PANDORA ID - 23
TIME TO WEBINAR IN INFECTIOUS DISEASES
19 Giugno 2023
NOVITA' DIAGNOSTICO-TERAPEUTICHE DELL'INFEZIONE DA HPV
VIRTUAL EDITION

Novel non-invasive markers for diagnosing HSIL lesions in PLWH (People leaving with HIV)

By Dall-E: "A scientist taking care of the microbiome. Digital art, pale colors"

Sergio Serrano Villar

ENFERMEDADES INFECCIOSAS
H.U RAMÓN Y CAJAL

INSTITUTO RAMÓN Y CAJAL DE INVESTIGACIÓN SANITARIA IRYCIS

Outline

1. Potential for the microbiome as a diagnostic tool in PWH

2. Current challenges to using the microbiome as a diagnostic tool.

3. The case of anal HPV-disease in PWH.

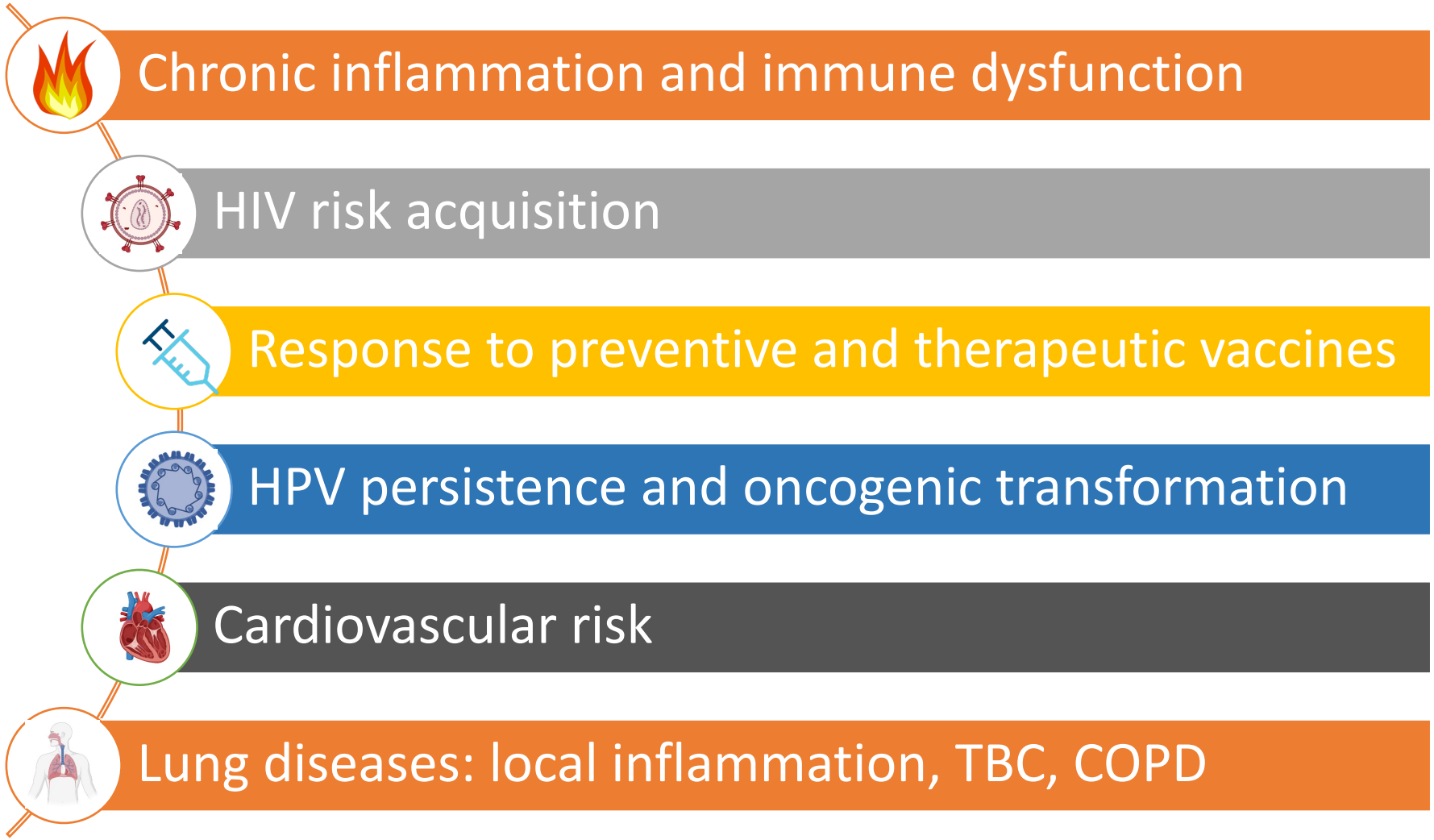
Outline

1 **Potential for the microbiome as a diagnostic tool in PWH**

2 Current challenges to using the microbiome as a diagnostic tool.

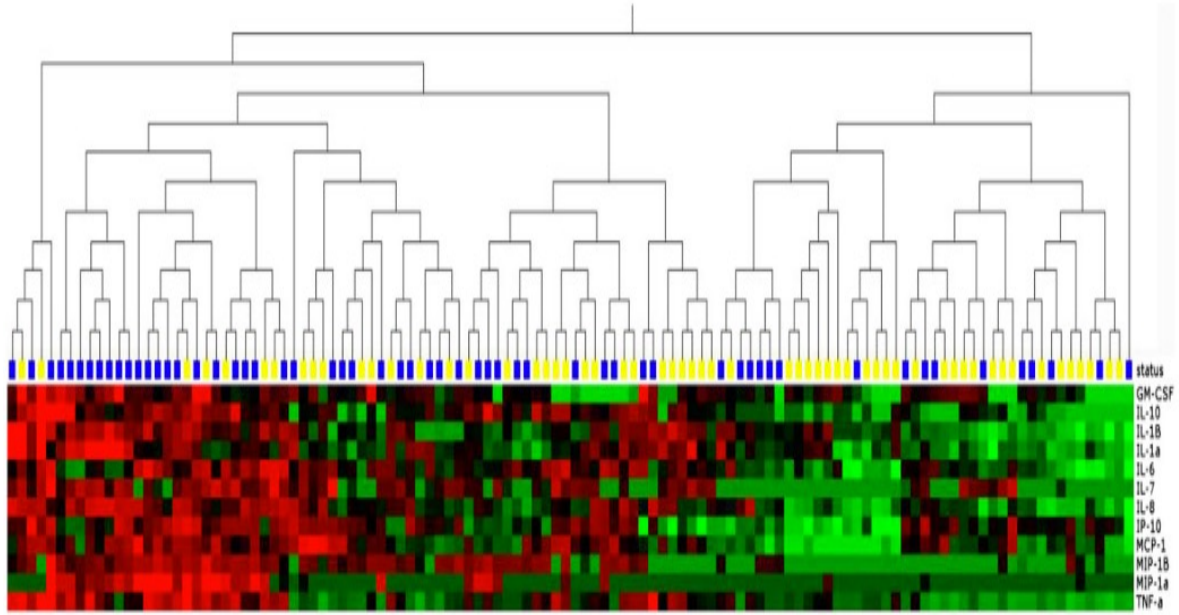
3 The case of anal HPV-disease in PWH.

Potential clinical implications of the microbiome under investigation



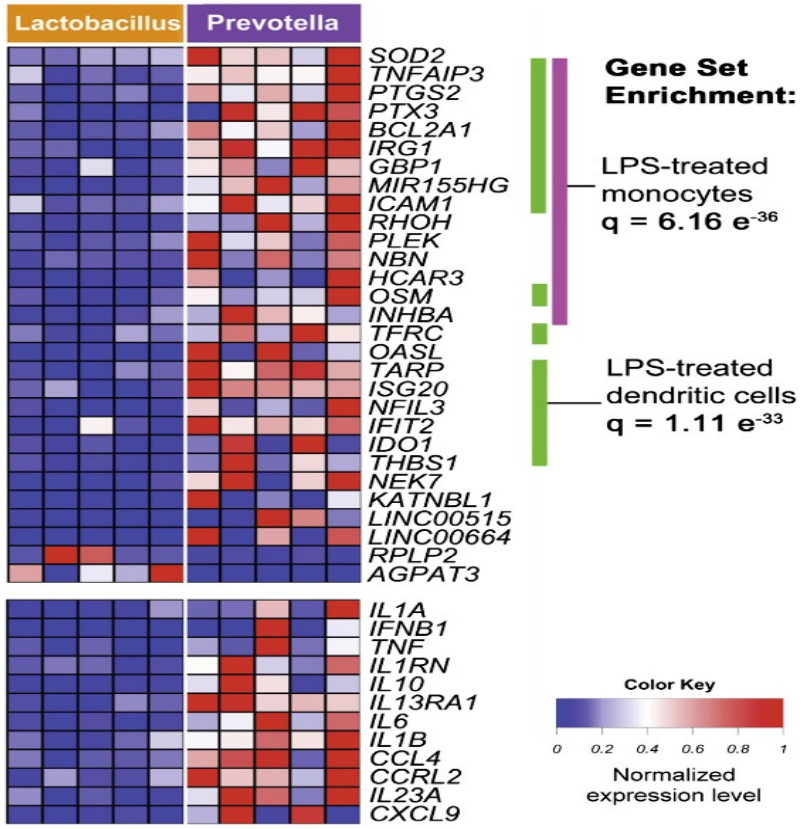
The Cervicovaginal Microbiota Influences the Risk of HIV Acquisition in Women

Women who later became HIV–infected had up-regulated preinfection cervicovaginal cytokine concentrations (risk x3)



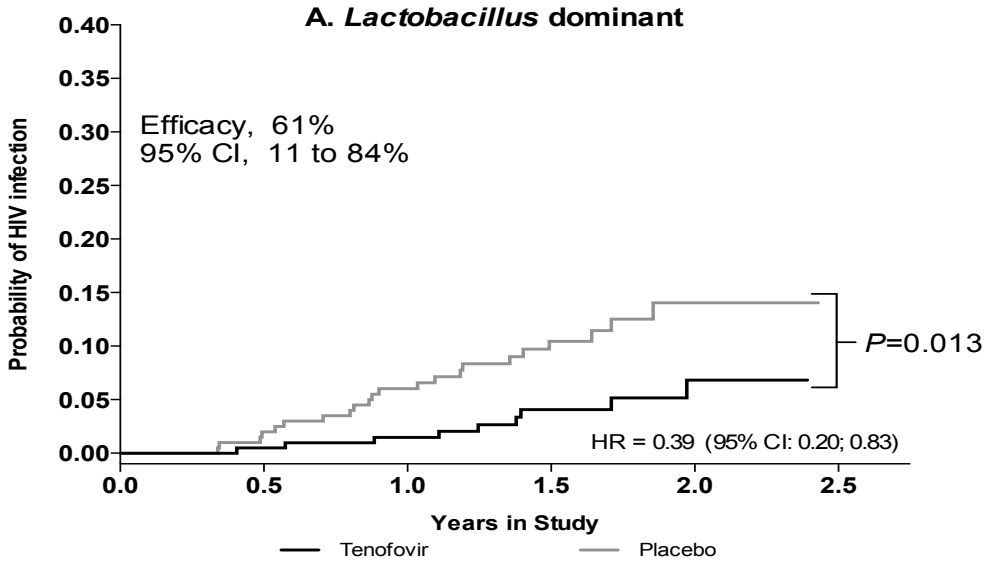
Masson et al. JID 2015

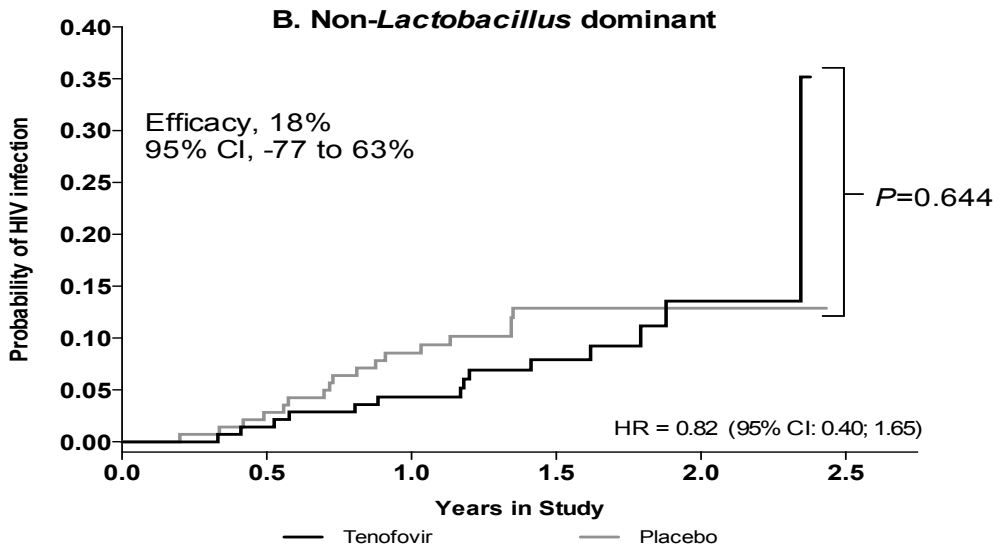
Bacterial vaginosis determines local inflammation via TLR-4 signaling



Anahtar et al. Immunity 2015

Tenofovir gel effective against HIV with Lactobacillus dominance



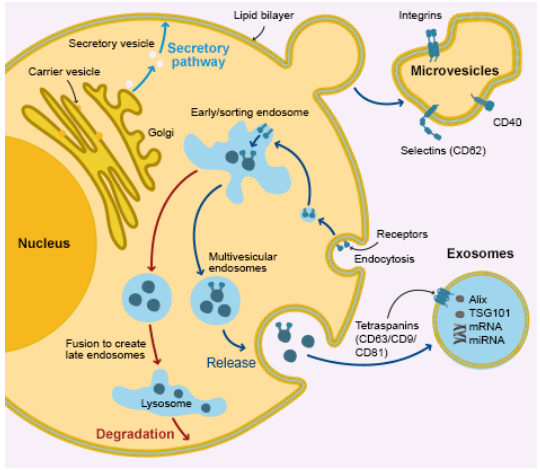


	Lactobacillus dominant		non- *Lactobacillus* dominant	
	Tenofovir	Placebo	Tenofovir	Placebo
# HIV-1 infections	9	22	14	17
HIV-1 incidence per 100 person-years	2.7	6.9	6.4	7.8
HIV-1 protection effectiveness	**61%**		**18%**	
95% CI, *P*-value	(11, 84), *p*=0.013		(-77, 63), *p*=0.644	

Klatt et al. Science 2017

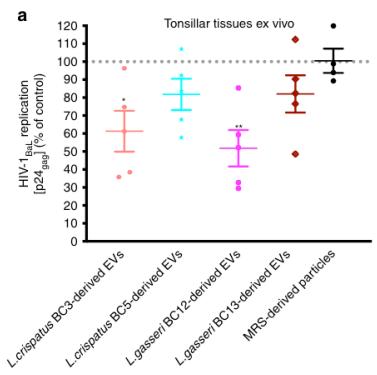
Extracellular vesicles from vaginal lactobacilli inhibit HIV-1 infection

"EVs released by lactobacilli into culture medium protect human T cells as well as human cervico-vaginal and tonsillar tissues ex vivo from HIV-1 infection, through the inhibition of viral attachment and entry in the target cells."



Lactobacilli cultures

Ultracentrifugation



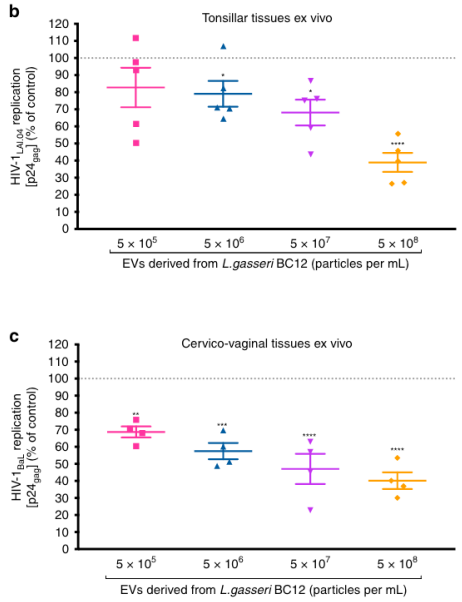


Table 1 Protein cargo of EVs derived from *L. gasseri* BC12 and *L. crispatus* BC5.

Lactobacillus-derived EV-associated proteins	Accession number
1. ATP synthase subunit beta	ATPB_LACGA
2. ATP synthase subunit alpha	ATPA_LACGA (+1)
3. Phosphonates import ATP-binding protein PhnC	PHNC_LACGA
4. ATP synthase subunit b	ATPF_LACGA
5. Enolase 2[a]	ENO2_LACGA
6. 60 kDa chaperonin[a]	CH60_LACGA (+1)
7. Enolase 1	ENO1_LACGA (+1)
8. Elongation factor Tu[a]	EFTU_LACGA
9. ATP synthase gamma chain[a]	ATPG_LACGA
10. Foldase protein PrsA 1[a]	PRSA1_LACJO
11. ATP synthase subunit beta	ATPB_LACAC
12. ATP synthase subunit delta[a]	ATPD_LACGA
13. 50S ribosomal protein L4[b]	RL4_LACAC
14. Pyruvate kinase[a]	KPYK_LACDE
15. 30S ribosomal protein S4	RS4_LACGA (+1)
16. Triosephosphate isomerase[a]	TPIS_LACGA (+1)
17. 50S ribosomal protein L21[b]	RL21_LACAC (+1)
18. 50S ribosomal protein L2[b]	RL2_LACGA (+2)

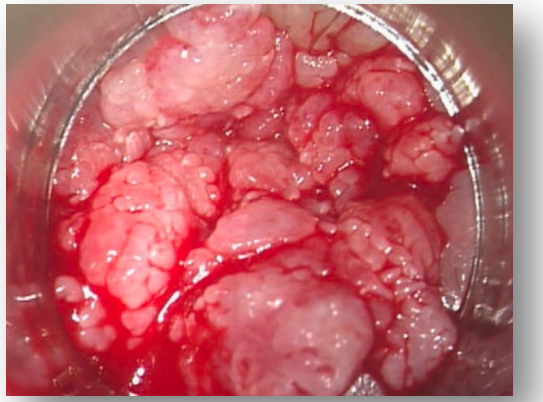
[a]Protein identified only in *L. gasseri* BC12-derived EVs
[b]Protein identified only in *L. crispatus* BC5-derived EVs

Ñahui Palomino et al. Nat Comm 2019

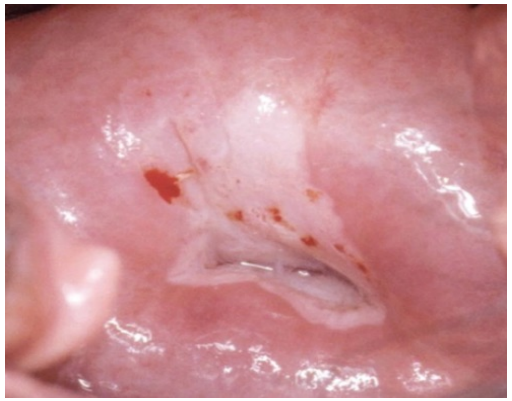
HPV Increases the risk of some cancers

Host's determinants remain unclear

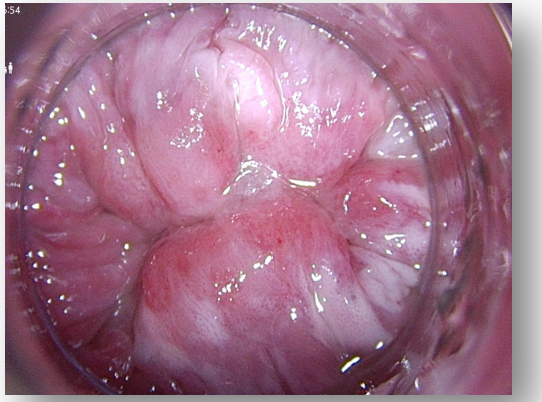
Anal condyloma



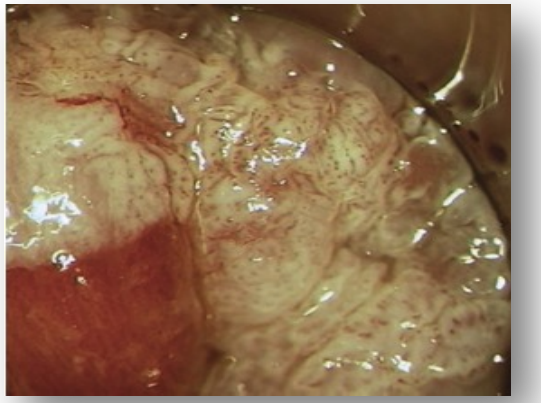
Cervical HSIL



Anal HSIL

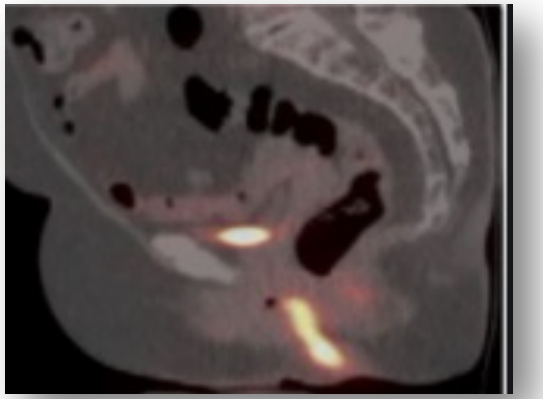


Anal HSIL

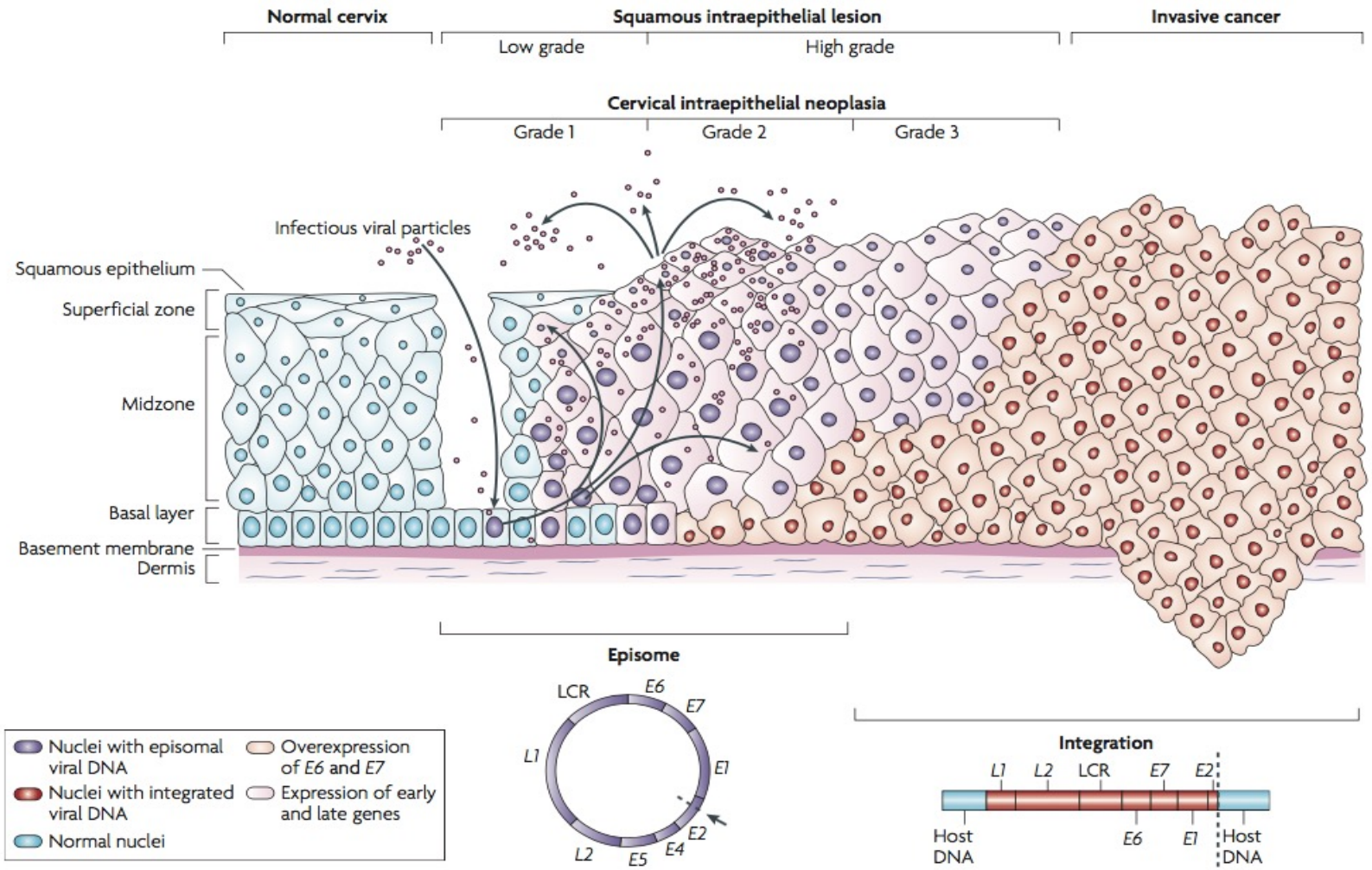


Oral cancer

Vulvar cancer

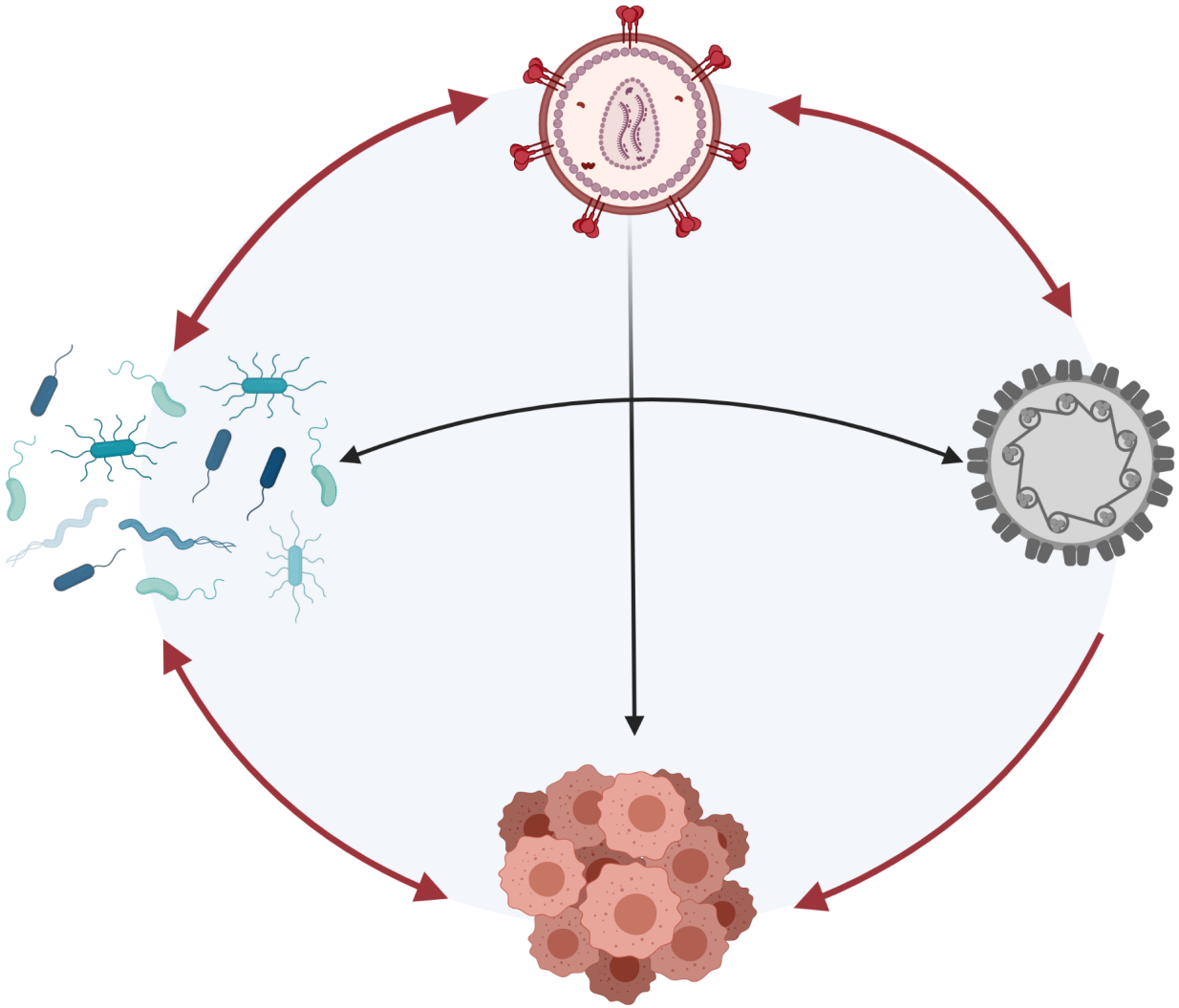


HPV infection is exclusively intraepithelial



Woodman CB. Nature reviews Cancer 2007

Challenges to address causal relationships between HPV-associated disease, HIV and the microbiome



Generated in biorender.com

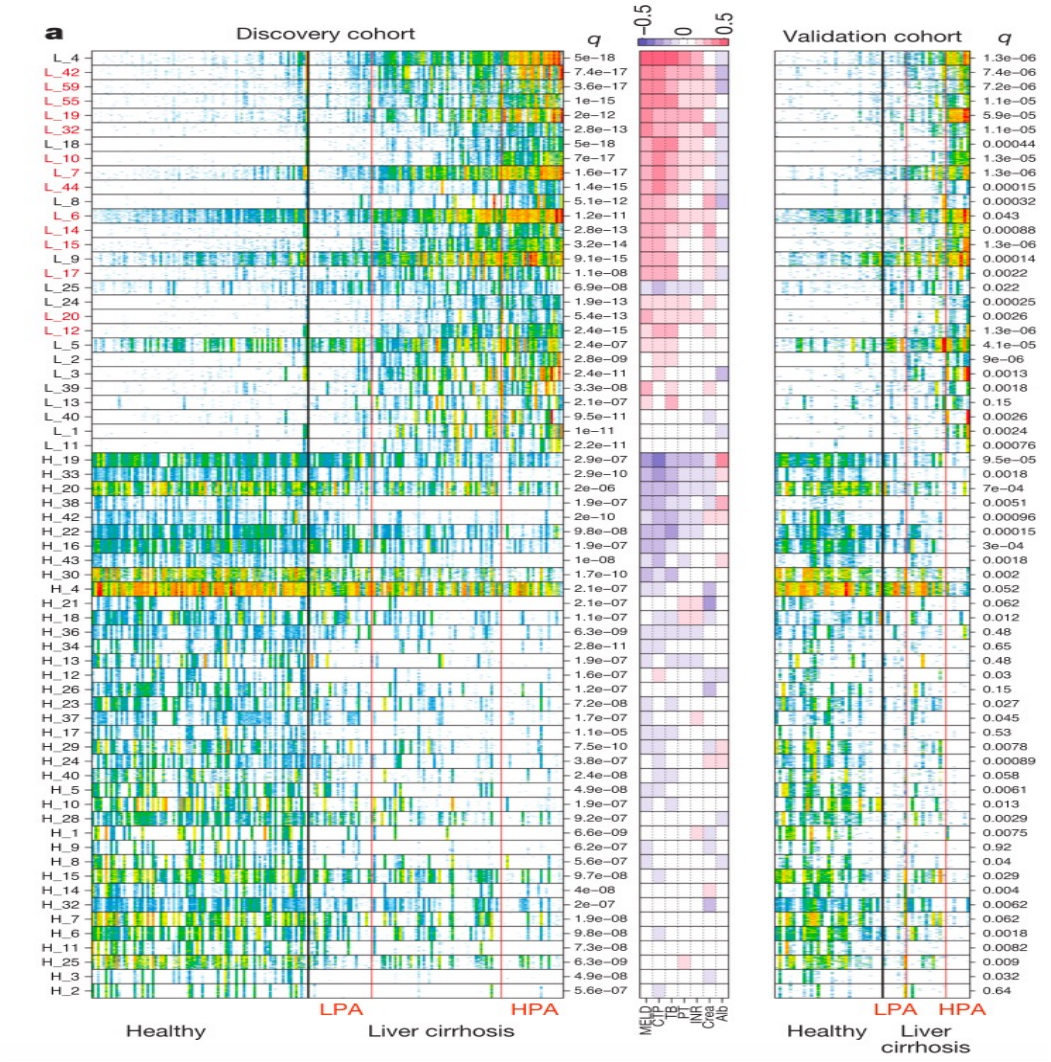
Outline

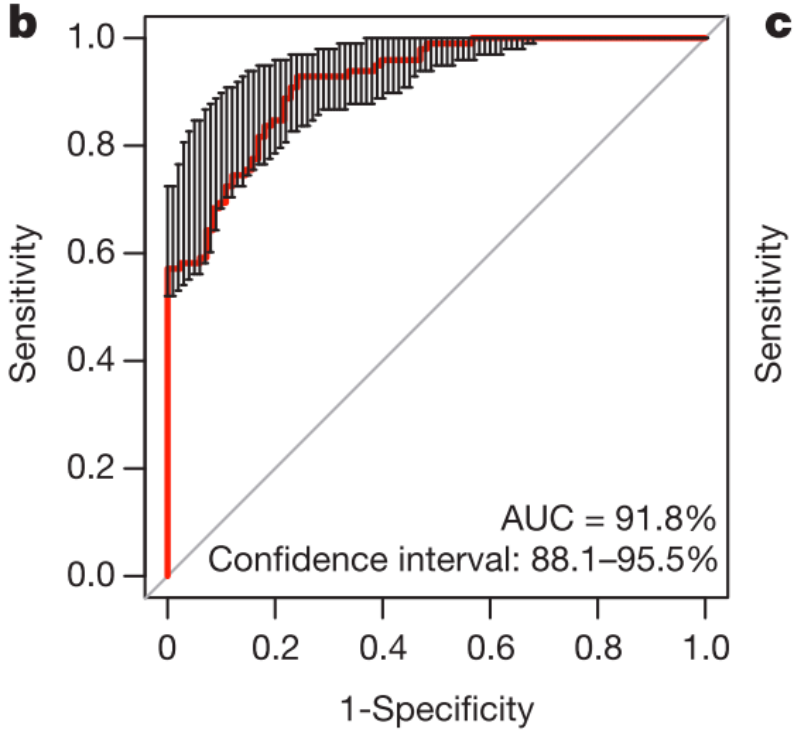
1 Potential for the microbiome as a diagnostic tool in PWH

2 Current challenges to using the microbiome as a diagnostic tool.

3 The case of anal HPV-disease in PWH.

The Microbiota as a Diagnostic Tool





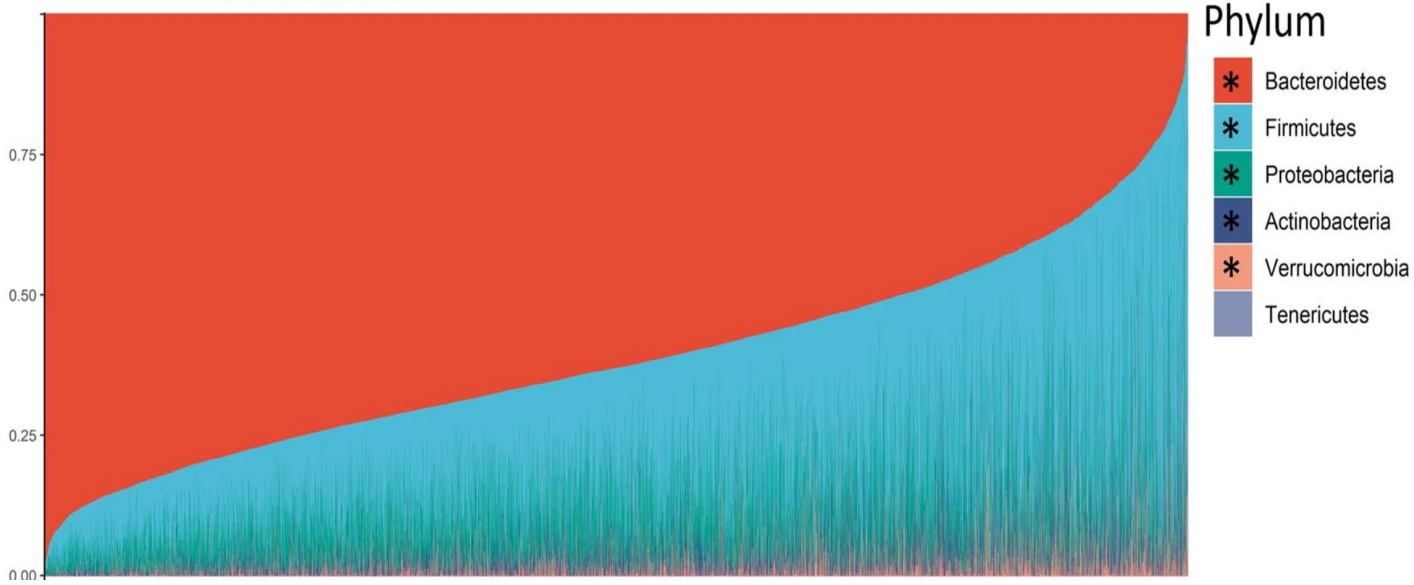
Qin. Nature 2014

The low number of “core-species” limits using microbiome signatures as a diagnostic tool

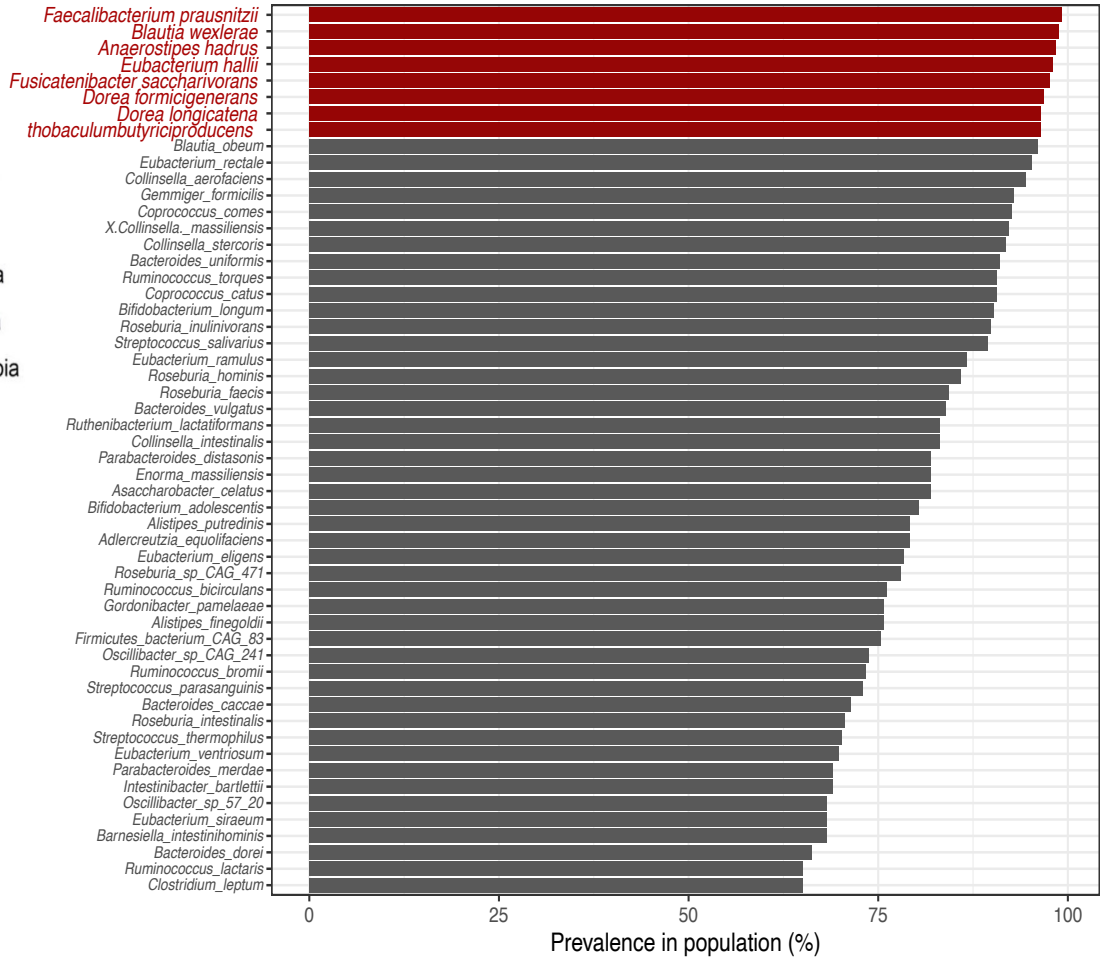
8208 participants.

1253 species identified.

Only 8 species found in >95% participants (“core microbiota”)



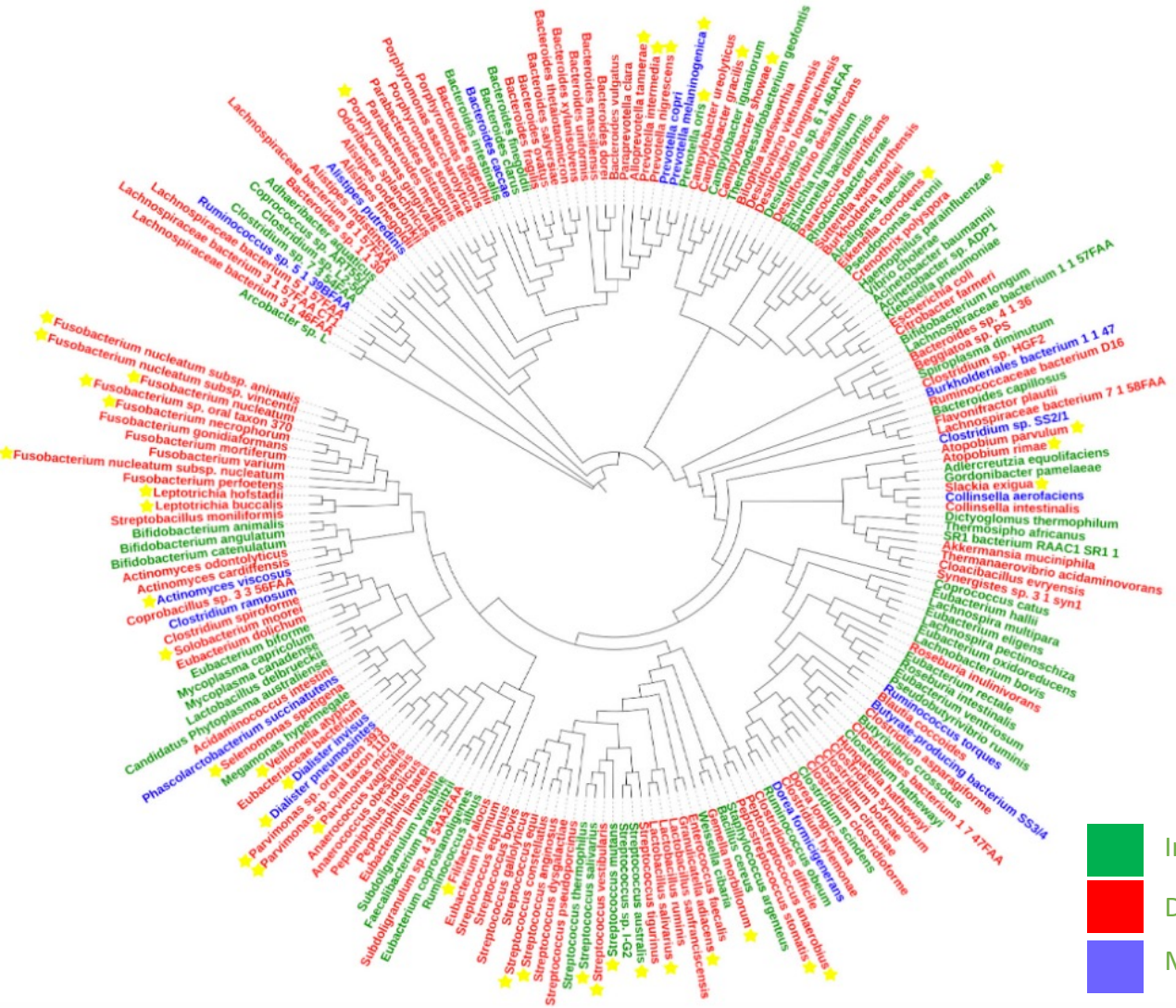
8,208 individuals



Adapted from: Gacesa et al. 2022 Nature

Challenges to leverage the microbiome as a diagnostic tool

Colorectal cancer (CRC)-related microbes across studies



Increased in CRC

Decreased in CRC

Mixed results

Combination of biomarkers
1. FIT/FOBT
2. Clinical features
3. Methylation/mutation

Robustness
1. Large-scale, cross-cohort studies
2. Regional marker

Cost evaluation
1. Test cost
2. Screening interval
3. Cost of subsequent evaluation

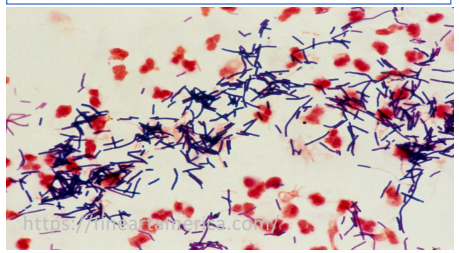
Longitudinal mechanism
1. Carcinogenesis research
2. Benefits of probiotics

Standardization
1. Inclusion and exclusion criteria
2. Sampling and storage
3. Statistical processing
4. Models and algorithms

Zhou et al. Frontiers Microbiol 2020

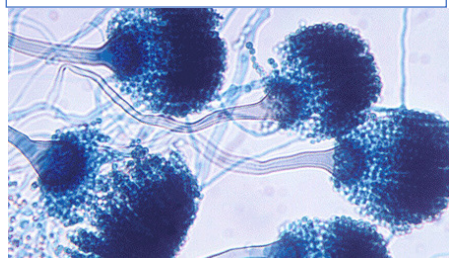
Microbiome ‘compartments’

Bacteria

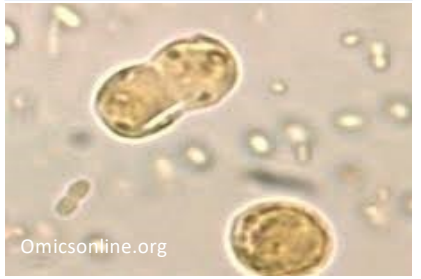


Archea

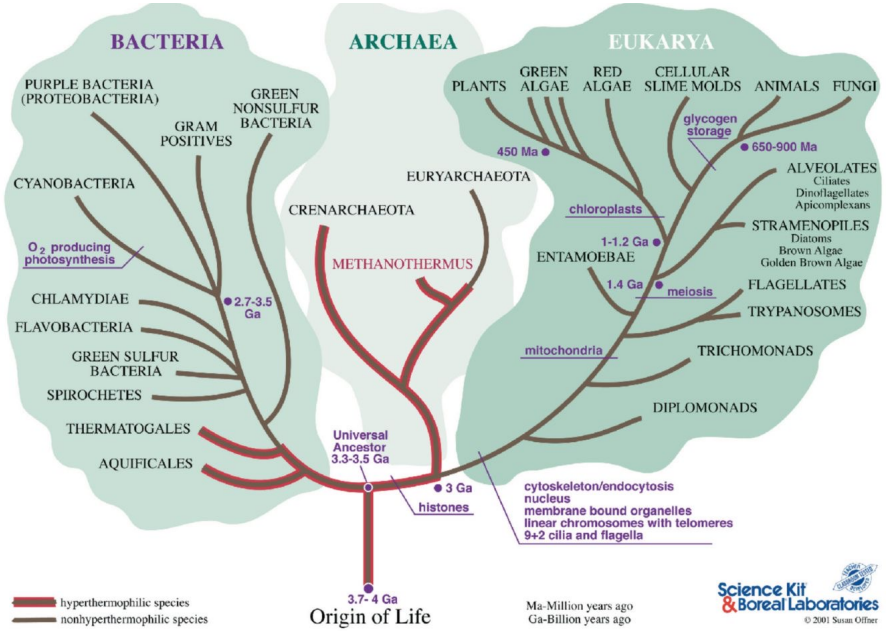
Funghi



Protists

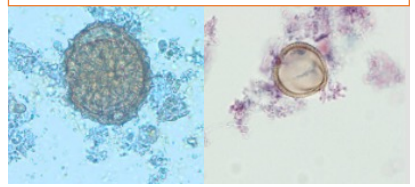


Phages



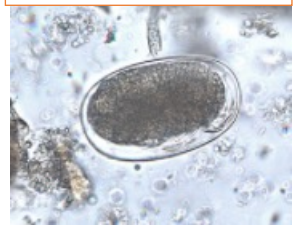
mx.vrw.com

Pollen spores

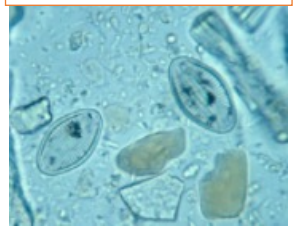


Nutrients

Mite eggs

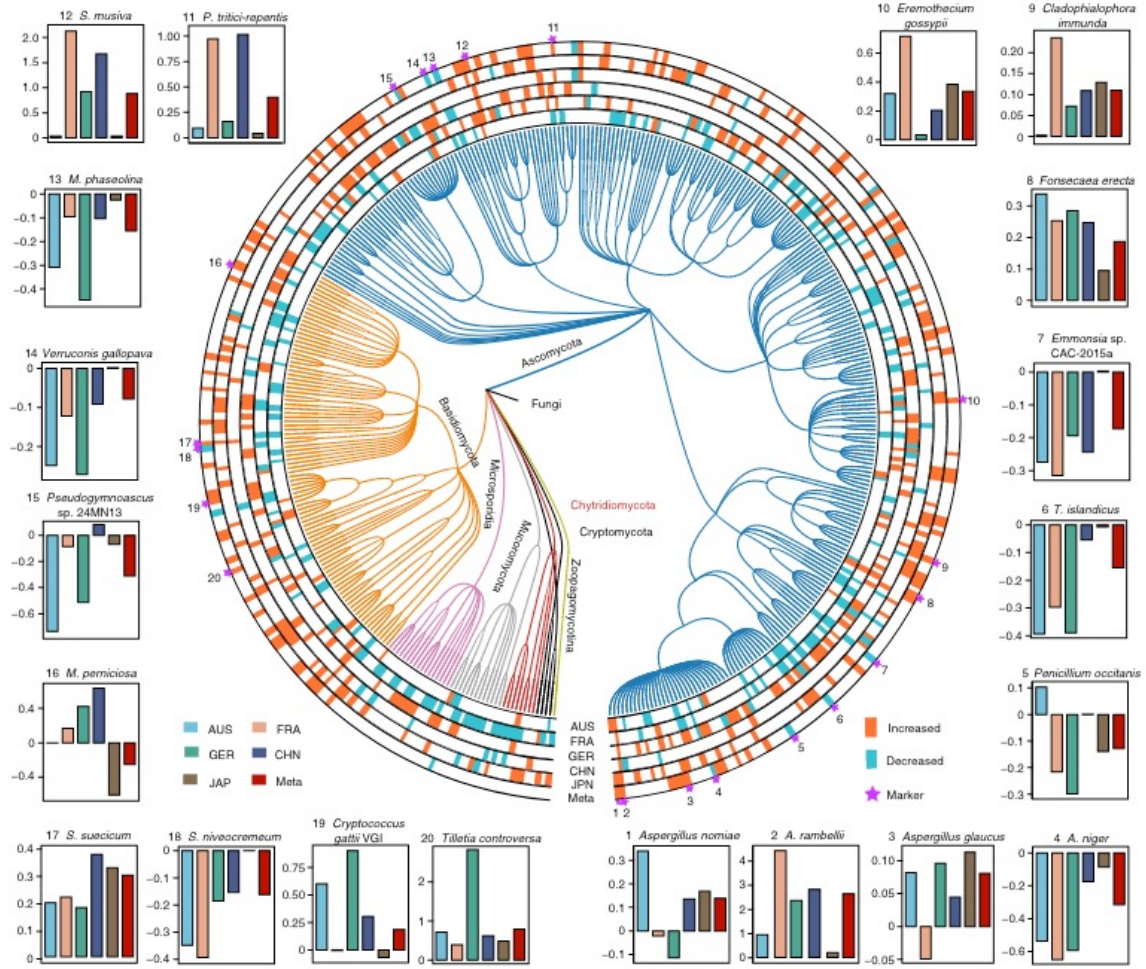


Diatoms

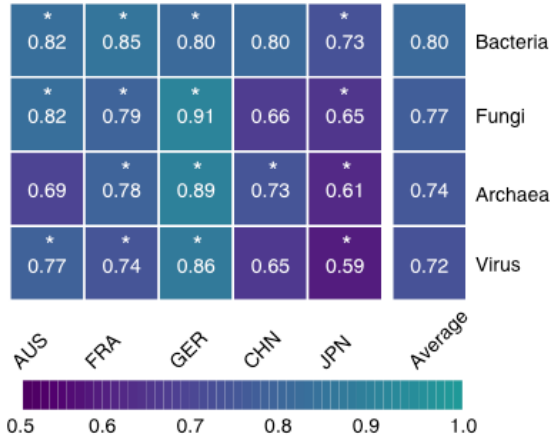


https://www.cdc.gov/dpdx/artifacts/index.html

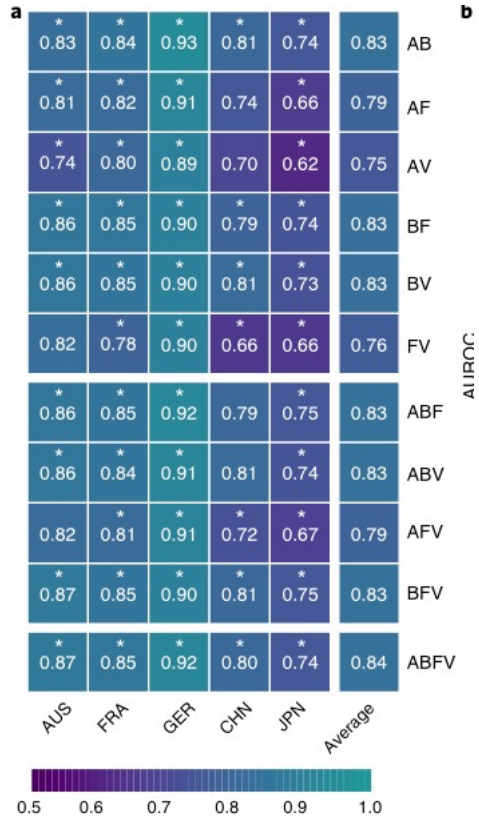
Multi-kingdom microbiome analyses identify biomarkers of colorectal cancer better than single-kingdom approaches



Single-kingdom prediction



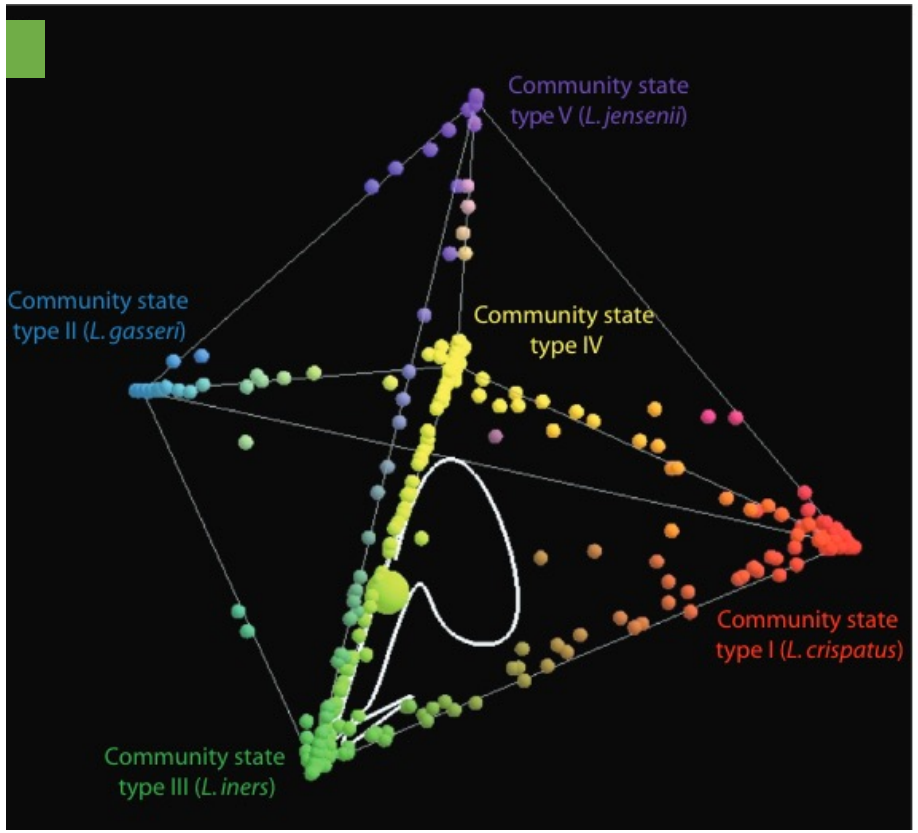
Multi-kingdom prediction



Liu et al. Nat Microbiol 2022

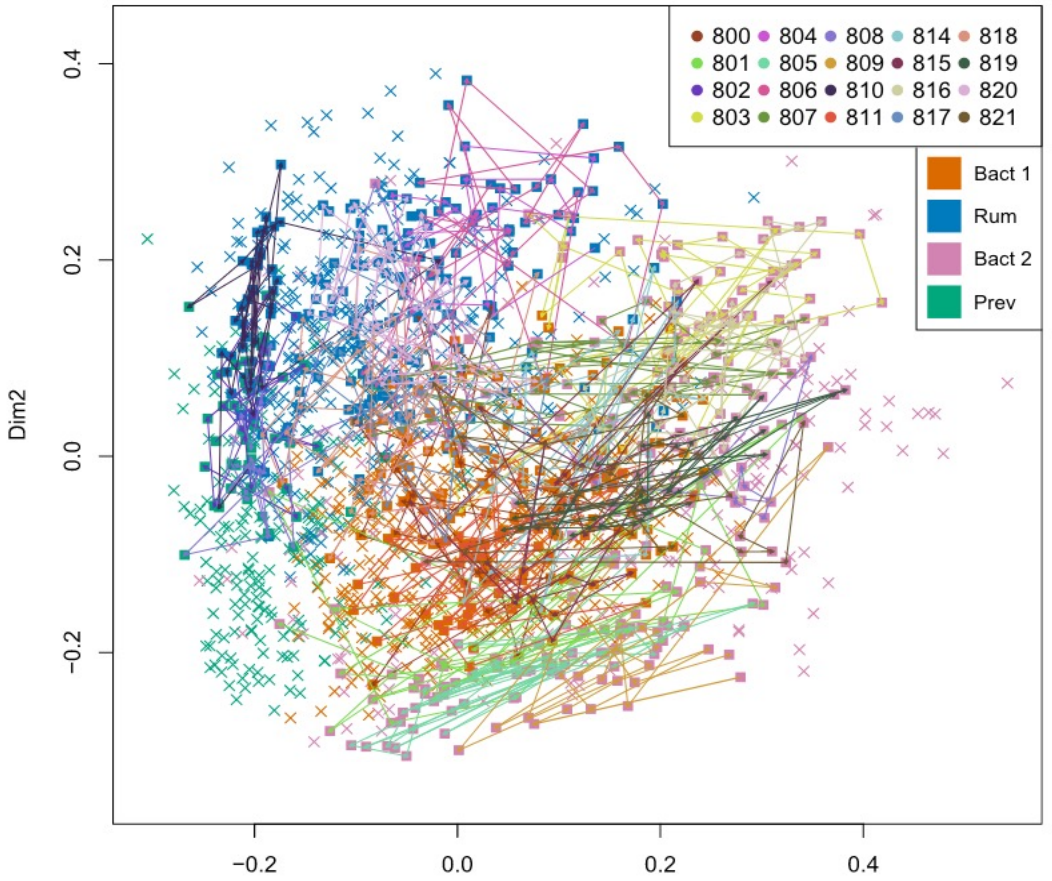
Need better understanding of the temporal variation of predictive microbial signatures

Vaginal microbiota



Gajer et al. Sci Transl Med 2012

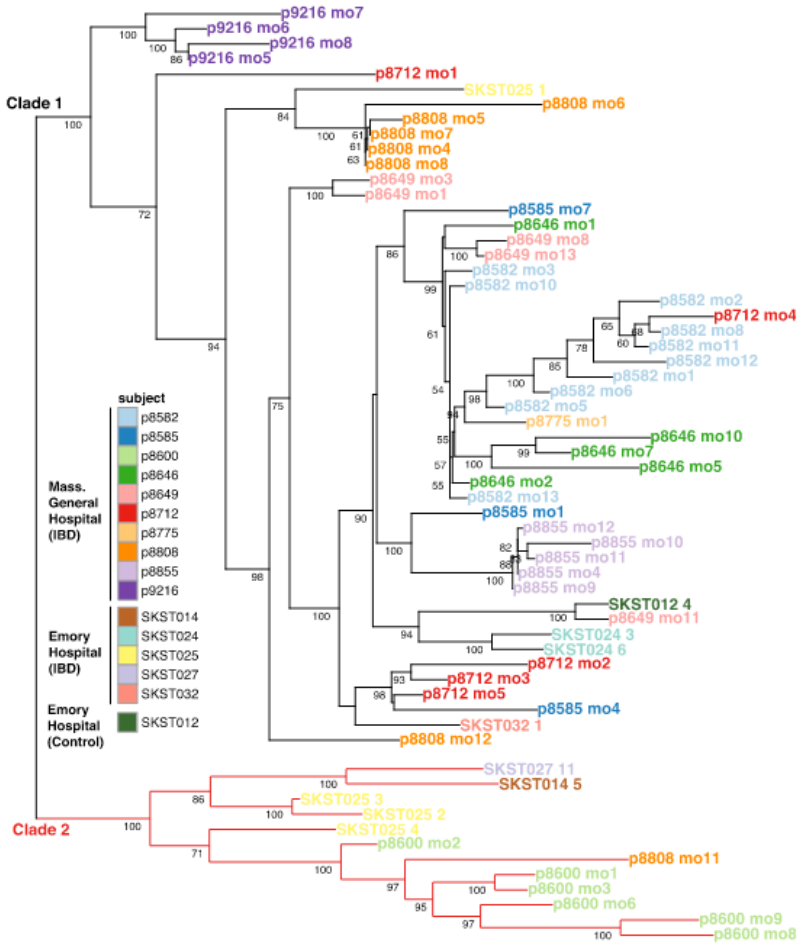
Fecal microbiota

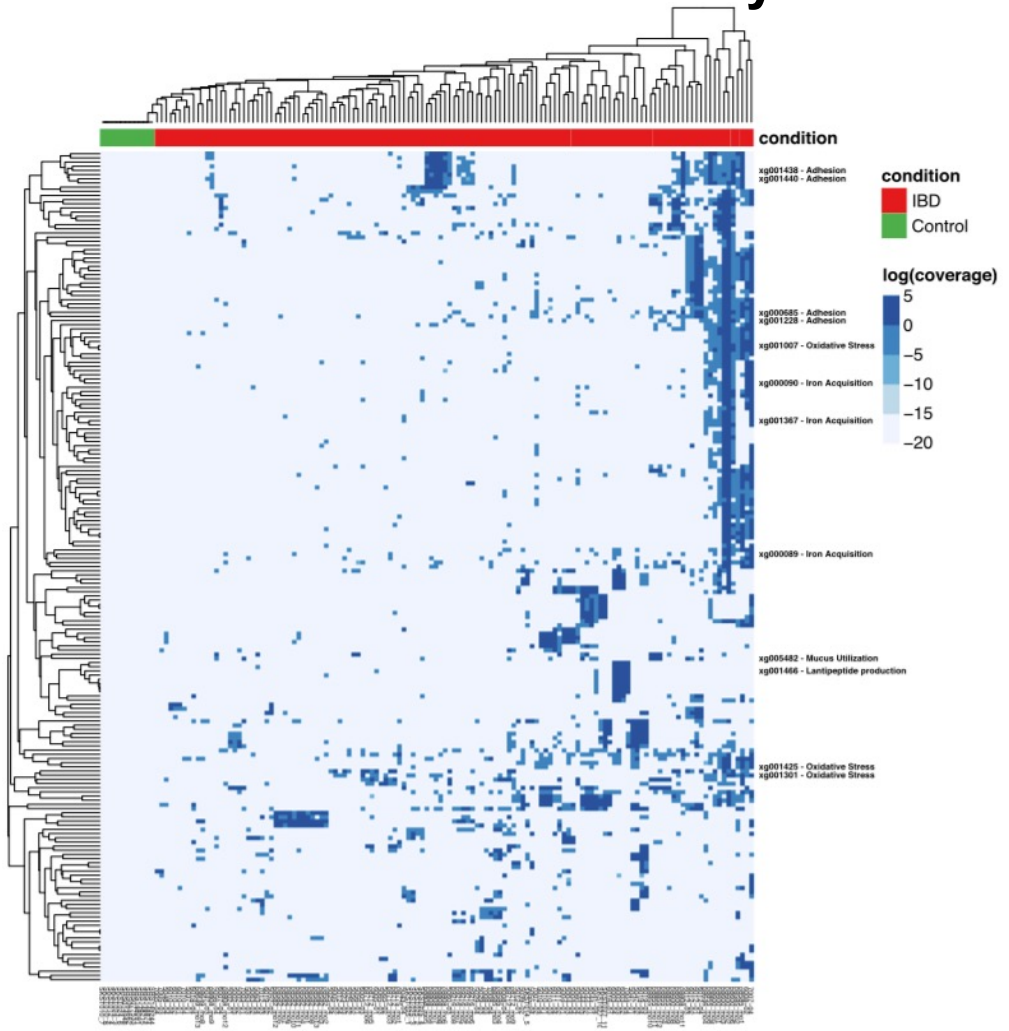


Vandeputte et al. Nat Comm 2022

Microbiome is complex at the sub-species level: shotgun metagenomics

Strain variation in *Ruminococcus gnavus* in individuals with IBD versus healthy controls





Hall et al. 2017 Genome Medicine

The Functional Hierarchy of the Microbiome

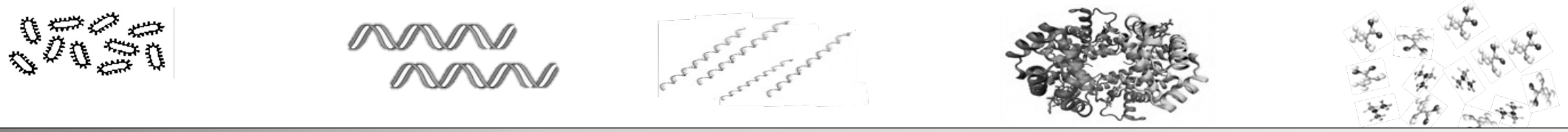
PHENOTYPING
Microbial DNA/RNA extraction
16S rRNA amplification and sequencing

METAGENOMICS
High throughput sequencing
PICRUSt: imputed functional analysis

METATRANSCRIPTOMICS
Gene regulation and expression

METAPROTEOMICS
Protein content, activity and post-translational modification

METABOLOMICS
Metabolite content and fluxes



Less functional information

More functional information

Serrano-Villar and Fererr. Curr Opin HIV AIDS 2017

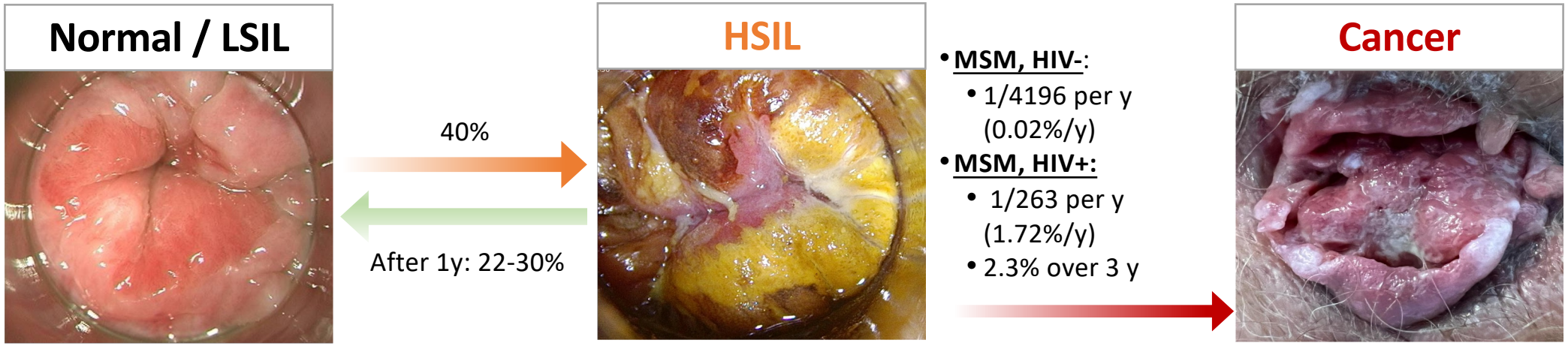
Outline

1 Potential for the microbiome as a therapeutic or diagnostic tool in PWH

2 Current challenges to using the microbiome as a diagnostic tool.

3 The case of anal HPV-disease in PWH.

Anal cancer is markedly increased in people with HIV



Tong W, *et al.* Progression to and spontaneous regression of high-grade anal squamous intraepithelial lesions in HIV-infected and uninfected men. AIDS 2013

Mathews WC, *et al.* Natural History of Anal Dysplasia in an HIV-Infected Clinical Care Cohort: Estimates Using Multi-State Markov Modeling. PLoS ONE. 2014. 9(8):e104116

Jongen VW et al. Anal Squamous Intraepithelial Lesions (SILs) in Human Immunodeficiency Virus-Positive Men Who Have Sex With Men: Incidence and Risk Factors of SIL and of Progression and Clearance of Low-Grade SILs. J Infect Dis. 2020;222(1):62.

Machalek DA, *et al.* Lancet Oncol. 2012;13(5):487.

Goldstone S, *et al*. Five-year cumulative incidence of invasive anal cancer among HIV-infected patients according to baseline anal cytology results: an inception cohort analysis. HIV Med 2015; 16:191–5.

Fazendin EA, *et al*. Condyloma acuminatum, anal intraepithelial neoplasia, and anal cancer in the setting of HIV: do we really understand the risk? Dis Colon Rectum 2017; 60:1078–82

Mette T. Faber *et a*l. Risk of Anal Cancer Following Benign Anal Disease and Anal Cancer Precursor Lesions: A Danish Nationwide Cohort Study, Cancer Epidemiol Biomarkers Prev 2020

Poynten *et al*. The Natural History of Anal High-grade Squamous Intraepithelial Lesions in Gay and Bisexual Men. Clin Infect Dis. 2021 Mar 1;72(5):853-861.

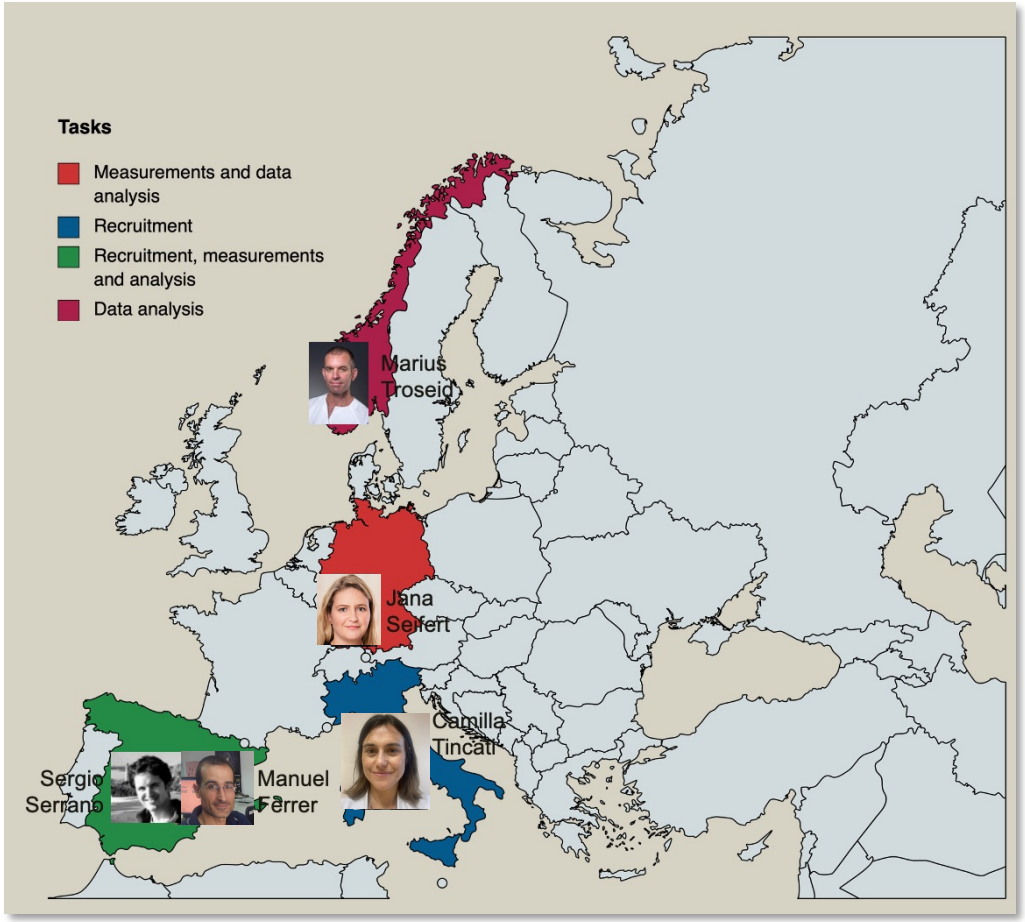
Microbiome-based SCReening of Anal Cancer in PWH

SCRATCH study

METHODS

- **Objectives:**
  - Identify anal bacterial biomarkers to improve the accuracy of anal cytology for HSIL diagnosis.
  - Potential bacterial biomarkers: bacteria, proteins and metabolites.
- **Study period: 2018-2022**
- **Prospective cohort study with discovery and validation cohorts.**
- **All experienced anoscopists and pathologists.**

https://www.transcanfp7.eu/index.php/abstract/scratch.html



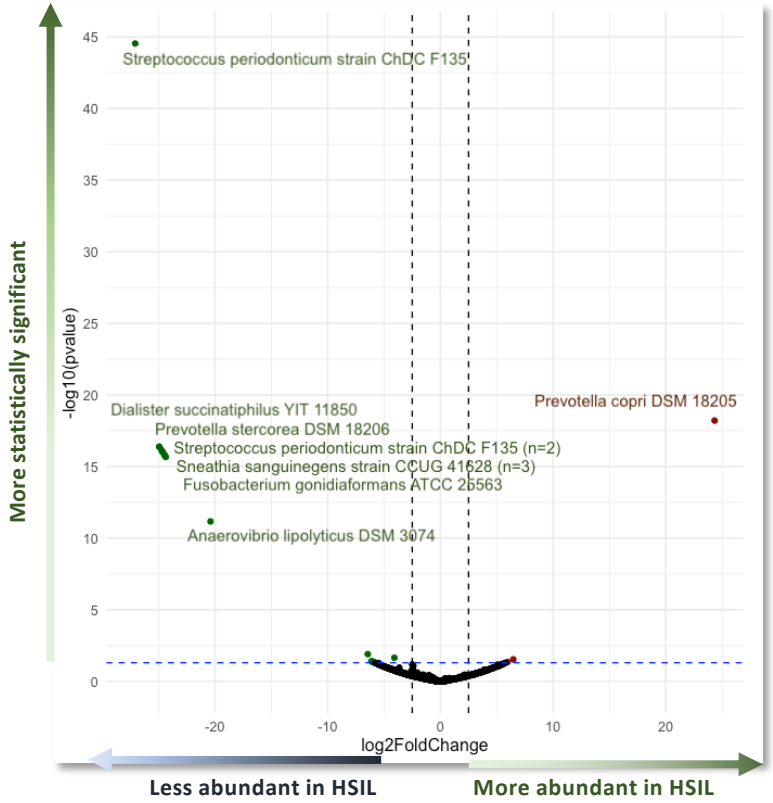
Microbiome-based SCReening of Anal Cancer in PWH

SCRATCH study

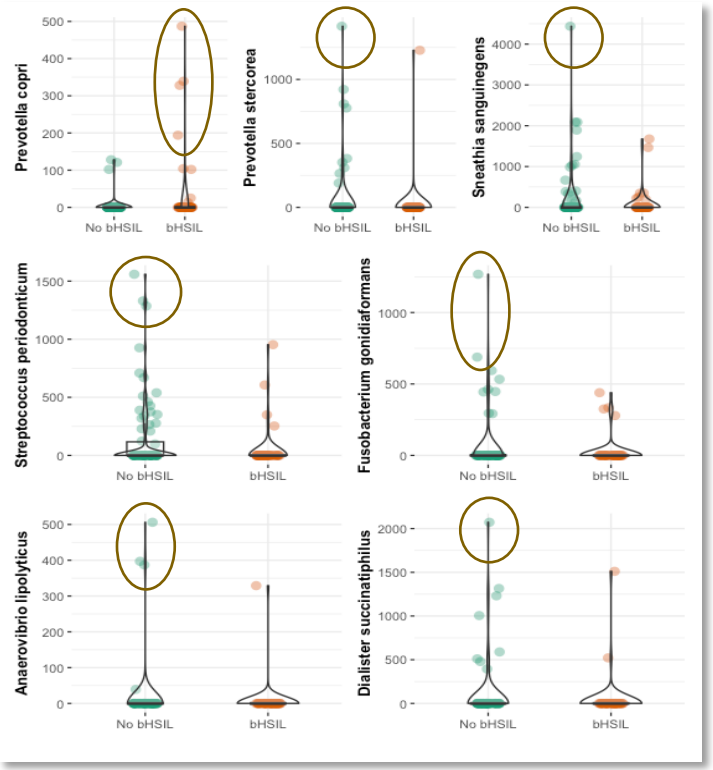
Anal microbiota composition did not consistently predict HSIL

Relevant differences in bacterial proteins and HSIL

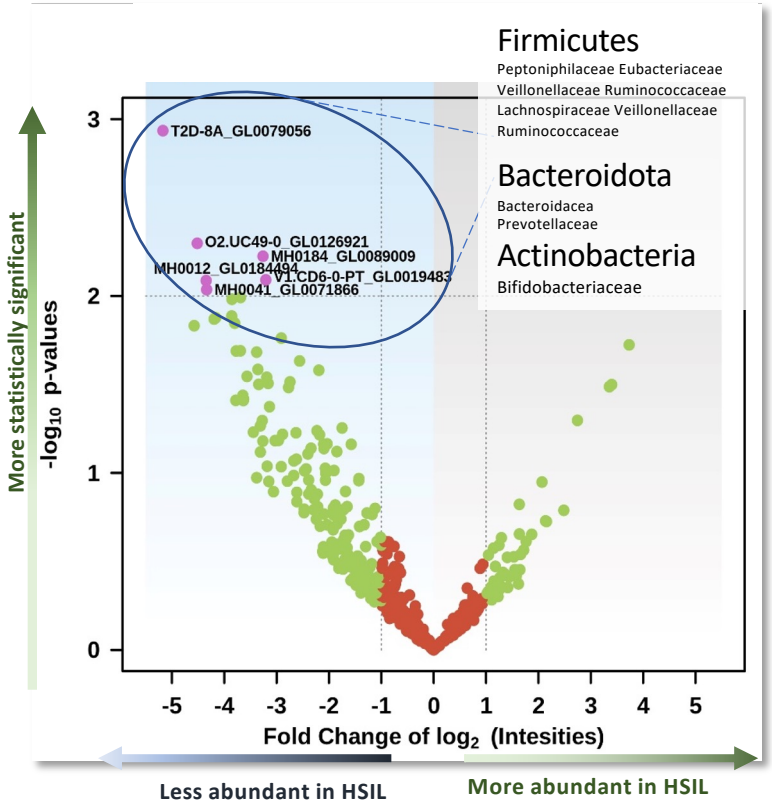
Differentially abundant bacteria



Significantly differential bacteria



Differentially expressed proteins



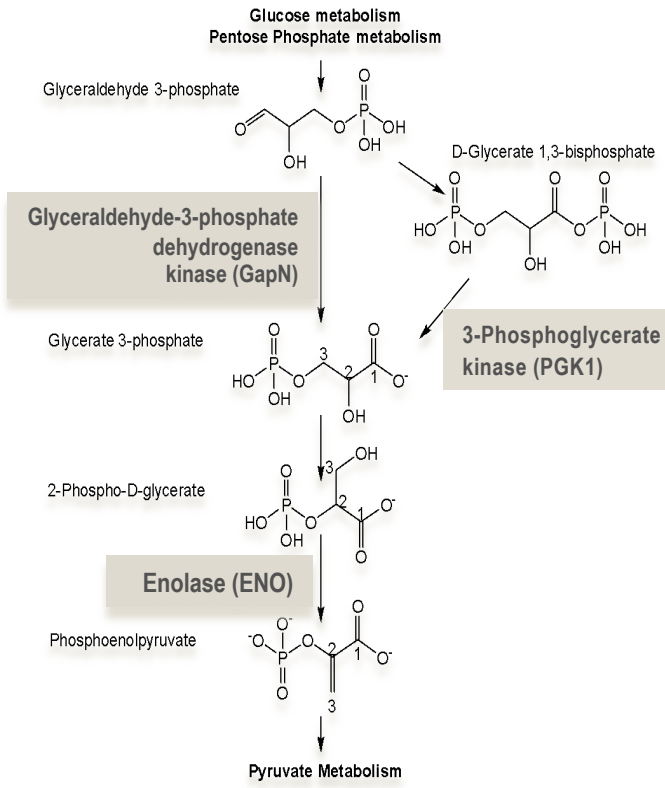
Serrano-Villar et al. Nature Medicine 2023 (coming soon)

Microbiome-based SCReening of Anal Cancer in PWH

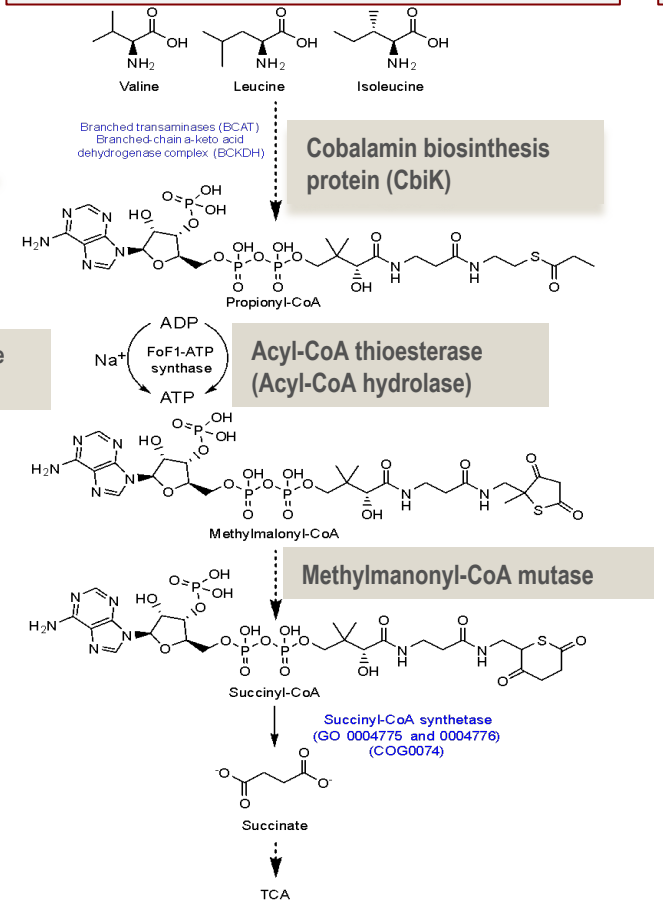
SCRATCH study

Proteins overexpressed in HSIL showed biological consistency and plausibility

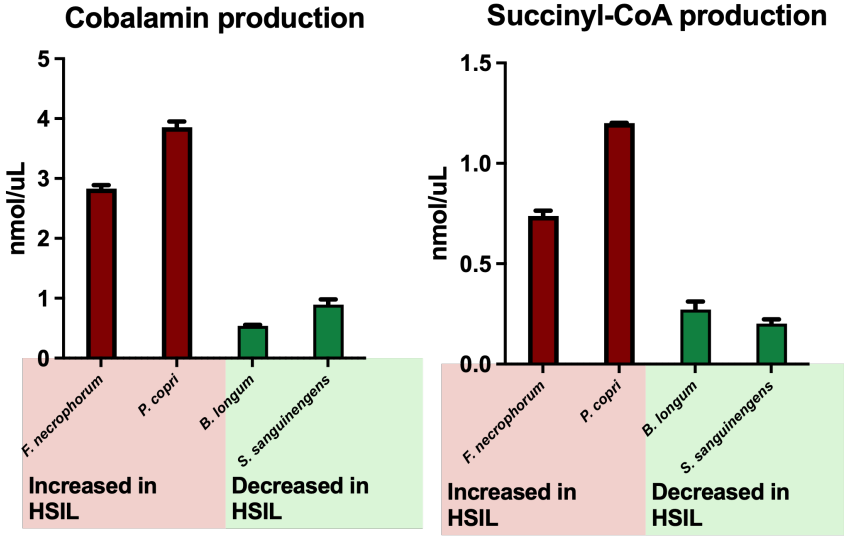
Pathway within the glycolysis and gluconeogenesis overexpressed in HSIL



Pathway within the cobalamin biosynthesis and connections with other proteins overexpressed in HSIL



Bacteria presumably implicated in progression to cancer produced greater concentrations of cobalamin and SucCoA than those presumably protective.



F. necrophorum: associated with cancer pathogenesis *(Shang, World J Gastrointest 2018)*

P. copri: *associated with HSIL in this study.*

B. longum: known anci-carcinogenic effects *(Fahmy, Nutr Cancer 2019)*

S. sanguinengens: decreased in HSIL in this and our previous study *(Ron, JID 2021)*

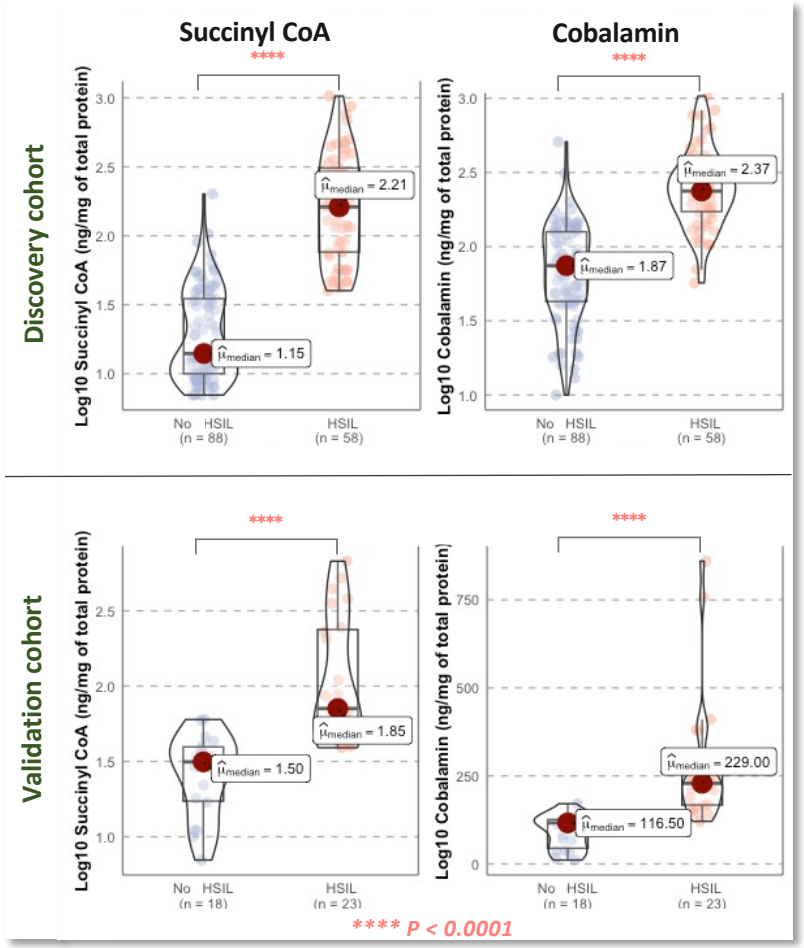
Serrano-Villar et al. Nature Medicine 2023 (coming soon)

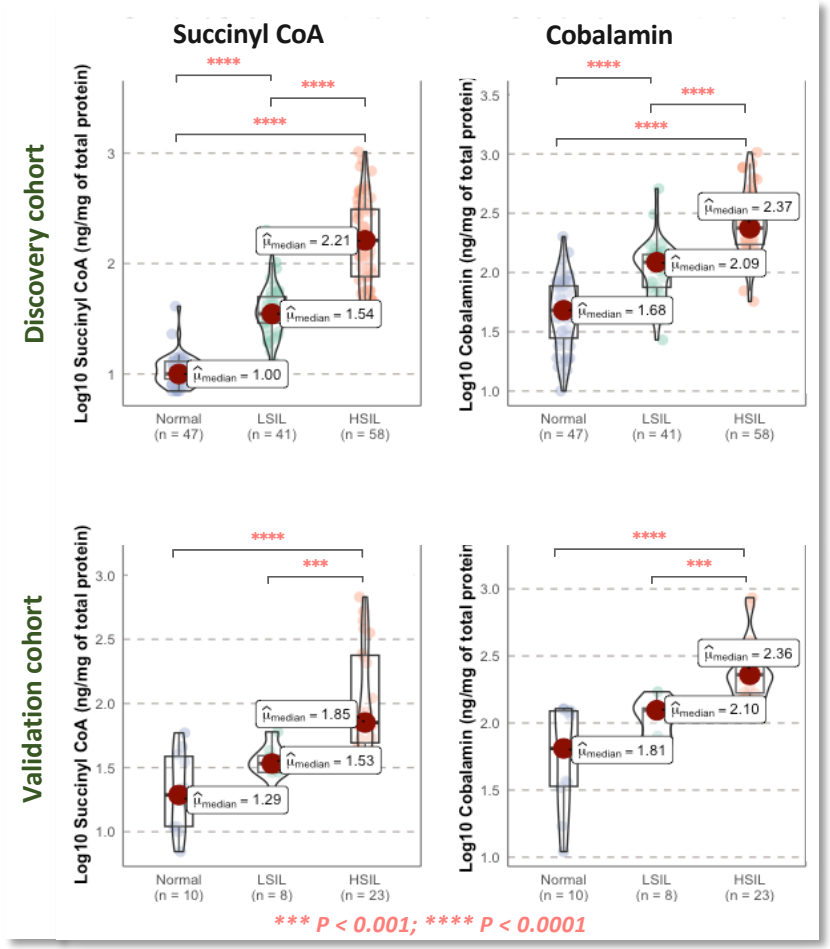
Microbiome-based SCReening of Anal Cancer in PWH

SCRATCH study

RESULTS: Targeted metabolite analysis directed by the proteomic findings

Succinyl-CoA and cobalamin are increased in anal cytologic samples from patients with HSIL





Microbiome-based SCReening of Anal Cancer in PWH

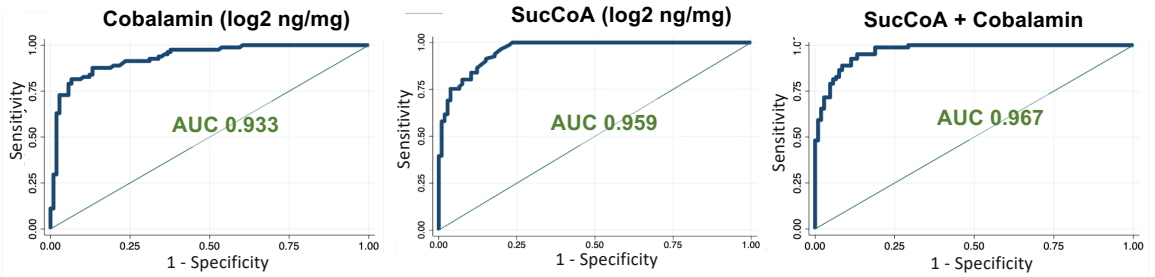
SCRATCH study

Diagnostic accuracy

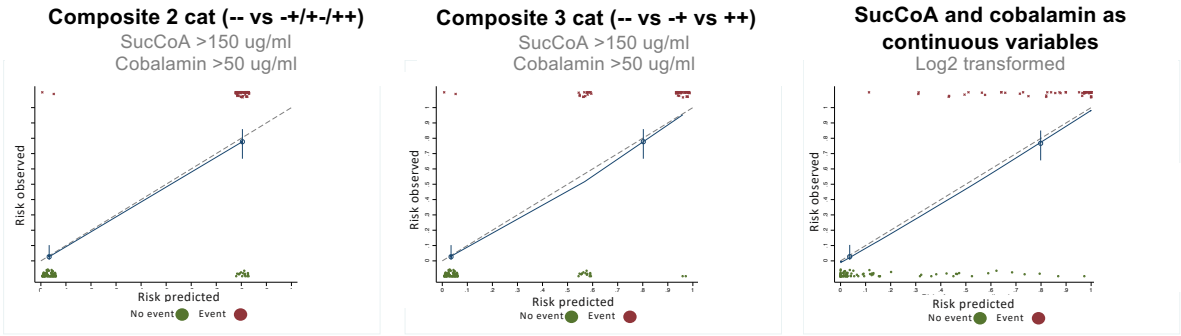
Measuring SucCoA and cobalamin overperforms the reference test (anal cytology)

	Abnormal cytology	SucCoA and Cobalamin (log2 continuous)	A: SucCoA >50 ng/mg	B: Cobalamine >150 ug/mg	A/B + vs. A&B -	A/B + vs. A&B -
Cohort	**Derivation**	**Derivation**	**Derivation**	**Derivation**	**Derivation**	**Validation**
Sensitivity	91.2%	87.9%	87.9%	79.3%	96.6%	95.7%
Specificity	34.1%	92.1%	87.5%	92.0%	81.8%	83.3%
Roc area	0.627	0.971	0.877	0.857	0.892	0.895
Positive predictive value	48.1%	87.9%	82.3%	86.8%	77.8%	88.0%
Negative predictive value	85.3%	92.1%	91.7%	87.1%	97.3%	93.8%
Accuracy (% correctly classified)	59.9%	90.1%	87.7%	87.0%	87.7%	90.2%

Model discrimination



Model calibration



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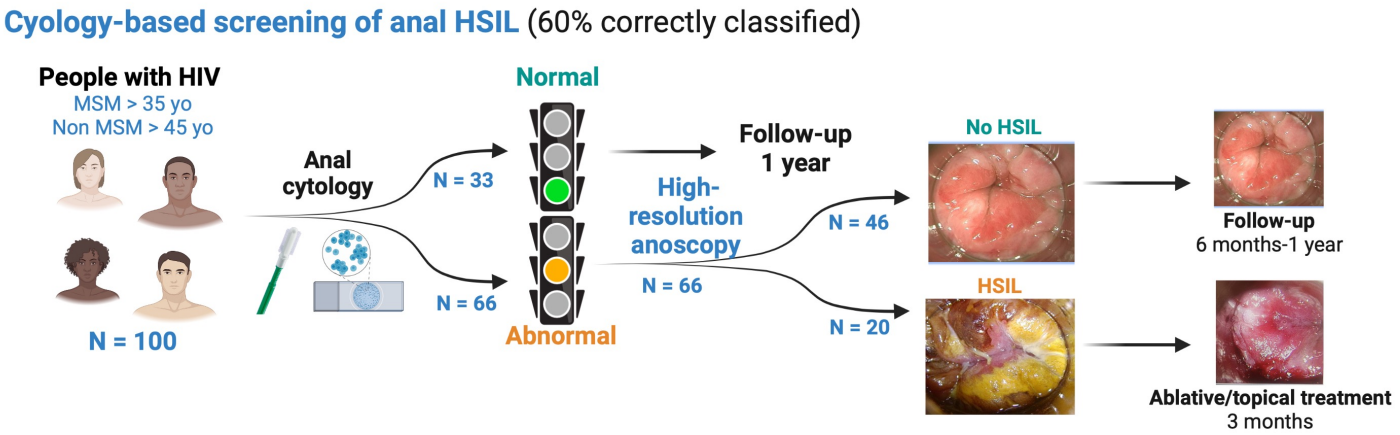
Microbiome-based SCReening of Anal Cancer in PWH

SCRATCH study

Succinyl CoA and cobalamin reclassified to true negatives >80% of cytologic false positive results.



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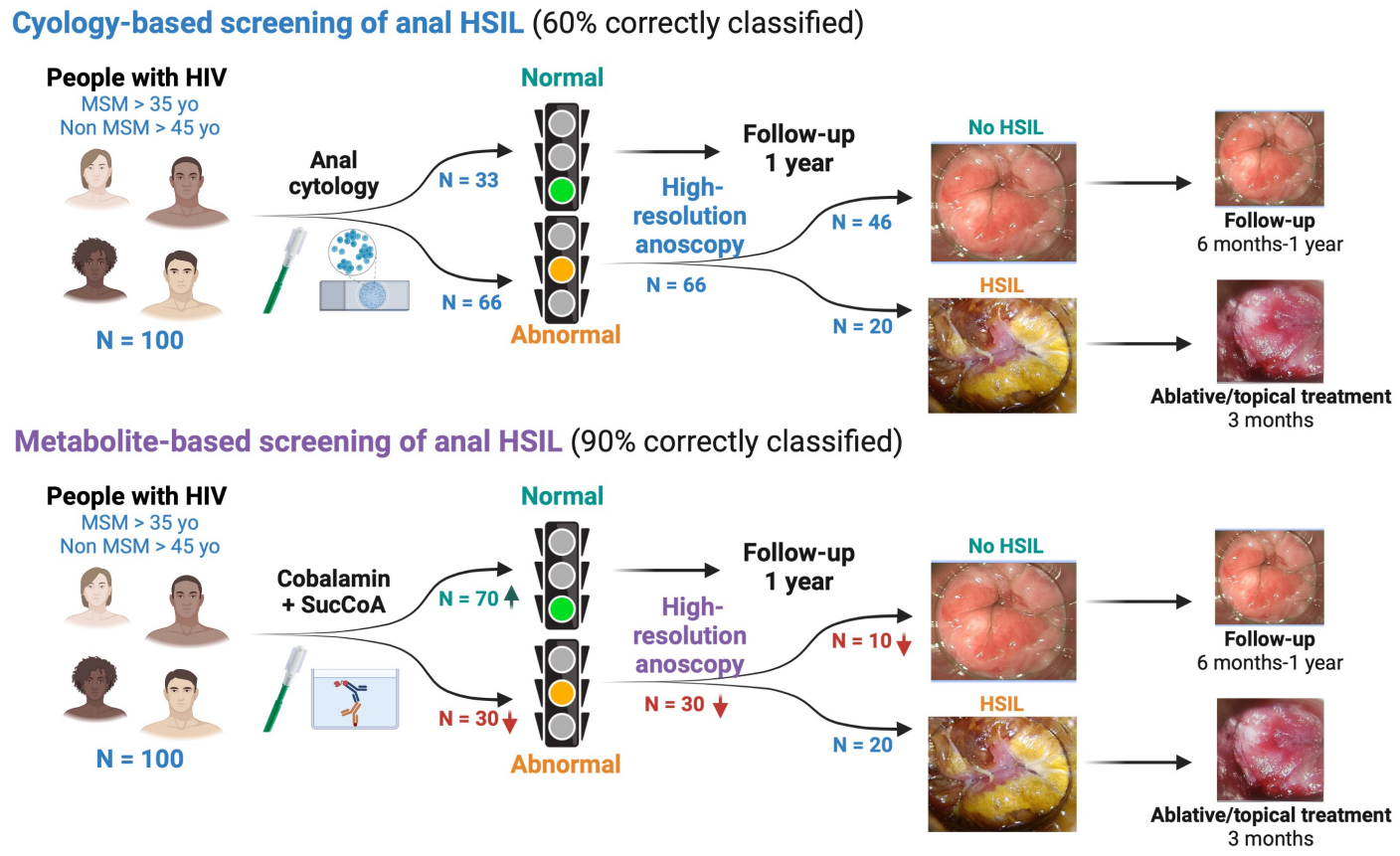


Serrano-Villar et al. Nature Medicine 2023 (coming soon)

Take-home messages

By Dall-E: "A scientist taking care of the microbiome. Digital art, pale colors"

1. Individual bacterial species are unlikely to become accurate diagnostic biomarkers.
2. Multi-omic studies can result into predictive signatures which are difficult to translate into the clinics...
3. ...but can inform hypothesis-driven approaches that allow discovering biomarkers with better properties to allow implementation.
4. Succynil-CoA and cobalamin could improve HSIL screening.
5. Emerging research opportunities (e.g., identify people at greater risk of HIV acquisition, HSIL progressing to cancer, prevent STIs, etc)

Thanks to all study participants

Hospital Ramón y Cajal and IRYCIS, Madrid

Infectious Diseases
- Santiago Moreno
- José A. Pérez-Molina
- Javier Martínez-Sanz
- Raquel Ron
- Clara Crespillo

Immunovirology Lab
- Elena Moreno
- Alba Talavera
- Claudio Diaz

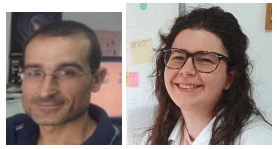
Microbiology
- Rosa del Campo

Pathology
- Amparo de Benito

Biostatistics
- Javier Zamora
- Alfonso Muriel

Proteomic and Metabolomic Platforms

Instituto de Catálisis, CSIC, Madrid
- Manuel Ferrer
- Laura Fernández



Bangor Universtity, United Kindom
- Rafael Bargiela

Hospital La Paz and IdiPAZ, Madrid

Dermatology
- Elena Sendagorta

Hospital Fund. Jiménez Diaz, Madrid

Infectious Diseases
- Alfonso Cabello

Oslo University
- Marius Troseid
- Sajan Rajy
- Johannes Roksund

Milano University
- Camilla Tincati

Stuttgart University
- Jana Seifert
- Johan S. Saez

Grants/Funding/Support
Instituto de Salud Carlos III
European Comission – ERANET
Asociación Española Contra el Cáncer

Questions? Ideas?

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