Safety of Estrogen Ring and/or Probiotics for Improving Vaginal Health in African/Caribbean/Black Women:

Results from a Prospective, Randomized, Open-label, Intervention Phase I Trial (CTN 308)

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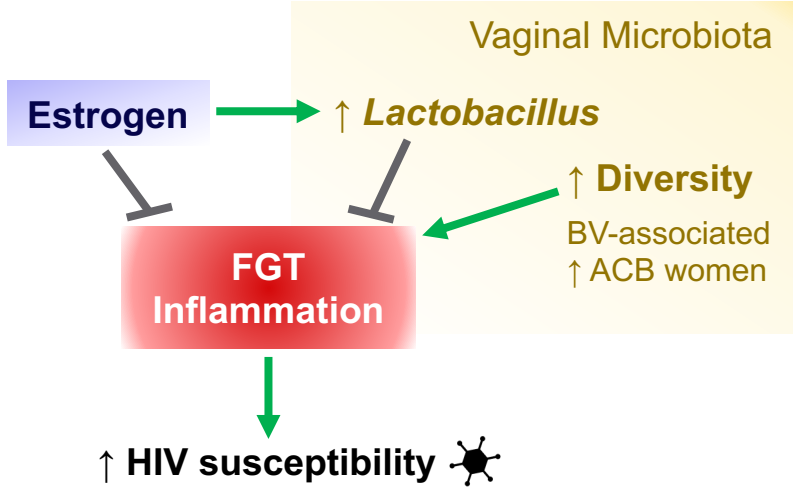
Estrogen and a *Lactobacillus*-dominant vaginal microbiota can promote vaginal health & reduce susceptibility to HIV.

Compared to men, women are disproportionately affected by human immunodeficiency virus (HIV), with young women at twice the risk of acquiring HIV[1]. Although there are various socioeconomic factors contributing to women's augmented risk, there are numerous biological factors, as well. Most women acquire HIV in the lower female genital tract (FGT) after a sexual encounter with an infected partner, making the vaginal barrier the first line of defence against HIV infection[2]. Accordingly, the function of the vaginal barrier and factors that affect its integrity are critical for mediating susceptibility to infection.

Female sex hormones can alter the immune milieu of the lower female genital tract (FGT), which in turn, affects the level of inflammation and integrity of the vaginal barrier[2]. Estradiol, specifically, is associated with both lower inflammation and susceptibility to infection[3]. Moreover, estrogen can enhance colonization of *Lactobacillus* in the FGT, bacteria which has been associated with fortification of the vaginal barrier and reduced susceptibility to infection[2,4]. A highly diverse, non-*Lactobacillus* dominant vaginal microbiota and elevated inflammation are features of bacteria vaginosis, a clinical condition associated with a higher susceptibility to HIV[2]. BV is more prevalent in African/Caribbean/Black (ACB) women, and it is estimated that ~40% of ACB women harbour a polymicrobial vaginal microbiota, putting them at higher risk of HIV infection.

To promote vaginal health and resilience towards HIV infection by targeting these hormonal and microbiota factors, we propose an innovate approach: local administration of low dose estradiol using an intravaginal ring alone or in combination with a probiotic containing *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuterii* RC-14. To determine if these interventions are safe in pre-menopausal ACB women, we conducted a prospective, randomized, open-label phase I intervention trial (CTN 308; clinicaltrials.gov NCT03837015).



References

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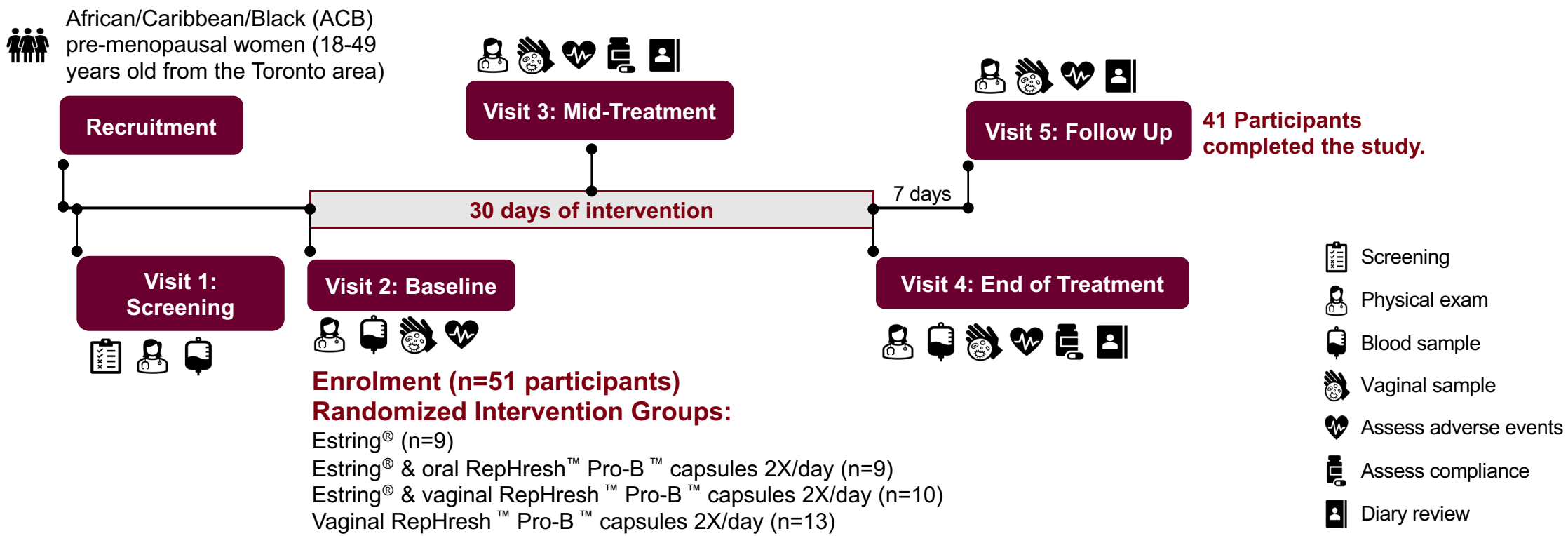
Image created by Dr. Christina Hayes

CTN 308: a Prospective, Randomized, Open-Label, Intervention Phase I Trial

Interventions: **Estring®:** intravaginal low dose estradiol ring (7.5µg/day)
RepHresh™ Pro-B™: 1x10^7 cfu of *Lactobacillus rhamnosus* GR-1 & 1x10^7 cfu *Lactobacillus reuteri* RC-14 probiotics per capsule

Study Design:



Enrolment (n=51 participants)
Randomized Intervention Groups:
Estring® (n=9)
Estring® & oral RepHresh™ Pro-B ™ capsules 2X/day (n=9)
Estring® & vaginal RepHresh ™ Pro-B ™ capsules 2X/day (n=10)
Vaginal RepHresh ™ Pro-B ™ capsules 2X/day (n=13)

Intervention Safety Assessments:

- Review adverse events (AEs) at visits 3, 4, & 5
- Physical examinations at visits 3, 4 & 5
- Bloodwork at visits 1 & 4 included comprehensive metabolic and lipid panels, as well as a complete blood count panel

Most adverse events (AEs) were mild, short-term, and non-recurring.

Table 1. Intensity, duration, and frequency of reported AEs.

	# Times AE Reported	AE Intensity				Ongoing at End of Study	Resolved	Total # Participants Reported AE	# Participants Reported AE		
		Mild	Moderate	Severe	Other				Once	Twice	Three Times
All AEs	**92**	**66 (72%)**	**18 (20%)**	**7 (8%)**	**1 (1%)**	**6 (7%)**	**86 (93%)**	**29 (57%)**	**N/A**	**N/A**	**N/A**
Vaginal irritation/itching/burning	20 (22%)	16 (*80%*)	4 (*20%*)	0 (*0%*)	N/A	2 (*10%*)	18 (*90%*)	14 (27%)	8 (16%)	6 (12%)	0 (0%)
Cramps/abdominal pain	15 (16%)	7 (*47%*)	6 (*40%*)	2 (*13%*)	N/A	0 (*0%*)	13 (*100%*)	9 (18%)	5 (10%)	2 (4%)	2 (4%)
Vaginal discharge	10 (11%)	9 (*90%*)	1 (*10%*)	0 (*0%*)	N/A	0 (*0%*)	11 (*100%*)	9 (18%)	8 (16%)	1 (2%)	0 (0%)
Headache	11 (12%)	7 (*64%*)	2 (*18%*)	2 (*18%*)	N/A	1 (*9%*)	10 (*91%*)	10 (20%)	9 (18%)	1 (2%)	0 (0%)
Nausea	8 (9%)	4 (*50%*)	3 (*38%*)	1 (*13%*)	N/A	0 (*0%*)	8 (*100%*)	8 (16%)	8 (16%)	0 (0%)	0 (0%)
Vaginal odour	6 (7%)	6 (*100%*)	0 (*0%*)	0 (*0%*)	N/A	0 (*0%*)	6 (*100%*)	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Vaginal dryness	5 (5%)	5 (*100%*)	0 (*0%*)	0 (*0%*)	N/A	0 (*0%*)	5 (*100%*)	4 (8%)	3 (6%)	1 (2%)	0 (0%)
Spotting	5 (5%)	5 (*100%*)	0 (*0%*)	0 (*0%*)	N/A	0 (*0%*)	5 (*100%*)	3 (6%)	2 (4%)	0 (0%)	1 (2%)
Breast tenderness	2 (2%)	2 (*100%*)	0 (*0%*)	0 (*0%*)	N/A	1 (*50%*)	1 (*50%*)	2 (4%)	2 (4%)	0 (0%)	0 (0%)
Light headedness	2 (2%)	0 (*0%*)	0 (*0%*)	2 (*100%*)	N/A	0 (*0%*)	2 (*100%*)	2 (4%)	2 (4%)	0 (0%)	0 (0%)
Insomnia, painful sexual activity, burning when urinating, swelling behind ear, acne	1 (1%)	1 (*100%*)	0 (*0%*)	0 (*0%*)	N/A	Insomnia, acne	Painful sexual activity, burning when urinating, swelling behind ear	1 (2%)	1 (2%)	0 (0%)	0 (0%)
Palpitations, generally unwell	1 (1%)	0 (*0%*)	1 (*100%*)	0 (*0%*)	N/A	0 (0%)	1 (100%)	1 (2%)	1 (2%)	0 (0%)	0 (0%)
Pregnancy	1 (1%)	N/A	N/A	N/A	1 (*100%*)	N/A	N/A	1 (2%)	1 (2%)	N/A	N/A

Data shown as n (% total AEs or % of enrolled participants or *% of the specific AEs*). N/A = not applicable.

No serious AEs were reported. No indications of inflammation of infection were observed during pelvic exams.

30 days of intervention did not induce any clinically significant changes in blood markers of health or immune cell counts.

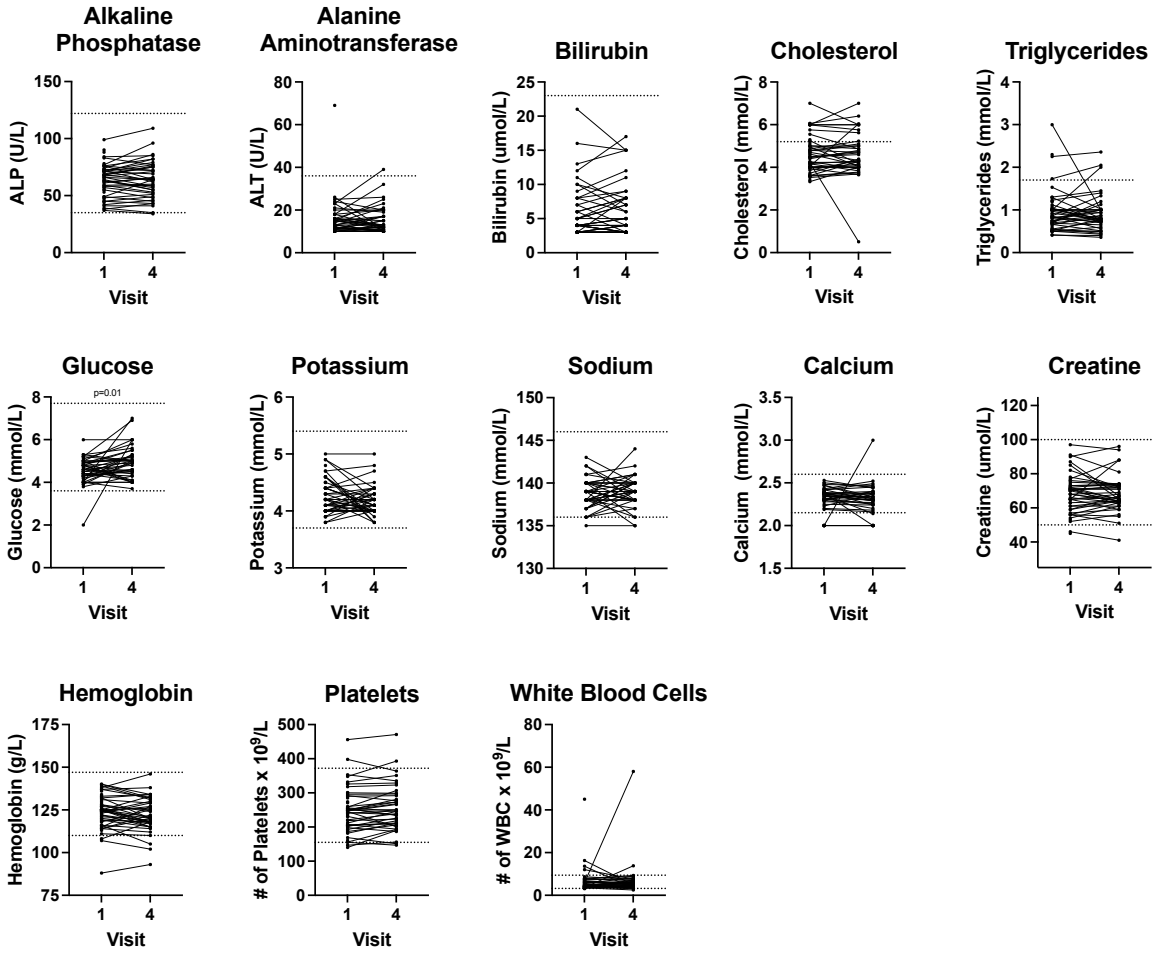


Figure 1. Blood markers of health and immune cells before and after 30 days of intervention. Metabolite, lipid and complete blood count panels from blood samples collected at visit 1 (screening) and visit 4 (end of intervention) were implemented to monitor for changes in indicators of overall health. Each data point represents a participant; dotted lines depict upper and/or lower limits of a normal/healthy range. Any statistically significant changes ($p<0.05$ determined by a paired student t test) are included on the graph.

Summary

- No serious AEs were reported
- 43% of participants did not experience any AEs
- Most AEs were mild (72%), short-term (93% resolved) and were not recurring
- 6% of participants experienced an AE of severe intensity, all of which resolved by study completion
- Vaginal irritation/itching/burning and cramps/abdominal pain were the most reported AEs
- 8% of participants had ongoing AEs at the end of the study, which included vaginal irritation/itching/burning, headache, insomnia, breast tenderness, and acne
- Blood panels did not reveal any clinically significant changes in blood markers of general health

Conclusion

Administration of intravaginal low dose estrogen alone or in combination with RepHresh™ Pro-B™ probiotics is safe.