Randomized Control Trial of *Lactobacillus crispatus* CTV-05 (LACTIN-V) to Lower HIV Risk in Young South African Women

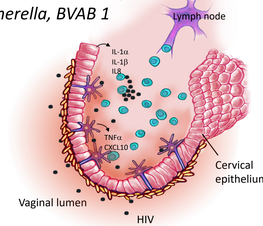
1138

Anke Hemmerling[*1], Caroline Mitchell[*2], **Suuba M. Demby**[*3], Musie Gebremichael[3], Joseph Elsherbini[3], Jiawu Xu[3], Nondumiso Xulu[3], Johnathan Shih[3], Krista Dong[2,4], Vaneshree Govender[5], Vanessa Pillay[4], Thomas P. Parks[7], Thumbi Ndung'u[6], Douglas S. Kwon[+2,3], Craig R. Cohen[+1]; [1]University of California San Francisco, San Francisco, CA, USA; [2]Massachusetts General Hospital, Boston, MA, USA; [3]Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA, USA; [4]FRESH Clinic, Umlazi, KZN, ZA; [5]Aurum Institute NPC, Johannesburg, ZA; [6]University of KwaZulu Natal, Durban, KZN, ZA; [7]Osel Inc., Mountain View, CA, USA. *Equally contributed, +Co-corresponding

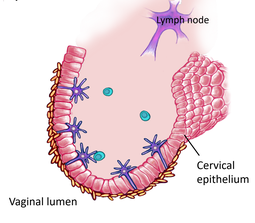
1 BACKGROUND

Genital inflammation associated with dysbiosis of vaginal bacteria is linked to adverse health outcomes in women, including HIV acquisition, HPV persistence, and cervical dysplasia. Promoting dominance by *Lactobacillus crispatus* is a potential strategy to reduce those risks. KwaZulu Natal in South Africa has an HIV prevalence above 40% in women aged 18-24 years. The FRESH LACTIN V Phase 2 clinical trial is the first trial of a *L. crispatus* live biotherapeutic product (LBP) in South African women.

Cervicotype 4: Diverse anaerobes: *Prevotella, Gardnerella, BVAB 1*



Cervicotype 1: *Lactobacillus crispatus*



2 TRIAL DESIGN

Visit 1: Enrollment

Women enrolled in the Females Rising Through Education, Support and Health (FRESH) study in Umlazi, KwaZulu Natal, ZA with a ***Lactobacillus* deficient microbiome**, (Nugent score between 4-10), were co-enrolled in the study.
(n = 57)

Visit 2: Bacterial Vaginosis (BV) Treatment (Pre-MTZ)

Eligible participants administered 7-Day BV treatment:
Oral metronidazole (MTZ) (500mg) twice daily
(n = 57)

Visit 3: Randomization and Mucosal Sampling (Post-MTZ)

Participants randomized **2:1** to LACTIN-V ($2x10^9$ CFU/dose) and placebo treatment arm.
(n = 45)

LACTIN-V

Week 1: Once daily for 5 days
Week 2: Once daily for 2 days
Week 3: Once daily for 2 days
Week 4: Once daily for 2 days

PLACEBO

Week 1: Once daily for 5 days
Week 2: Once daily for 2 days
Week 3: Once daily for 2 days
Week 4: Once daily for 2 days

Cessation of administration of both LACTIN-V and PLACEBO (Week 4)

Mucosal sampling after treatment cessation

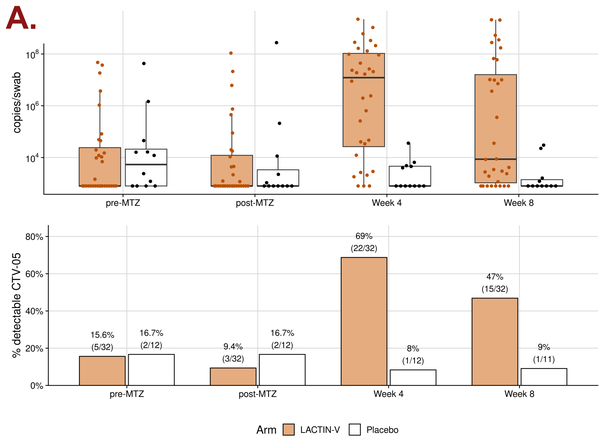
Mucosal Sampling (Week 8)

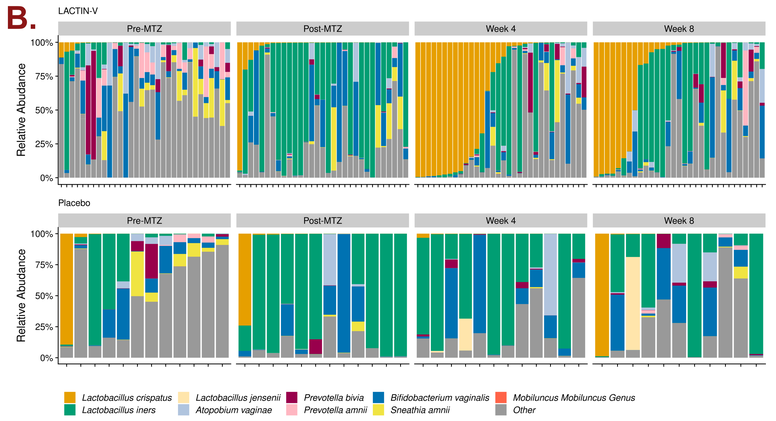
Mucosal sampling 4 weeks after treatment cessation

**Mucosal sampling included cervicovaginal lavage (CVL) with sterile saline, cervicovaginal swabs, and a cytobrush*

Participants administered a *L. crispatus* vaginal probiotic were 5.2x more likely to transition to an optimal microbiome 4 weeks after treatment compared to placebo in a cohort of South African women (*p = 0.009*).

3 RESULT I: MICROBIOME SIGNIFICANTLY IMPACTED BY LACTIN-V

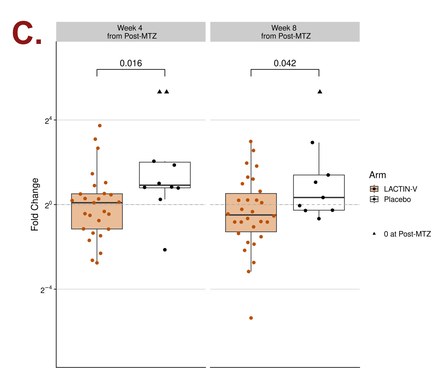


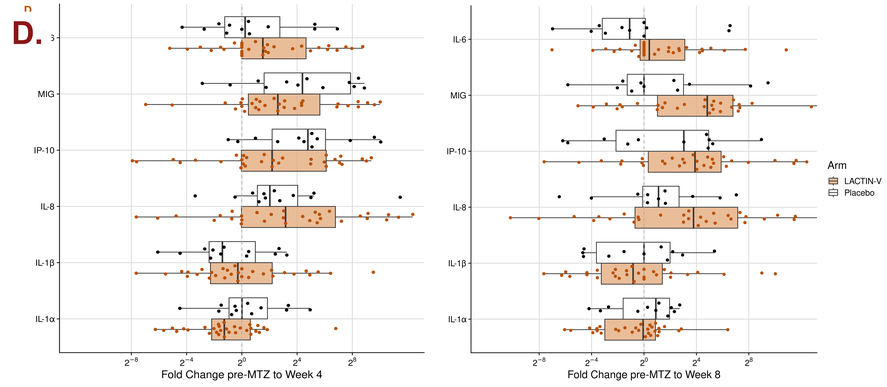


A: By qPCR, the median quantity of *L. crispatus* was significantly higher at Week 4 in the LACTIN-V arm vs. placebo (*p < 0.001)*. A higher proportion of the LACTIN-V arm vs. placebo had the *L. crispatus* CTV-05 strain at Week 4 (*p <0.001)* and Week 8 (*p = 0.033)*.

B: Diverse vaginal microbiota (marked by <50% *Lactobacillus* relative abundance by 16S rRNA sequencing) was common at baseline (90.7%). A non-*L crispatus, Lactobacillus* dominant community was common post-MTZ treatment (58%). After treatment with LACTIN-V, 40.6% of participants transitioned to *L. crispatus* dominance at Week 4, and 25.8% through Week 8.

4 RESULTS II: IMMUNOLOGICAL CHANGES THROUGHOUT TRIAL





C: The median change in the proportion of endocervical activated HIV target cells (CD4+, HLA-DR+, CD38+) out of total T cells (CD3+) increased post-MTZ to Week 4 in the placebo arm but not the LACTIN-V arm: 0.916 (IQR 0.791, 2.004) vs. 0.086 (IQR -1.155, 0.51) median log2 fold change; *p = 0.02.*

D: Change in 13 cytokines and chemokines (6 shown) between pre-MTZ and Week 4 by multiplex ELISA was not statistically different across treatment arms in cervicovaginal lavage fluid.

5 RESULTS III: SAFETY AND ACCEPTABILITY

Adverse events (AEs), all mild, occurred in 77.8% of all participants. Local solicited AEs, most commonly vaginal discharge, were grade 1 with no significant difference by arm. 75% of participants in both arms "strongly agreed" or "agreed" they would use the product again.

6 CONCLUSION

FRESH LACTIN-V was the first study of a vaginal live biotherapeutic in Africa.

In a region with low prevalence of *L. crispatus* dominant vaginal microbial communities, LACTIN-V was able to colonize in 69% of South African participants and establish dominance that persisted after cessation of treatment in 40.6% of participants. Treatment was also associated with a lack of rebound in the proportion of HIV target cells in the endocervix compared to placebo.

This study highlights the importance of the continued study of the vaginal microbiome to improve gynecological health and reduce HIV risk in women.

7 ACKNOWLEDGEMENTS/FUNDING

Special thanks to the participants and staff at the FRESH clinic and the researchers of the HIV Pathogenesis Programme at the University of KwaZulu Natal, South Africa.

Grant #: NICHD 1R01HD098978