# *Bacterial Dysbiosis Persists in Women Living With HIV-1 After ART-Treatment*

**Katherine M. Bricker**[1], Andrea Lisco[1], Alessandra Borgognone[2], Maura Manion[1], Andrew Redd[1,4], Frances Galindo[1], Sarah Kilibbala[3], Aggrey Anok[3], Stephen Tomusange[3], Steven Reynods[1,4], Marc Noguera-Julian[2,5,6], Irini Sereti[1]
[1]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA, [2]IrsiCaixa AIDS Research Institute; Hospital Universitaria Germans Trias I Pujol, Barcelona, Catalonia, Spain; [3]Rakai Health Sciences Program, Kalisizo, Uganda; [4]Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD, USA; [5]CIBERINFEC, Madrid, Spain; [6]University of Vic-Central University of Catalonia (Uvic-UCC), Vic, Catalonia, Spain

## BACKGROUND

The cervicovaginal (CV) microbiome has a critical role in women's health and disease, including HIV acquisition and progression. A healthy cervicovaginal (CV) microbiome is dominated by a single Lactobacillus species that helps maintain a low vaginal pH, prevent outgrowth of harmful bacteria, and regulate immune homeostasis. Bacterial vaginosis (BV) occurs when Lactobacillus is depleted and replaced by an outgrowth of facultative or obligate anaerobes. Women living with HIV (WLWH) have high prevalence of BV-associated bacteria that are linked to vaginal inflammation, higher genital HIV viral loads, and greater HIV shedding. However, the impact of antiretroviral treatment (ART) on the CV microbiome has not been described in depth. **In this study, we sought to evaluate the CV microbiome of ARV-naïve WLWH over their first year of ART.**

## METHODS

| **Characteristic** | |
|---|---|
| Age, *mean (range)* | 31 (19 – 59) |
| Sex, % (n) | |
| *Female* | 100% (83) |
| Race or Ethnicity, % (n) | |
| *Black* | 100% (83) |
| *Hispanic/Latinx* | 2.4% (2) |

- Cervico-vaginal pellets were collected from 83 ART-naïve cisgender Ugandan women living with HIV-1 longitudinally at week 0, 8, 24, and 52 following ART initiation.
- DNA was extracted from CV pellets and V3-V4 region of bacterial 16s rRNA was amplified by PCR.
- Extracts were sequenced through Illumina NovaSeq platform and paired-reads were analyzed using DADA2 pipeline in R.
- Reads were binned into ASVs and taxonomically annotated with the SILVA taxonomic framework.
- Results were analyzed through Phyloseq and DeSeq2 packages in R.

ART-treatment does not significantly alter the CV microbiome in ART-naïve women living with HIV-1:

*-Suppressive ART does not significantly impact Shannon Diversity of CV microbiome.*

*-However, specific BV-associated bacteria are significantly less expressed at week 52 compared to week 0.*

## RESULTS

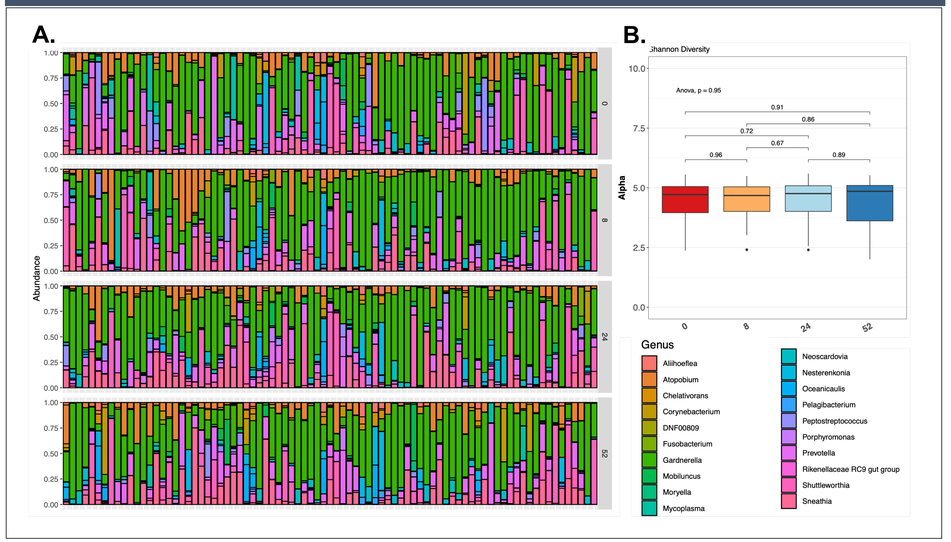



**Figure 1.** CV relative abundance of top genera and alpha diversity in ART-naïve women over their first year of ART treatment.

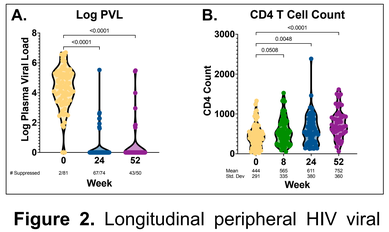



**Figure 2.** Longitudinal peripheral HIV viral loads and CD4+ T cell counts.

| ***Genus*** | **log2 FC** | **p-value** |
|---|---|---|
| *Peptostreptococcus* | - 4.01 | $1.63 \times 10^{-10}$ |
| *Howardella* | - 3.55 | $4.20 \times 10^{-6}$ |
| *Mycoplasma* | - 3.51 | $1.06 \times 10^{-5}$ |
| *Fusiobacterium* | - 3.35 | $1.56 \times 10^{-2}$ |
| *Streptococcus* | - 2.65 | $1.14 \times 10^{-2}$ |
| *Porphyromonas* | - 2.49 | $3.62 \times 10^{-3}$ |
| *Prevotella* | - 1.09 | $1.19 \times 10^{-2}$ |

**Table 2. L**og2 FC value and p-value of significantly altered genera.

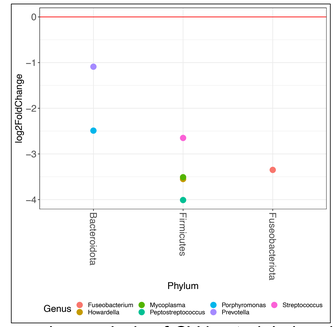



**Figure 3.** Differential expression analysis of CV bacterial abundance following 52 weeks of suppressive ART.

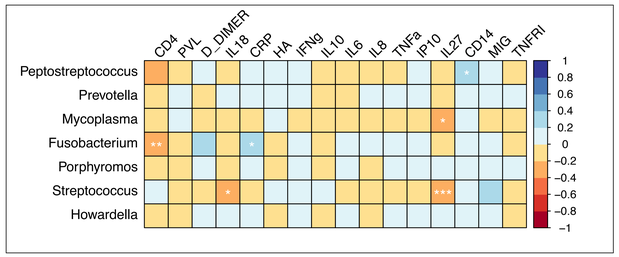



**Figure 4.** Correlation of CV bacterial abundance and clinical characteristics following one year of suppressive ART.

## CONCLUSIONS

- ART resulted in undetectable viremia in 90.5% and 86% of participants at week 24 and 52, respectively, and a median CD4 T+ cell count of 687 cells/µl at week 52.
- Gardnerella was identified as the most abundant family in our dataset at all timepoints, with a median relative abundance of 35, 46, 35, and 35% at weeks 0, 8, 24, and 52, respectively.
- Seven genera were identified as significantly less abundant at week 52 compared to baseline.
- Our findings indicate that ART alone does not effectively modulate compositional diversity of the CV microbiome. These data support exploration of targeted therapeutics to restore the CV microbiome.

## ACKNOWLEDGEMENTS

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Contact: Katherine.Bricker@nih.gov