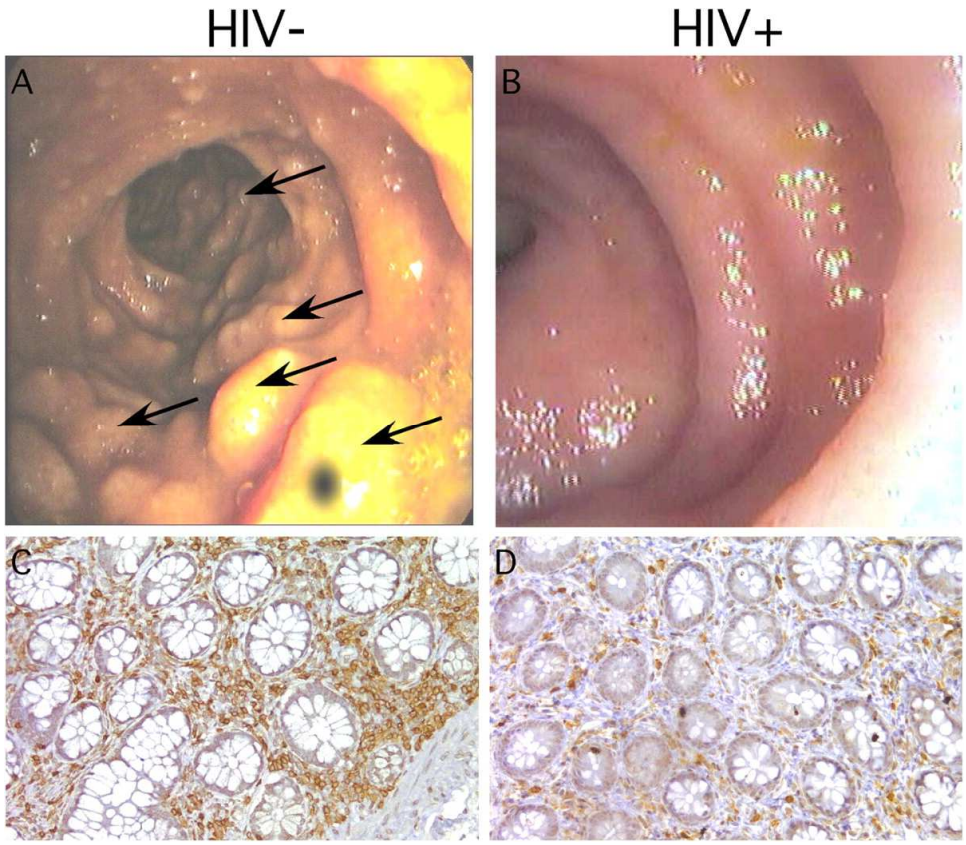
- Accelerated ageing
- Immune senescence
- Hypersensitivity reactions
- Autoimune disorders

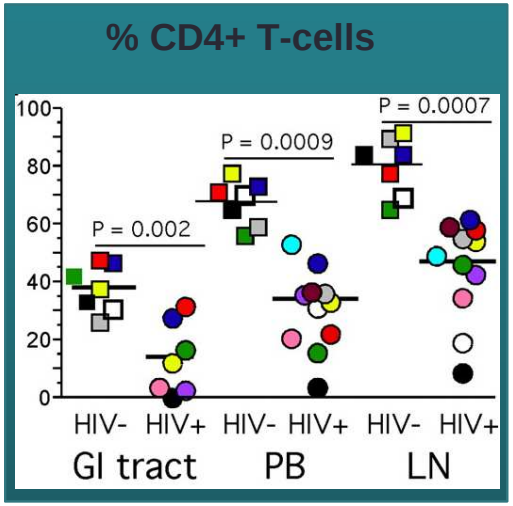
- To eliminate cancer cells
- To improve preventive vaccine responses
- To cure HIV by purging the reservoirs



THE MICROBIOME IN HIV

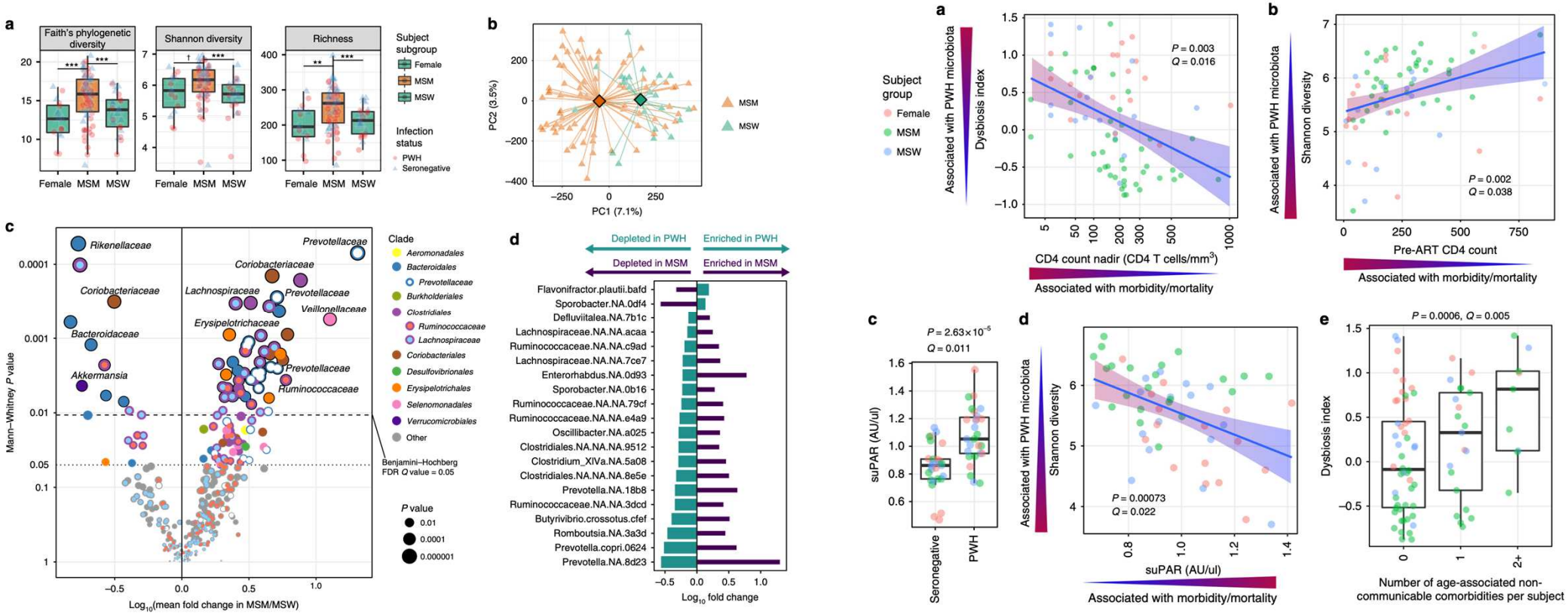
HIV DESTROYS THE GALT





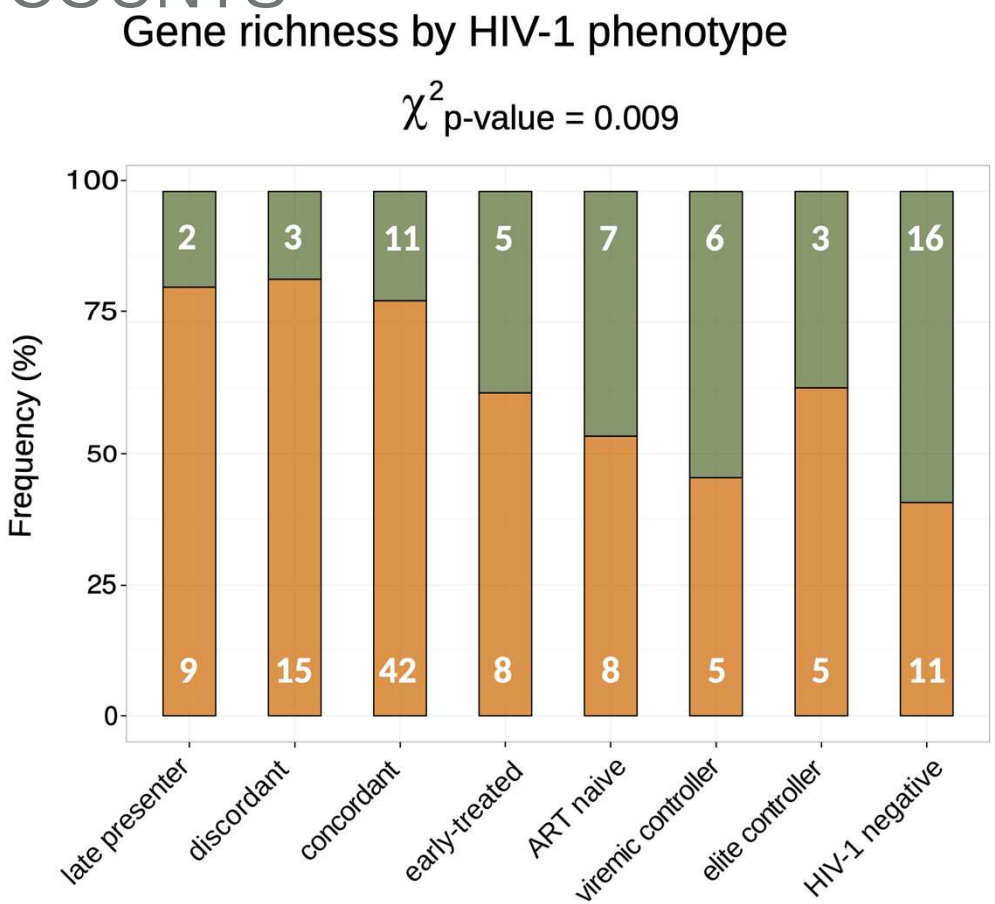
Brenchley J M et al. J Exp Med 2004;200:749-759

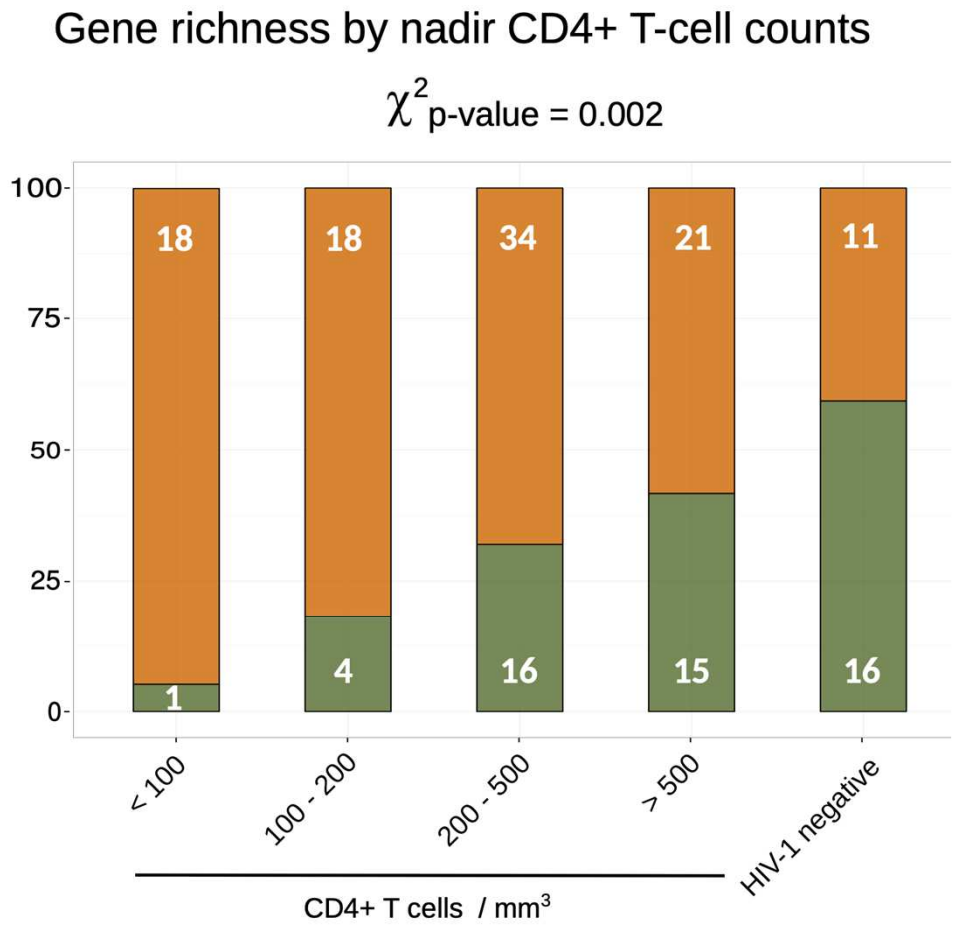
INDEPENDENT HIV-SPECIFIC GUT DYSBIOSIS



Vujkovic-Cvijin I, et al. Nat Comm 2020

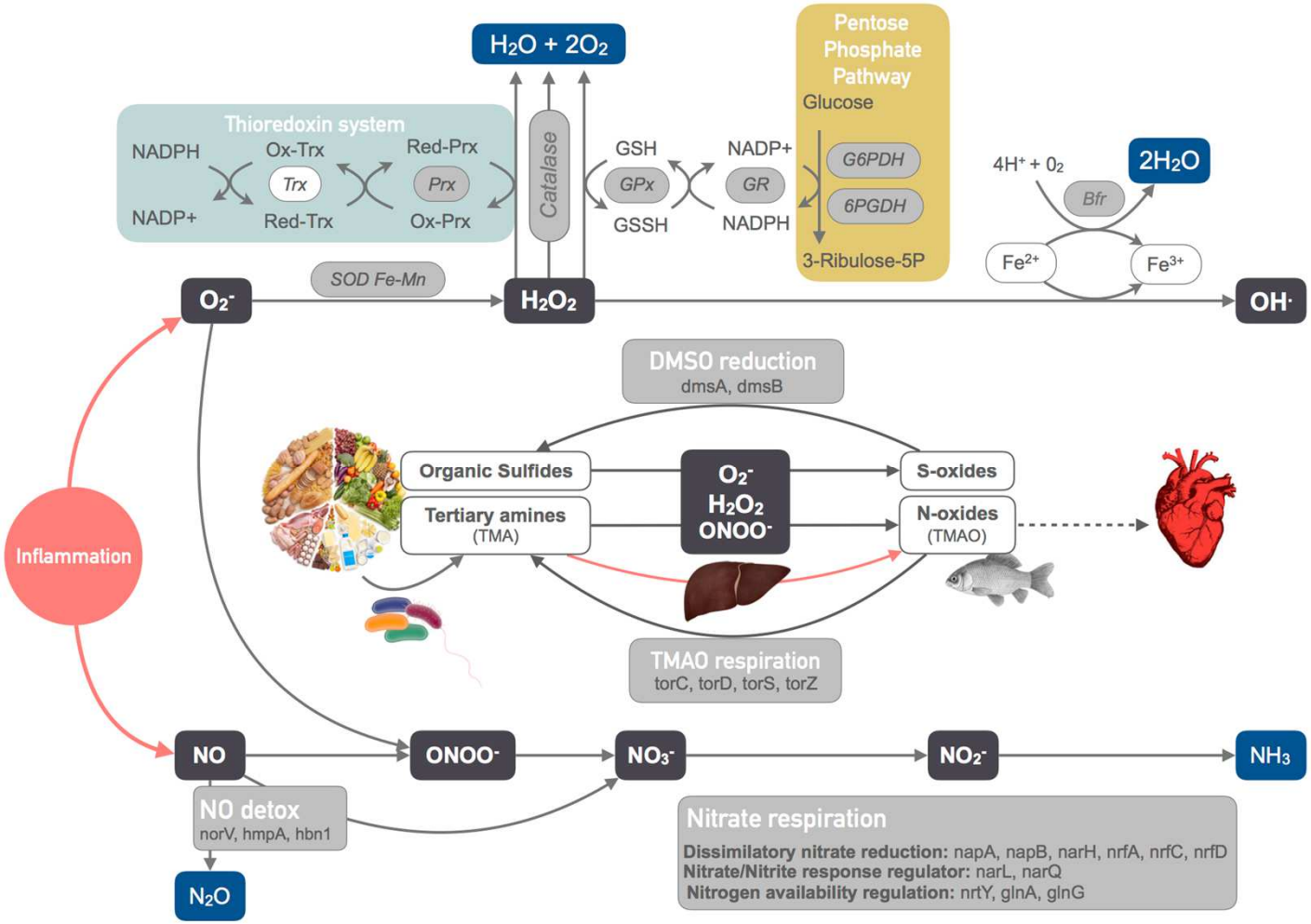
MICROBIAL GENE RICHNESS LINKED TO LOW CD4+ T-CELL COUNTS





Guillen Y et al. Mucosal Immunology 20

ADAPTATION TO OXIDATIVE STRESS

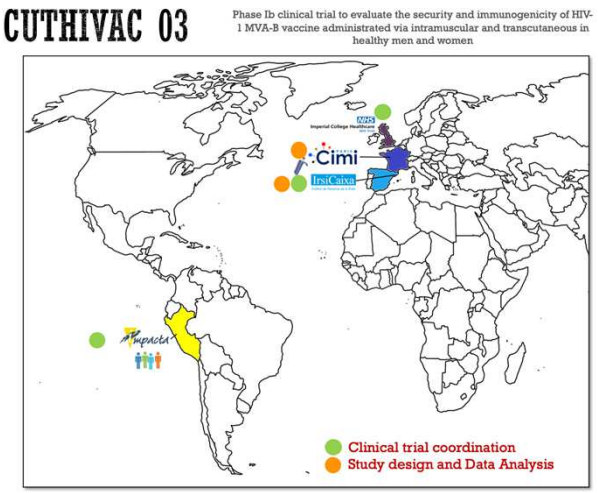


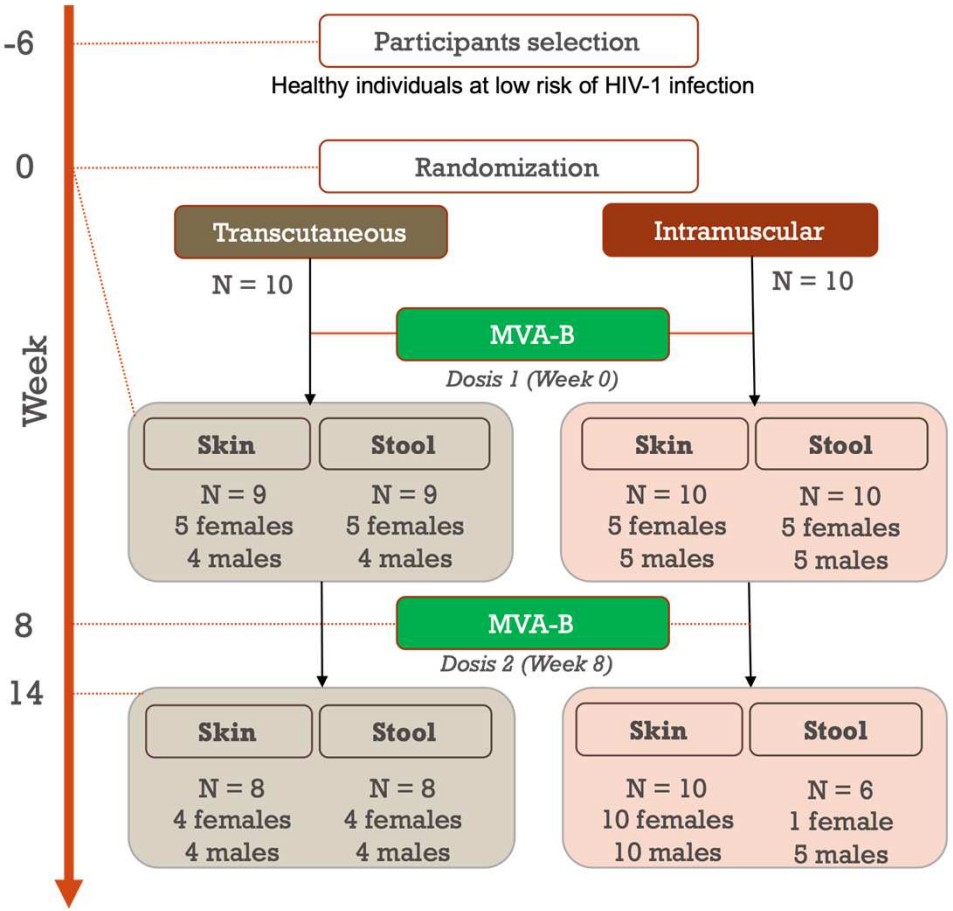
Guillen Y et al. Mucosal Immunology 20

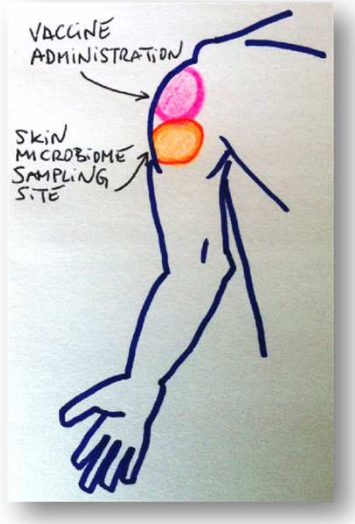
THE MICROBIOME AND HUMORAL IMMUNITY

GUT & SKIN MICROBES LINKED TO HUMORAL RESPONSES
MICROBIOME PLUS TRANSCRIPTOMICS ACHIEVE BETTER PREDICTIONS

CUTHIVAC 03 STUDY





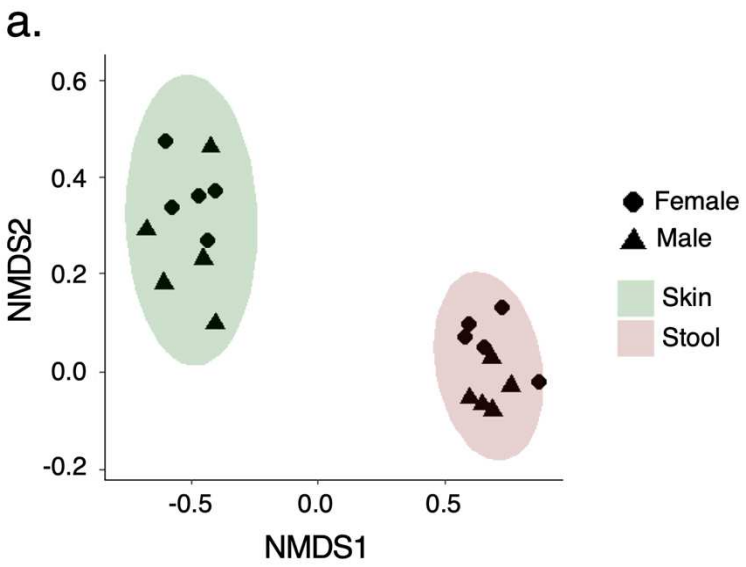


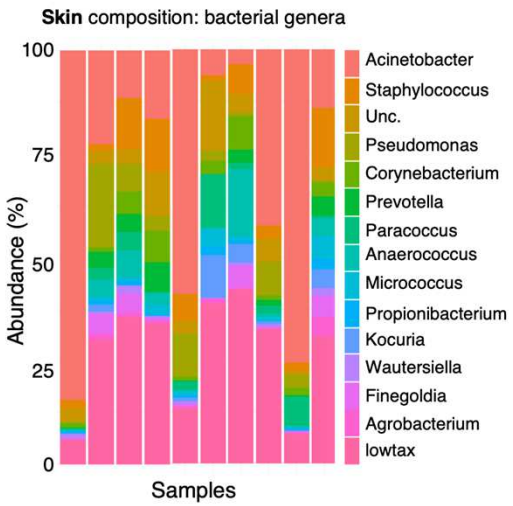
36 Skin Samples (Run 1)
+ 2 NC

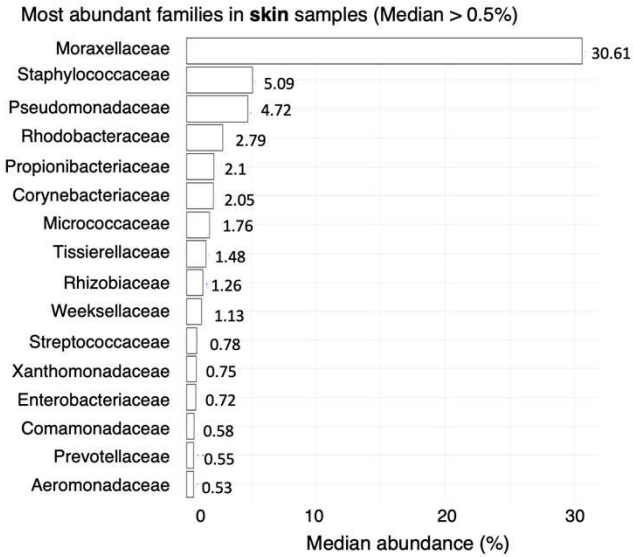
33 Stool Samples (Run 2)
+ 2 NC (3 duplicates)

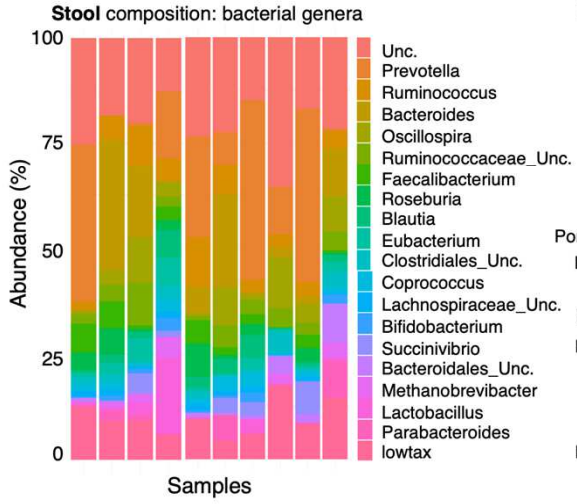
Guillén Y et al, unpublished

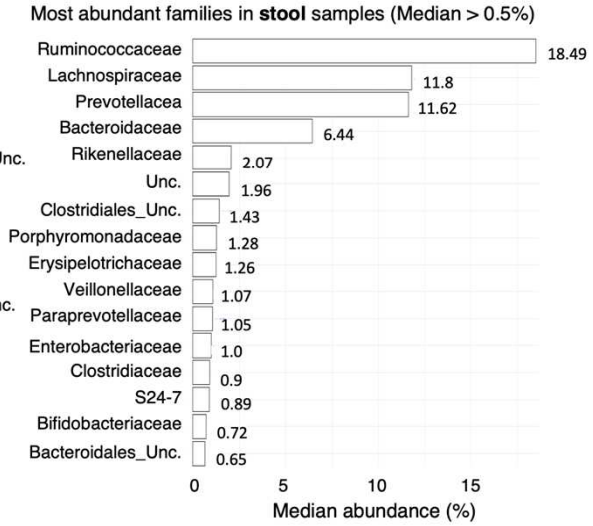
MICROBIAL COMPOSITION OF FECAL AND SKIN SAMPLES











Guillén Y et al, unpublished

SKIN AND STOOL GENUS DIVERSITY CORRELATIONS WITH BLOOD GENE EXPRESSION

BLK B lymphocyte kinase
EBF1 Early B-cell factor
IGLV8-61 immunoglobulin lambda variable 8-61

STOOL	Genus diversity sd_invsimpson		Genus diversity shannon	
Genes	r	P-value	r	*P*-value
AHDC1	-0.47	0.0361	*ns*	*ns*
BLK	**-0.66**	**0.0015**	**-0.64**	**0.0024**
EBF1	**-0.46**	**0.0418**	*ns*	*ns*
EIF4E2	-0.57	0.0090	-0.54	0.0145
EMX2	-0.48	0.0322	*ns*	*ns*
FAM149B1	0.52	0.0182	0.47	0.0375
FLT1	0.49	0.0264	0.44	0.0500
FOXD4L3	0.52	0.0181	0.47	0.0361
IGLV8-61	**-0.85**	**2.7116 e-06**	**-0.81**	**1.3721 e-05**
MEI4	0.48	0.0335	*ns*	*ns*
MIR3677	0.47	0.0375	*ns*	*ns*
NDUFC1	0.44	0.0500	*ns*	*ns*
OSER1	*ns*	*ns*	-0.44	0.0500
SNAPC2	-0.63	0.0027	-0.56	0.0100
TMEM17	0.56	0.0105	0.48	0.0322
TOMM70	0.61	0.0043	0.52	0.0182
TRBJ2	-0.45	0.0466	*ns*	*ns*
TRIP11	0.46	0.0434	*ns*	*ns*
ZDHHC2	0.50	0.0254	0.45	0.0450

SKIN	Genus diversity sd_invsimpson		Genus diversity shannon	
Genes	r	P-value	r	*P*-value
AHDC1	-0.46	0.0389	-0.55	0.0121
AQP2	-0.4679	0.0375	-0.52	0.0174
BAIAP3	-0.48	0.0335	-0.46	0.0403
BLK	**-0.67**	**0.0011**	**-0.60**	**0.0048**
DAAM1	*ns*	*ns*	-0.5102	0.0215
EBF1	**-0.55**	**0.0127**	**-0.53**	**0.0166**
FAM149B1	*ns*	*ns*	0.46	0.0418
GPC6	*ns*	*ns*	-0.50	0.0254
HMGA2	0.47	0.0375	0.59	0.0067
IGLV8-61	**-0.56**	**0.0095**	**-0.54**	**0.0133**
LOC1001311655	-0.47	0.0348	*ns*	*ns*
MCMBP	0.49	0.0264	0.4679	0.0375
RPL34	0.51	0.0225	0.58	0.0078
SLC46A3	0.50	0.0234	0.5253	0.0174
SNAPC2	-0.49	0.0275	-0.53	0.0152
SPHK2	*ns*	*ns*	-0.51	0.0215
TMEM17	0.60	0.0051	0.68	0.0009
TOMM70	0.50	0.0234	0.57	0.0082
TRBJ2	-0.49	0.0286	*ns*	*ns*
TRBV7	*ns*	*ns*	-0.44	0.0500
UGT2A1	0.50	0.0244	0.55	0.0121
ZDHHC2	*ns*	*ns*	0.48	0.0310

Guillén Y et al, unpublished

MICROBIAL ABUNDANCE CORRELATES WITH MVA-NAB

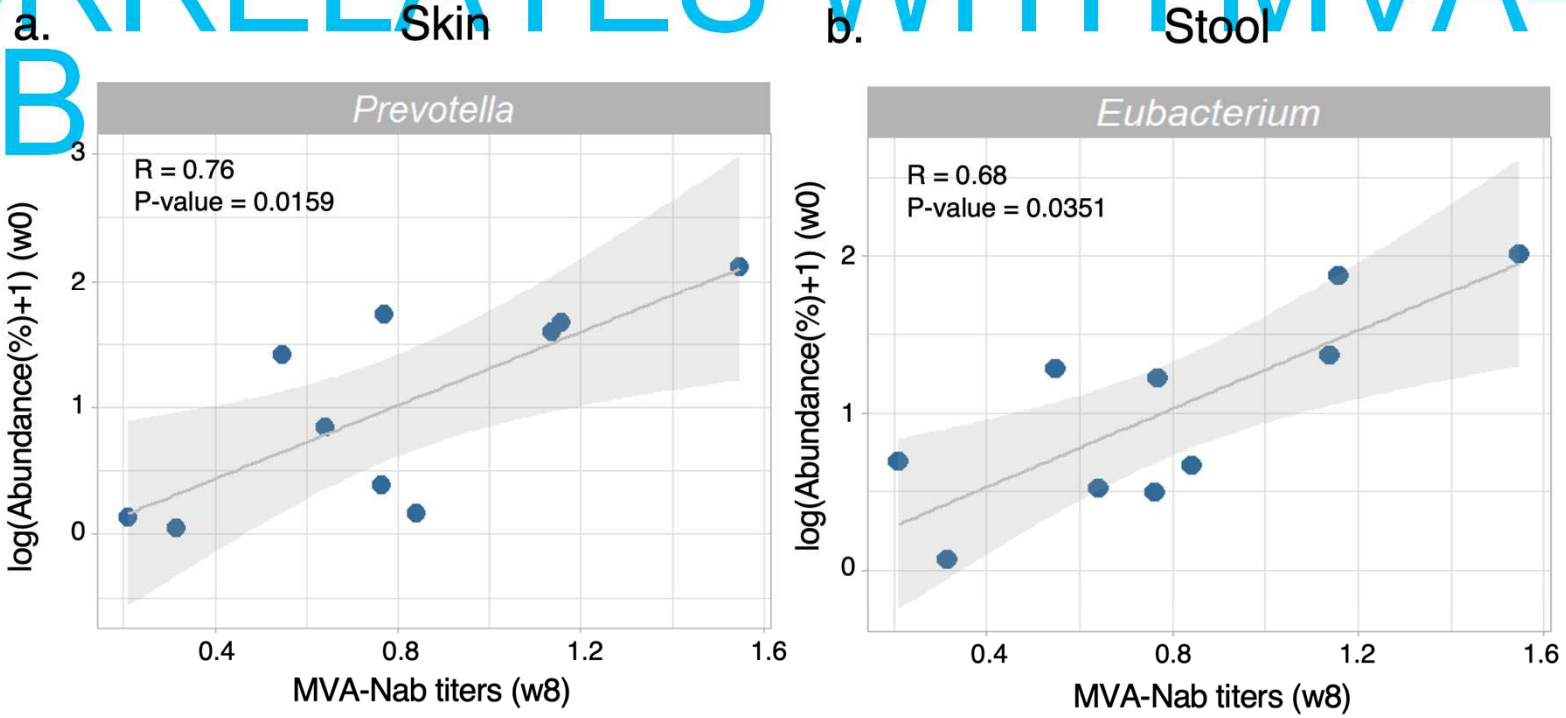


Figure 1: **Microbial abundance before vaccination is correlated with MVA-Nab responses**
a. Abundance of *Prevotella* in skin and **b.** abundance of *Eubacterium* in stool are correlated with MVA-specific neutralizing antibody titers at w8 (log1/EC50). Spearman rank sum test was applied with a *P*-value < 0.05. All genera were filtered by a minimum median abundance of 0.1% across the samples.

Guillén Y et al, unpublished

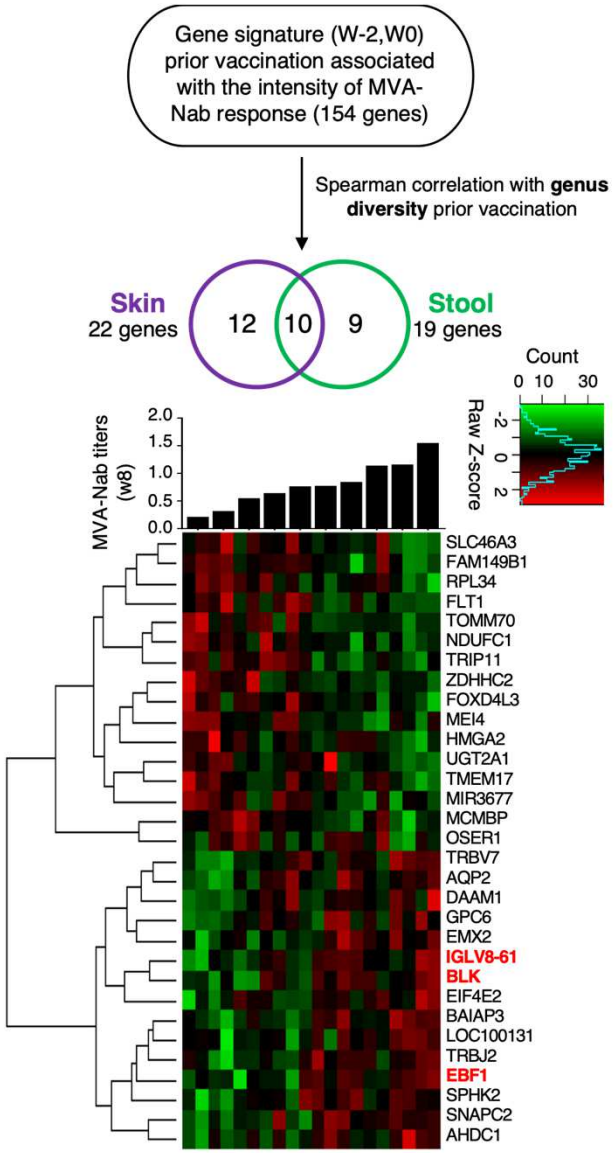
BLOOD GENE EXPRESSION COMBINED WITH PRE-VACCINE HOST MICROBIOTA PREDICT M… RESPONS…

PROTEIN TRANSMEMBRANE TRANSPORT, TRANSLATION AND TRANSCRIPTION REGULATION, CELL DIVISION, MIGRATION, PROLIFERATION, AND DIFFERENTIATION, OXIDATION REDUCTION AND METABOLIC PROCESSES

IGLV8-61, BLK, AND EBF1

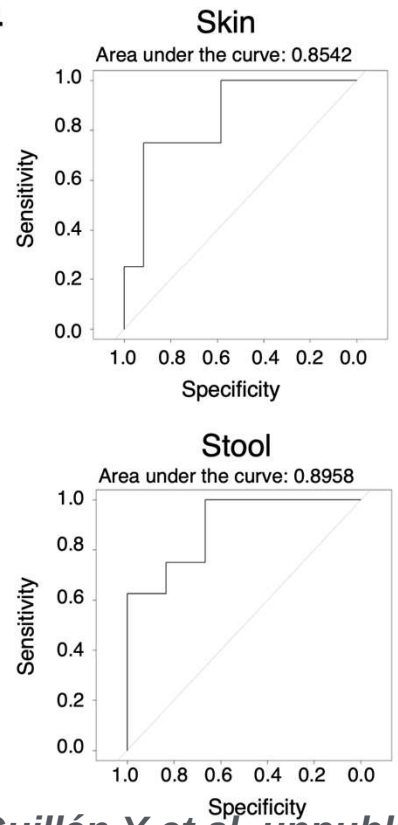
ANTIGEN RECOGNITION, B CELL DEVELOPMENT, PROLIFERATION, AND DIFFERENTIATION, AND POSITIVE REGULATION OF TRANSCRIPTION IN B CELL AND B CELL RECEPTOR SIGNALING

CELL HOMEOSTASIS AND MIGRATION, CELL GROWTH, DIVISION, PROLIFERATION, REGULATION OF GENE EXPRESSION, APOPTOTIC PROCESS, EXOCYTOSIS, AND INTRACELLULAR SIGNAL TRANSDUCTION



	Prevotella abundance		Eubacterium abundance	
	R	P-value	R	P-value
IGLV8-61	-0.64	0.0035	-0.54	0.0145
BLK	-0.66	0.0014	-0.56	0.0105
EBF1	-0.58	0.0078	-0.58	0.0078

C.

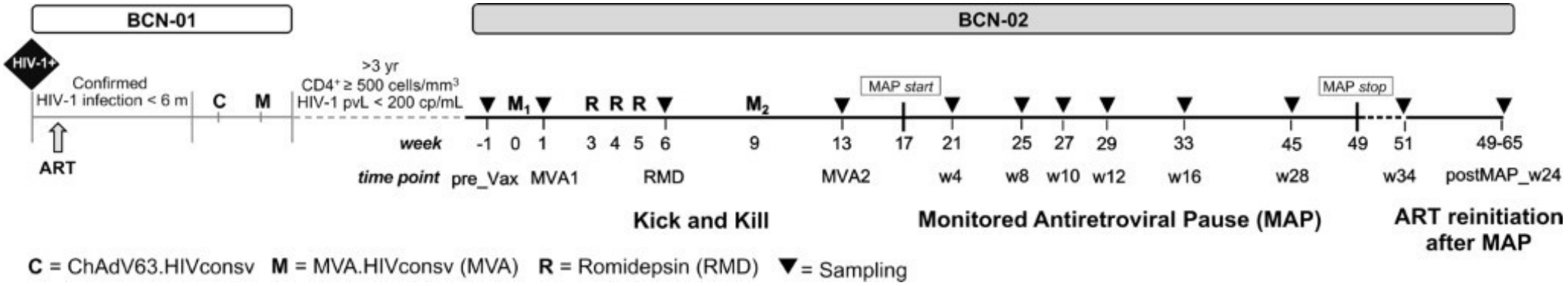


Guillén Y et al, unpublished

STRATIFYING T-CELL HIV VACCINE RESPONSES

GUT MICROBIOME BIOMARKERS MAY HELP PREDICT HIV VIRAL CONTROL

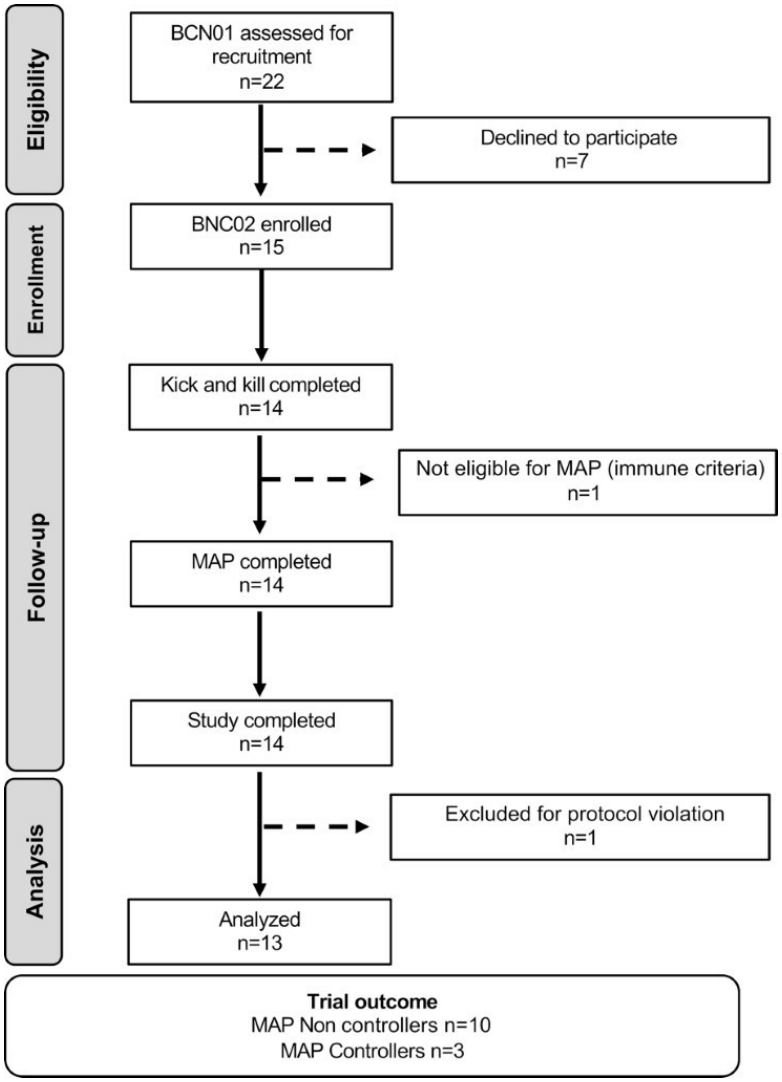
BCN02-ROMI STUDY DESIGN



Borgognone A et al, unpublished

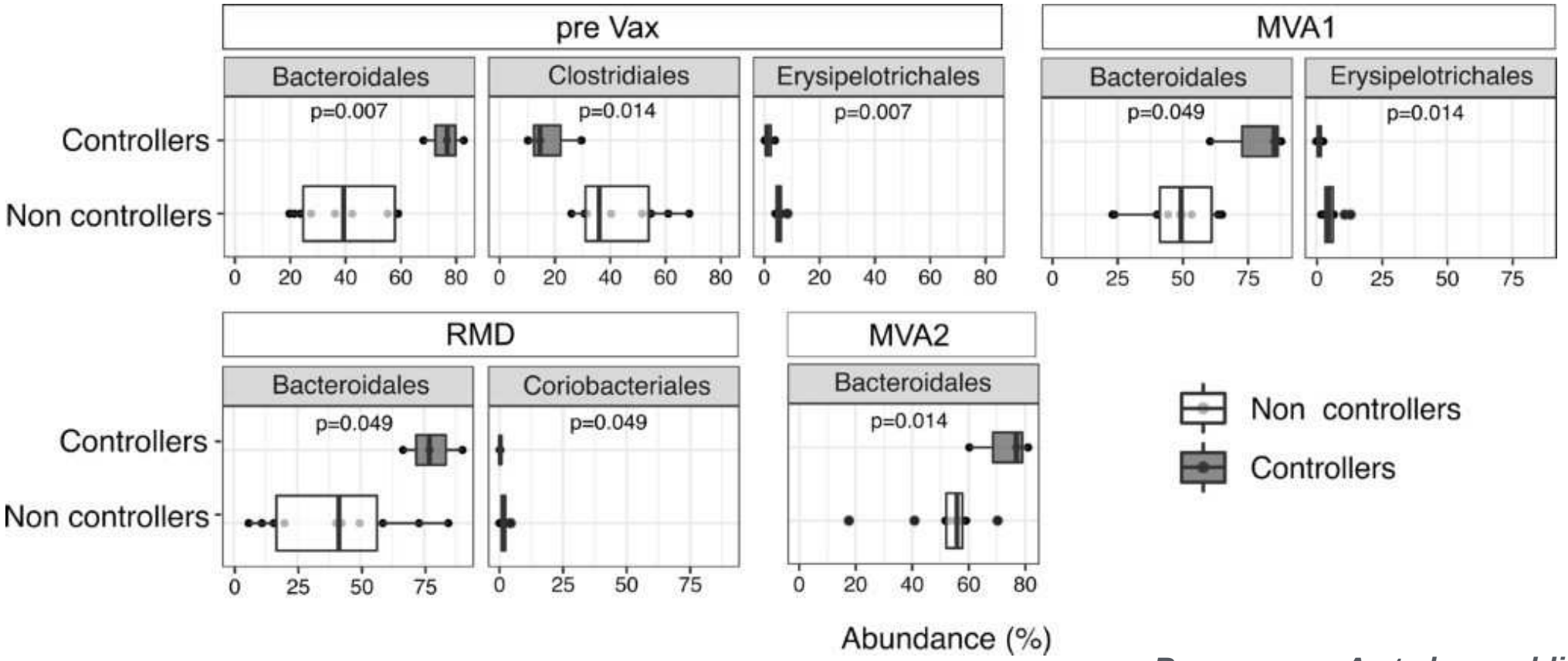
BCN02-ROMI STUDY PATIENTS

Variable	All subjects (n=13)	Non_controllers (n=10)	Controllers (n=3)
Demographics			
Gender (M/F), *n*	12/1	9/1	3/0
Risk group (MSM/HTS), *n*	12/1	9/1	3/0
Ethnic group (Caucasian/Latin), *n*	12/1	9/1	3/0
Age (years)	42 (39-47)	43 (39-47)	34 (33-38)
BMI (Kg/m^2)	22.9 (20.9-24)	22.3 (21.1-23.4)	24.3 (22.2-25)
Treatment and clinical characteristics			
ART regimen, *n* (TDF_FTC_RAL/ABC_3TC_RAL/ABC_3TC_DTG)	2/9/2	2/6/2	0/3/0
Viral reservoir (HIV-1 DNA cp/10^6 CD4$^+$ T-cells)	140 (65-361)	165 (76.2-415.7)	65 (62.5-116.5)
CD4$^+$ T-cell (cells/mm^3)	728 (648-1182)	839 (581.8-1293.8)	657 (652.5-814)
CD4$^+$ T-cell (%)	42.9 (42.2-49.3)	43.4 (42.3-48.1)	42.2 (38.4-48.1)
CD4/CD8 T-cell counts ratio	1.4 (1.2-1.6)	1.4 (1.2-1.5)	1.3 (1.1-1.6)



Borgognone A et al, unpublished

DIFFERENTIAL BACTERIAL ORDER ABUNDANCE BETWEEN HIV CONTROLLERS AND NON-CONTROLLERS

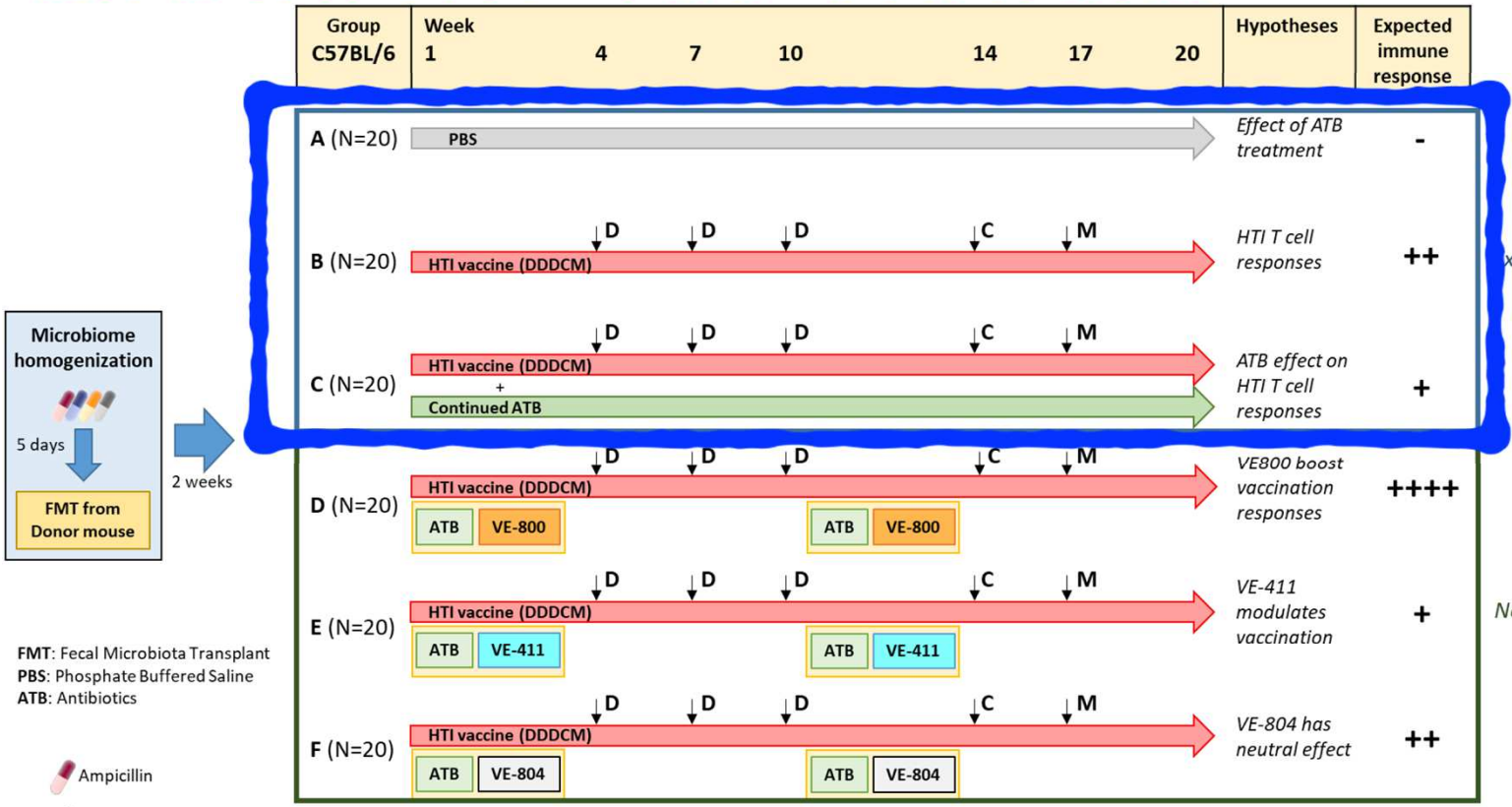


Borgognone A et al, unpublished

MODULATING T-CELL HIV VACCINE IMMUNOGENICITY WITH BUGS

GUT MICROBIOTA CHANGES MAY MODIFY HIV VACCINE IMMUNOGENICITY

MICROBIOME INTERVENTIONS TO MODULATE HIV VACCINE



xperiments already done

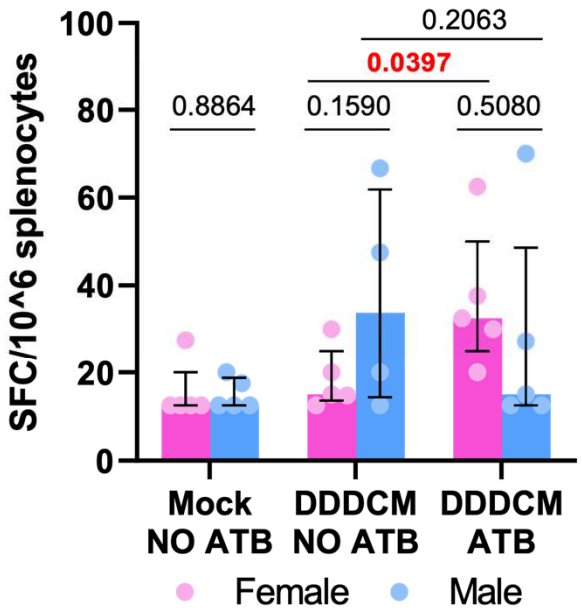
Next experiments

Olvera A & Elizalde A et al, unpublished

GENDER-BASED RESPONSES

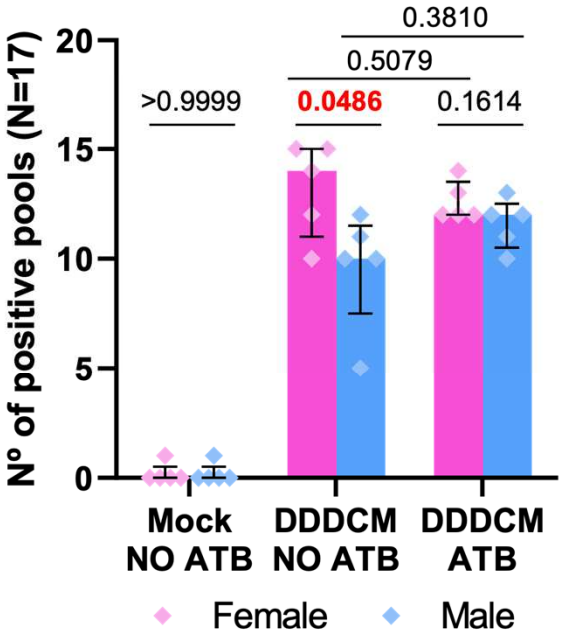

Threshold

(Basal activation of splenocytes)



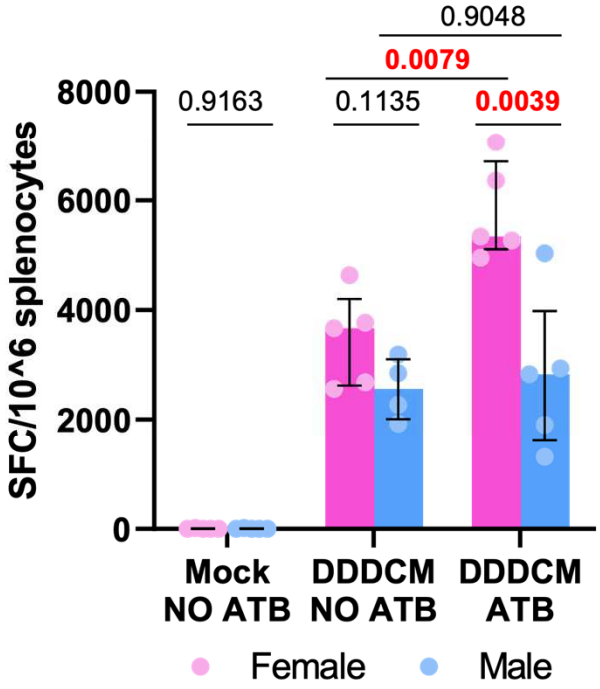
Breath

(Nº of regions of HTI targeted)



Magnitude

(Sum of splenocytes that produce INFg)

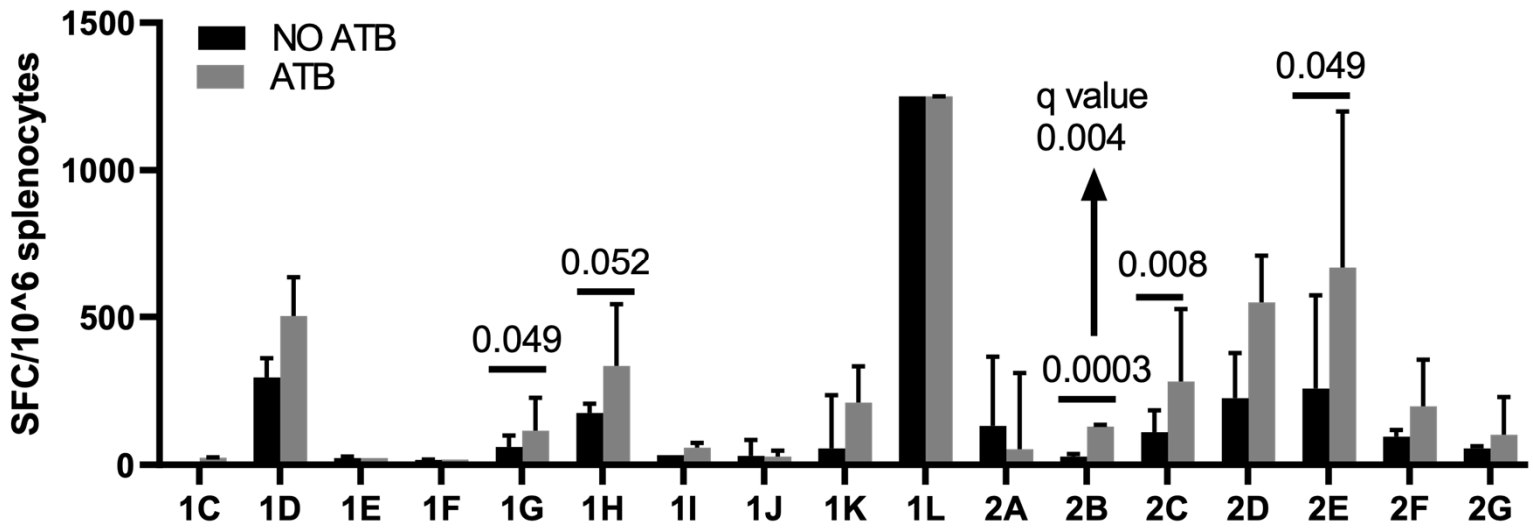


Olvera A & Elizalde A et al, unpublished

MAGNITUDE - MALE & FEMALE

Immune readout
INFg producing splenocytes (SFC) in response to HTI
-Assay: INFg ELISPOT
-Stimulus: 17 peptide pools covering the HTI sequence (2-11 peptides/pool)
-Pool description:

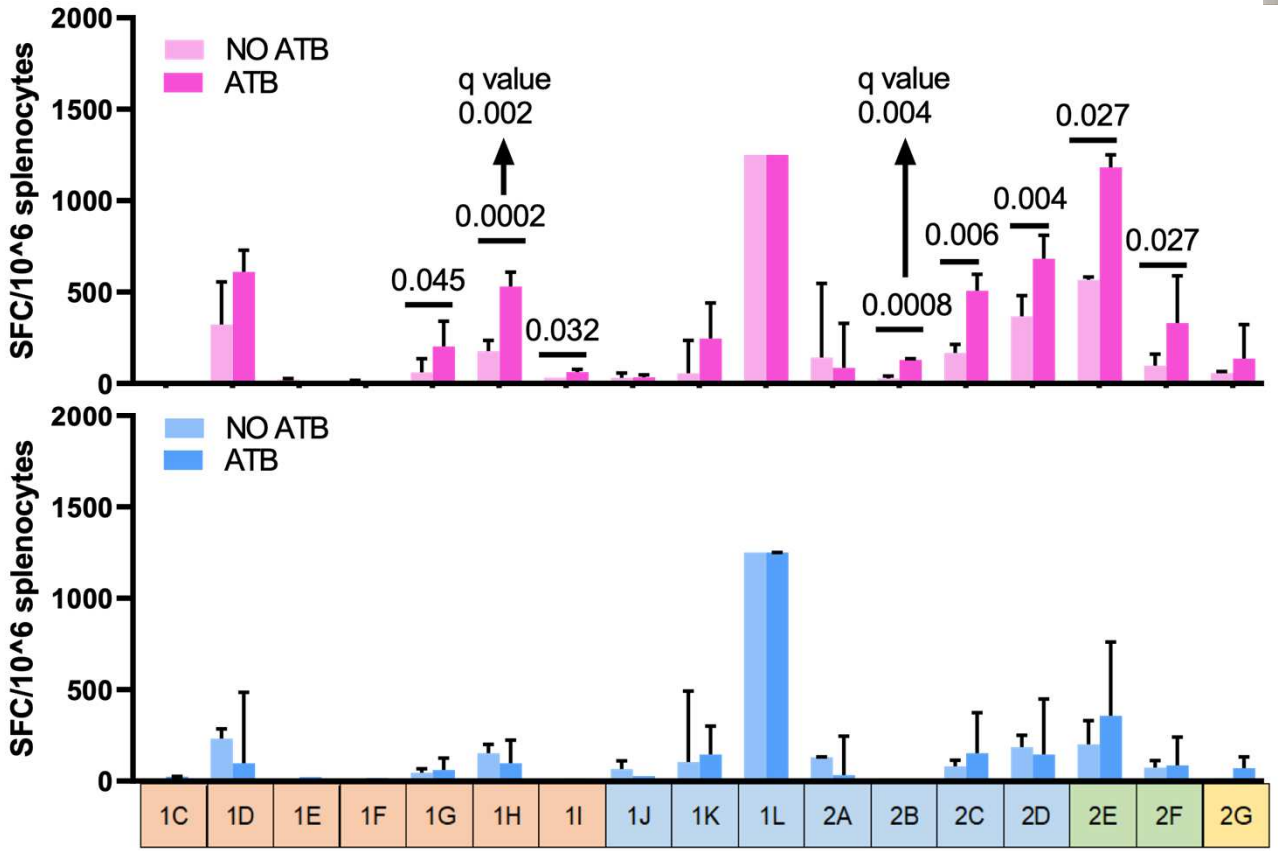
ID	Source protein	Protein subunit	HTI Segment	% in HTI
1C	Gag	p17	S1	45%
1D		p17	S1	
1E		p24	S2/3	
1F		p24	S4	
1G		p24	S4	
1H		p24	S5/6	
1I		acc	S7	
1J	Pol	PRT	S8	44%
1K		PRT	S8	
1L		RT	S9/10	
2A		RT	S10	
2B		RT	S11	
2C		Int	S12	
2D		Int	S13	
2E	Vif	-	S14	8%
2F		-	S15	
2G	Nef	-	S16	3%



Olvera A & Elizalde A, ongoing experiments

MAGNITUDE BY GENDE

ID	Source protein	Protein subunit	HTI Segment	% in HTI
1C	Gag	p17	S1	45%
1D		p17	S1	
1E		p24	S2/3	
1F		p24	S4	
1G		p24	S4	
1H		p24	S5/6	
1I		acc	S7	
1J	Pol	PRT	S8	44%
1K		PRT	S8	
1L		RT	S9/10	
2A		RT	S10	
2B		RT	S11	
2C		Int	S12	
2D		Int	S13	
2E	Vif	-	S14	8%
2F		-	S15	
2G	Nef	-	S16	3%



Females

Males

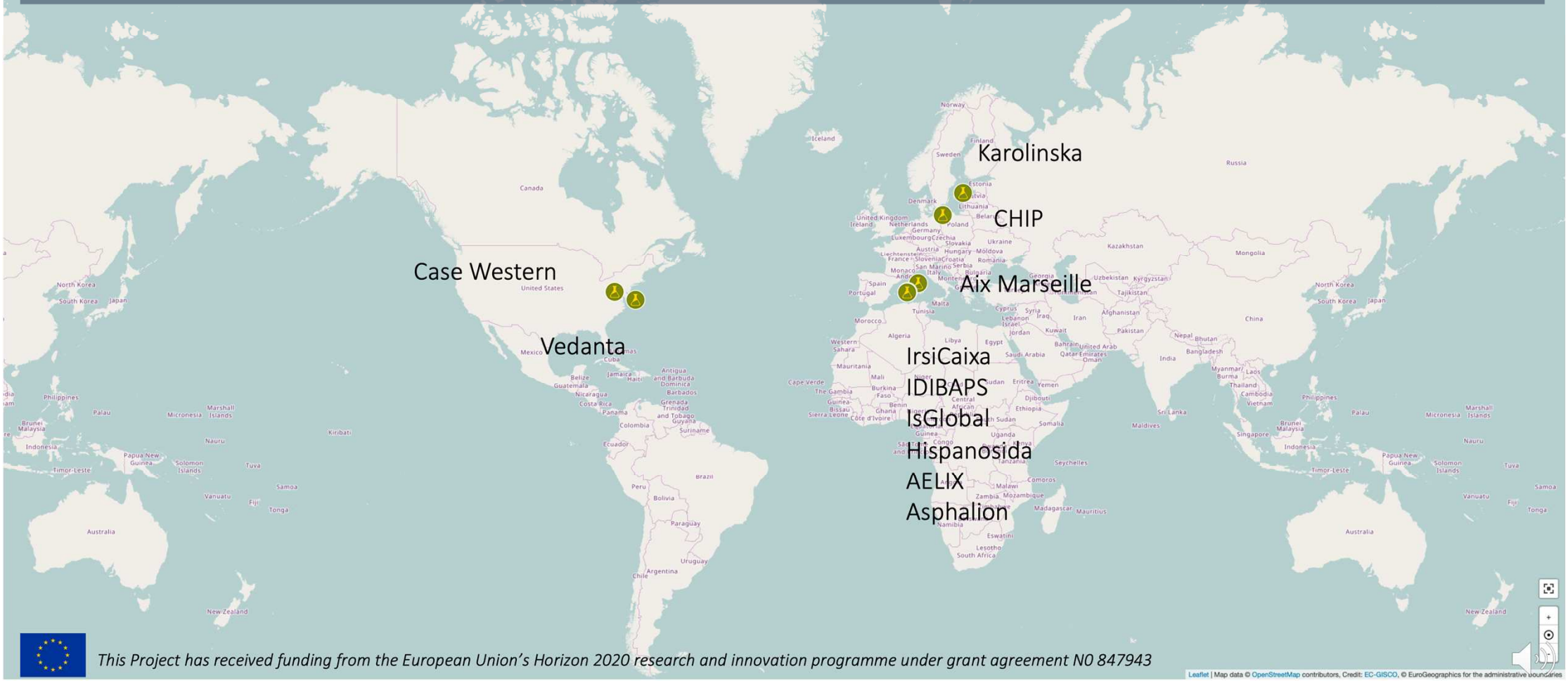
Olvera A & Elizalde A et al, unpublished

MISTRAL

MICROBIOME-BASED STRATIFICATION OF INDIVIDUALS AT RISK OF HIV-1 ACQUISITION, CHRONIC CLINICAL COMPLICATIONS, ANTIMICROBIAL DRUG RESISTANCE, AND UNRESPONSIVENESS TO THERAPEUTIC HIV-1 VACCINATION

MISTRAL

Microbiome-based stratification of individuals at risk of HIV-1 acquisition, chronic clinical complications, antimicrobial drug resistance, and unresponsiveness to therapeutic HIV-1 vaccination



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Participant No	Participant organisation name	Lead PI	Country
1 (Coordinator)	Fundació Privada Institut de Recerca de la Sida-Caixa (IrsiCaixa)	Roger Paredes	Spain
2	IHU Méditerranée Infection (Aix-Marseille University)	Grégory Dubourg	France
3	CHIP - Centre of Excellence for Health, Immunity and Infections (RegionH)	Jens D. Lundgren	Denmark
4	Case Western Reserve (Case)	Rafick-Pierre Sékaly	USA
5	Karolinska Institutet (KI)	Kristina Broliden	Sweden
6	Projecte dels NOMS-HISPANOSIDA (Hispanosida)	Michael Meulbroek	Spain
7	Consorci Institut d'Investigacions Biomèdiques A Pi i Sunyer (IDIBAPS)	José María Miró	Spain
8	Institut de Salut Global de Barcelona (IsGlobal)	Jordi Vila	Spain
9 (SME)	Asphalion, S.L. (ASPH)	Oriol Penón	Spain
10 (SME)	AELIX Therapeutics, S.L. (AELIX)	Jordi Naval	Spain
11 (SME)	Vedanta Biosciences, Inc. (Vedanta)	Bruce Roberts	USA

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Objectives

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- To identify **novel signatures** in any dimension of the host-microbiome interaction associated with:
  - Protection from HIV-1 acquisition
  - Responses to state-of-the-art therapeutic HIV-1 vaccination
  - Development of AIDS/non-AIDS clinical complications
  - Systemic immune reconstitution following ART initiation
  - Systemic inflammation and CD4+ recovery in ART-treated PLWH following exposure to synbiotic products

- To provide strong scientific rationale to design **microbiome-based interventions** aimed at preventing HIV-1 acquisition, avoid chronic clinical complications and improve the efficacy of HIV immunotherapy

- To define the phenotypic and clinical correlates of shotgun metagenomics-based gut **microbial resistome** analyses, and their relationship with immune reconstitution and antiretroviral therapy

- To develop a practical **cloud-based software tool** for clinicians and researchers which enables and facilitates microbiome-based patient stratification

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Methods

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HIV transmission

WP1. Correlates of HIV protection and control

HIV immunotherapy

WP2. Microbiome-based modulation of HIV immunotherapy (HTI vaccine) in C57/bl6 mice

WP3. HTI vaccine RCT (AELIX-002)

Clinical complications

WP4. Microbiome correlates of severe AIDS/non-AIDS events

WP5. Impact of ART in the gut resistome

WP6. RCT to modulate the microbiome in PLWH:

- RCT1: ART initiation in late presenters
- RCT2: Synbiotics in HIV-infected subjects with CD4<500 cells/mm^3

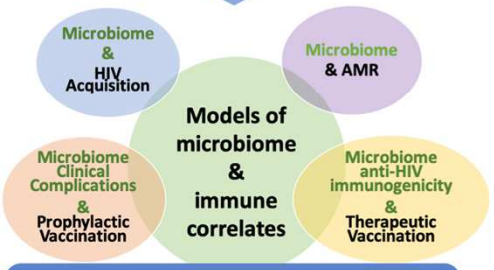
WP7. Data integration & systems biology
WP8. Microbiome-based patient stratification software

WP9. Regulatory procedures
WP10. Management, Exploitation and Communication

Mouse models | Human cohort | Randomized Clinical Trial

Data types

Clinical & epidemiological
Virology and reservoir
High-throughput immunology
Host single-cell transcriptomics
HIV-1 Reservoir assays
16S RNA Sequencing
Shotgun sequencing
Fecal proteome
Plasma metabolome
Phenotypic AMR



SC1-BHC-14-2019: Stratified host-directed approaches to improve prevention, treatment and/or cure of infectious diseases

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Innovation & impact

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Microbiota-derived biomarkers

HIV-1 acquisition
HIV-1 progression
Vaccine responses
Immune reconstitution
Clinical complications
Antimicrobial DR

Open-Access Research Data repository (iVIHome)

Subject stratification software (Enthera®)

New medical models

Microbiota-derived treatments & interventions

Prevent HIV-1 acquisition
Slow HIV-1 progression
Improve vaccine responses
Accelerate immune reconstitution
Prevent clinical complications
Monitor antimicrobial DR

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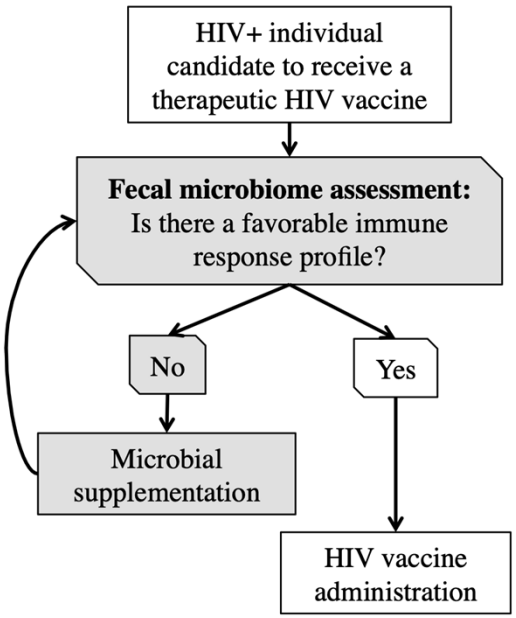
CONCLUSIONS

- The human microbiome can be used to stratify subjects according to their predicted response to HIV immunotherapy
- This will allow identifying subjects who might not respond to immunotherapy
- Interventions in the gut microbiome hold the potential to modify HIV vaccine immunogenicity
- Much more work is needed to identify the specific molecules and mechanisms

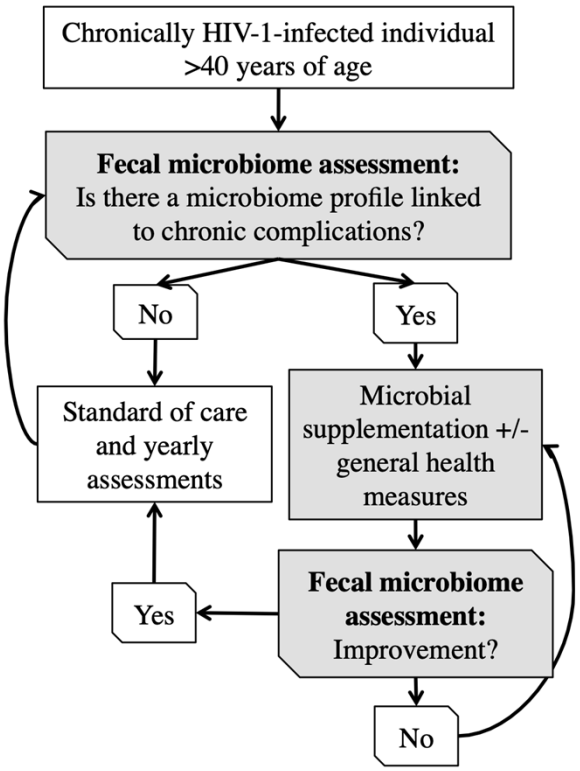
A VIEW INTO THE CLINICAL

New organizational models

Therapeutic HIV vaccine



Chronic complications



From bugs to drugs

THANKS

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Mariona Parera
Carmen Fuentes
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Laura Planells

Cellular Immunity & Host Genetics
Christian Brander
Beatriz Mothe
Alex Olvera

Virology & Cellular Immunology
Julià Blanco
Jorge Carrillo

Bonaventura Clotet

UVIC-UCC
Malu Calle

Universitat de Girona
Vera Pawlovsky-Glahn
Juan José Egozcue

Hospital Clínic Barcelona
José Maria Miró

isGlobal
Jordi Vila
Andrea Vergara
Denise Naniche
Inacio Mandomando

BCN Checkpoint
Michael Meulbroek
Jorge Saz

Imperial College
Carolina Herrera

Karolinska Institute
Kristina Broliden
Piotr Nowak
Anders Sönneborg

Univ Manitoba
Adam Burgener
Alicia Berard

Case Western Reserve
Rafick Sékaly

NIH
Jason Brenchley
Ivan Vujkovic-Cijin
Irini Sereti

Imperial College
Carolina Herrera

US Military HIV Research Program
Alexandra Schuetz

Persimune / CHIP
Jens D Lundgren
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