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BACKGROUND

During use of an ethinyl estradiol (EE)/ etonogestrel (ENG) intravaginal ring (IVR) over 3 weeks

• Efavirenz-based ART (EFV) significantly decreased EE levels by 53% and ENG levels by 76%

• Atazanavir/ritonavir-based ART (ATV/r) decreased EE levels by 29%, but increased ENG levels by 71%¹

- We explored the role of the IVR on
- vaginal microbial communities and

vaginal short chain fatty acids (SCFA)

- We explored the role of vaginal microbes/SCFA
- on EE/ENG concentrations

METHODS

Of the 74 PK-evaluable participants

• 25 ART Naïve, 25 EFV, 24 ATV

64 were vaginal environment evaluable

• 19 ART Naïve, 21 EFV, 24 ATV/r

IVR was placed at Entry (week 0) and removed at week 3 • 16S rRNA sequencing of the V4 region was performed. Sequences were processed using DADA2, species assigned using SPINGO with SILVA database, and Lactobacillus classified using BLAST.

- Vaginal aspirate SCFA were measured by Metabolon[®]
- Negative binomial and linear regression models identified differentially abundant microbiome and SCFA features
- Spearman correlation assessed relationships between microbiome relative abundance and weekly EE/ENG concentrations



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A COMBINED ESTROGEN/PROGESTIN VAGINAL RING IMPROVES MICROBIAL COMMUNITIES

RESULTS ART Naive Efavirenz based ART <u>.... AAA a</u> 40000 20000 2e+06 · 4e+05 2e+05 · 1000

Figure 1: Heatmap of vaginal microbiome Community State Type (CST), Country, Visit week, Hormone concentrations, Progesterone Levels, Bacterial species and Short chain fatty acid levels for 64 subjects by treatment arm and participant



Figure 2: Community State Type (CST) transitions during the course of the study. Start of IVR was associated with transitions to CST I-III. IVR removal was associated with a shift to CST IV

	Atazanavir based ART		
		CST	N
		Country Visit	٩g
		EE E	3N = c
<u> </u>	<u>, an al. 1111 an 1111 an 1111 an 1111 an 110 an 110 an 111 1111 an 110 an 110 an 111 an 110 an</u>		EN
		ENG F	7 7
		Progesterone	<n CD</n
		Lactobacillus iners Lactobacillus crispatus Gardnerella vaginalis Lactobacillus gasseri johnsonii	CE
2 X X		Atopobium vaginae Leptotrichiaceae sp. Megasphaera massiliensis Lactobacillus iensenii)a
		Mycoplasma girerdii Streptococcus sp. Sneathia sanguinegens Prevotella amnii	
		Mycoplasma hominis Shuttleworthia satelles Lactobacillus delbrueckii Prevotella bivia	
		Other Finegoldia magna Anaerococcus sp. Peptoniphilus sp.	ance
		Gemella asaccharolytica Aerococcus christensenii Saccharofermentans acetigenes Prevotella timonensis	pund
		Lactobacillus jensenii crispatus Fusobacterium sp. Prevotella buccalis Peptoniphilus lacrimalis	ive A
		Lactobacillus gasseri Ureaplasma sp. Prevotella ihumii Prevotella melaninogenica	Relat
		Megaira polyxenophila Fusobacterium nucleatum Prevotella colorans Parvimonas micra	
		Corynebacterium sp. Porphyromonas uenonis Ercella succinigenes Dialister sp. Dialister sp.	
		Staphylococcus sp. Lactobacillus reuteri Sutterella sp. Anaerococcus lactolyticus	
		Sneathia sp. Falsiporphyromonas endometrii Parvibacter caecicola Peptostreptococcus anaerobius	
		Prevotella histicola Prevotella disiens	
		2–Methylbutryic Acid	
		Acetic Acid	
<u>, , , , , , , , , , , , , , , , , , , </u>		Butyric Acid)
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	<u></u>		
		Isobutyric acid	
		Isovaleric acid	
		Lactic acid	•
		Propionic acid	
		Valeric acid	
ST) Cour	ntry Visit week Hormone concentrations		•

Lactobacillus crispatus Lactobacillus gasseri Lactobacillus iners Mixed anaerobic community 01059

CLINICAL CHARACTERISTICS

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EFV-based ART

ge (years) VII (kg/m2) (pg/mL) NG (pg/mL asma HIV VA (log10) D4 count ells per ul)

ART-Naïve 19 31 [27, 34] 27.1 [23.7, 33.2] 19.6 [14.7, 28.3] 1900 [1390, 2420]

3.42 [2.87, 4.20]

623 [477, 909] ata shown as median, [IQR]

EGEND



36[28, 45] 37.0 [32, 42.5] < 0.001 25.4 [24.1, 32.3] 26.6 [23.5, 29.3] 0.825 16.0 [10.9, 19.8] < 0.001 10.4 [6.4, 13.1] 3405 [2748, 4023] 430 [278*,* 534] < 0.001 undetectable < 0.001 undetectable 0.109 752 [603, 866] 671 [494, 933] accharofermentans acetigenes Prevotella disiens Peptoniphilus sp. Ercella succinigenes Anaerococcus sp. negoldia magna

ATV/r-based ART

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Figure 3: Associations between bacterial species and plasma hormonal concentrations (blue line = negative correlation, red line = positive correlation)

UMMARY

Use of the IVR was associated with an increased probability of transitioning to Lactobacillus-dominant (CST I-III) community types (p=0.09), suggesting a potential therapeutic role

Removal of the IVR was associated with

- a shift from CST I-III to CST IV (p=0.13)
- a decrease in lactic acid levels (p<0.003)
- no differences in species-level abundances
- Vaginal shedding of HIV-1 RNA/DNA was assessed at weeks 0 and 3 and will be presented in Themed Discussion 15 • Lactic acid levels are highly correlated with *Lactobacillus species* (p<0.001)
- Levels of all other SCFA are highly correlated with non-Lactobacillus mixed anaerobes (p<0.001)
- Specific microbes are associated with lower levels of EE and ENG (see Figure 3)
- For women on ATV-based regimens, higher levels of Saccharofermentans acetigenes and Sneathia sanguinegens are associated with lower levels of EE
- Further investigation is needed to fully assess the interactions and safety of vaginal hormonal contraception in women living with HIV