Clinical and Translational Report

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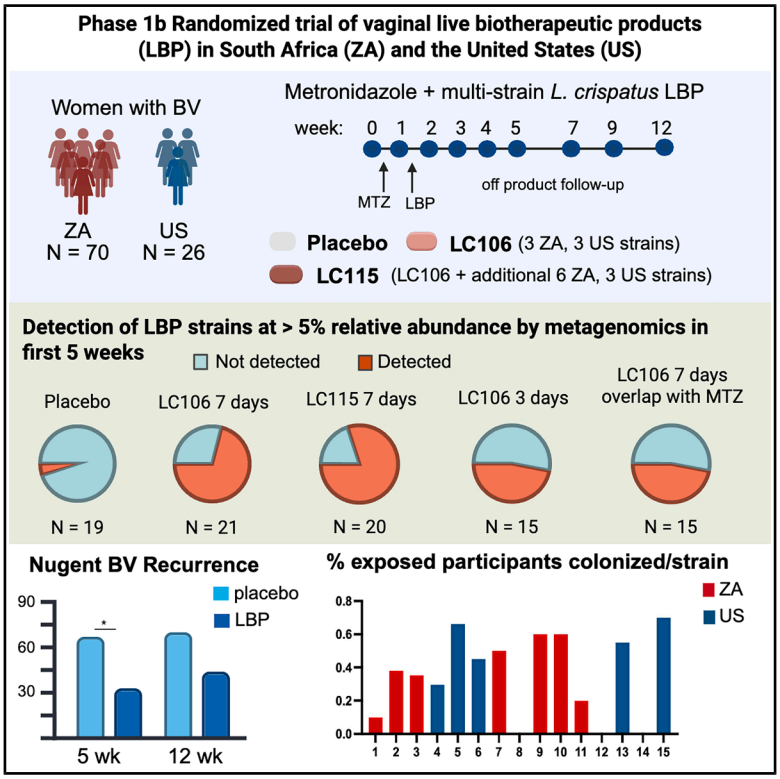
VIBRANT: A phase 1 randomized trial of multi-strain vaginal *L. crispatus* live biotherapeutic products in people with bacterial vaginosis

Graphical abstract



Highlights

- Multi-strain vaginal *Lactobacillus crispatus* LBPs colonized 66% of participants
- Colonization persisted for up to 12 weeks following only 3–7 days of dosing
- The same three strains most frequently colonized participants in the US and South Africa
- Study products reduce risk for recurrent BV and are safe and well tolerated

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In brief

In this phase 1 randomized trial of women with bacterial vaginosis in South Africa and the United States, Potloane et al. show that a multi-strain vaginal *Lactobacillus crispatus* live biotherapeutic administered for just 3–7 days following antibiotics can achieve vaginal colonization lasting up to 12 weeks.

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VIBRANT: A phase 1 randomized trial of multi-strain vaginal *L. crispatus* live biotherapeutic products in people with bacterial vaginosis

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SUMMARY

Bacterial vaginosis (BV) is characterized by high microbial diversity. High recurrence rates following antibiotics may stem from poor recolonization by protective *Lactobacillus* species. This phase 1 randomized trial in the United States and South Africa evaluated two vaginally delivered live biotherapeutic products (LBPs) containing multiple *Lactobacillus crispatus* strains. After metronidazole treatment for BV, participants received either a placebo or 3 or 7 days of active LBPs. LBP strains were detected by metagenomics in 66.1% (47/71) of participants in the active arms in the first 5 weeks. Among those, nearly half (49%, 23/47) remained colonized at 12 weeks despite the short initial treatment course. Participants were most often colonized by one of three component strains, with no geographic differences in strain colonization observed. LBPs were safe, acceptable, and well tolerated, with no serious adverse events (AEs) reported. These results provide a foundation for the development of transformational interventions aimed at optimizing the vaginal microbiome.

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INTRODUCTION

An optimal vaginal microbiome is characterized by low microbial diversity and dominance by *Lactobacillus* species. This "healthy" state is associated with reduced risk of adverse reproductive health outcomes.[1–4] By contrast, a non-optimal vaginal microbiome is marked by high diversity and an abundance of obligate and facultative anaerobes, including species in the genera *Gardnerella, Fannyhessea*, *Prevotella*, and *Mobiluncus*. Women with such microbiota are often diagnosed with bacterial vaginosis (BV), generally have high levels of genital inflammation, and face an increased risk for adverse outcomes such as HIV acquisition and transmission, human papillomavirus (HPV) infection and persistence, cervical dysplasia, miscarriages, and preterm births.[5–10] Globally, BV affects approximately 30% of women.[11] It may present with clinical symptoms, including malodorous vaginal discharge, itching, or irritation, or may remain asymptomatic.[12,13] Since the 1980s, the same two antibiotic classes have remained the standard treatment for clinical BV.[14,15] While effective in providing short-term symptom relief and reducing BV-associated bacterial burden, recurrences remain unacceptably high. Up to 60% of women experience recurrence within 6 months,[16–18] likely due to the failure of protective lactobacilli, such as *L. crispatus*, to recolonize the vagina after treatment.[19–21]

Interventions to initiate and promote colonization of the vaginal environment with beneficial *L. crispatus* are therefore a key therapeutic goal. Such strategies may reduce BV recurrence and mitigate the associated adverse reproductive health outcomes. Phase 2 trials of LACTIN-V, a live biotherapeutic product (LBP) containing a single naturally occurring vaginal strain of *L. crispatus* (CTV-05), showed reduced recurrence rates of clinical BV compared with placebo.[22,23] However, only 44% of participants in the Lactin-V arm still had detectable levels of the isolate at the final visit, after cessation of product use, suggesting that inoculation with a single strain of *L. crispatus* may be insufficient for durable colonization of the vagina with beneficial lactobacilli.[24]

To address this limitation, we designed two multi-strain, *L. crispatus*-containing vaginal LBPs using strains isolated from women in the US and South Africa who were stably dominated by *L. crispatus*. These LBP were formulated as 1 g vaginal tablets with a total dose of 2×10^9 colony-forming unit (CFU)/tablet, whether including 6 (LC106) or 15 (LC115) strains. The primary objective of this phase 1 trial was to assess the safety, tolerability, and colonization kinetics of these vaginally administered LBPs after a single, short course of treatment.

RESULTS

Women 18–40 years old diagnosed with BV were recruited from Vulindlela, South Africa, and Boston, United States. Once enrolled, a total of 96 participants were randomized to one of five arms (placebo, 7 days of LC106 [LC106-7], 3 days of LC106 [LC106-3], 7 days of LC106 overlapping with metronidazole [LC106-o], and 7 days of LC115 [LC115]). All participants were treated with 7 days of oral metronidazole. Participants self-administered the study product using a vaginal applicator. Between randomization and the primary outcome visit at week 5, participants collected daily vaginal swabs at home and were seen in person weekly. During the dosing period, participants were asked to collect the vaginal swab immediately prior to inserting the next dose of study product. Six randomized participants either withdrew after randomization or never used the study product (Figure 1). The modified intent-to-treat population therefore included 90 participants randomized to the five study arms: placebo (N = 19), LC106-7 (N = 21), LC106-3 (N = 15), LC106-o (N = 15), and LC115 (N = 20). The South African site enrolled the majority of participants (N = 70), evenly distributed across arms (N = 14/arm, Figure S1). At the US site, two arms were discontinued early due to slow enrollment. Prior to that change, one participant had been randomized to each of these arms, while the remainder had been assigned to placebo (N = 5), LC106-7 (N = 7), and LC115 (N = 6) (Figure S2).

Demographic characteristics were similar across arms, with notable differences between the two sites in self-identified race/ethnicity and contraceptive type (Tables 1, S1, and S2). Food insecurity, assessed using the short form of the United States Department of Agriculture (USDA) Food Security Questionnaire,[25] was more common at the South African site.

Colonization with LBP strains was detected in over half of participants

Overall, 66% (47/71; 95% confidence interval [CI]: 54%–77%) of women in the active arms achieved the primary outcome: detection of a single LBP strain at $\geq$5% relative abundance, or 2 or more at $\geq$10% total relative abundance as established by metagenomic sequencing of weekly swabs in the first 5 weeks of study participation (Table 2). Detection was highest at week 1 (during dosing) in the LC106-o arm and at week 2 (immediately post-dosing) in the other active arms (Figures 2A and 2B). LBP strain detection rates were similar across the active arms, although the study was underpowered to detect differences between the different arms (Table 2). At each site, one placebo participant had an LBP strain detected during follow up, perhaps reflecting misclassification of a native *L. crispatus* strain. Overall, 35% (24/68; 95% CI: 24%–48%) of women in the active arms met the primary outcome at the final 12-week visit. Among active arm participants who met the primary outcome in the first 5 weeks, 49% (23/47; 95% CI: 34–64%) remained colonized at week 12, without notable differences between arms (Figure 2C), demonstrating durable colonization despite a short dosing regimen.

At the visit immediately post-dosing in the 7-day arms, some participants had no detectable LBP strains by metagenomics despite reporting use of at least 6/7 product doses. Applicator staining for 77 participants in the modified intent to treat (mITT) analysis population who returned their used applicators revealed that 81% (62/77) of participants had high concordance between self-reported use and positive staining. Because 13 mITT participants did not return all applicators, product exposure was further assessed by a post hoc analysis using strain-specific qPCR on daily home-collected swabs during the dosing period, applying a strict detection threshold ($>10^7$ copies/swab of at least half of the expected LBP strains). With this approach, 90% (64/71) of participants in active arms had detection of LBP strains on at least one dosing day, and 75% (53/71) on at least half of dosing days (Figure S3). Among those randomized

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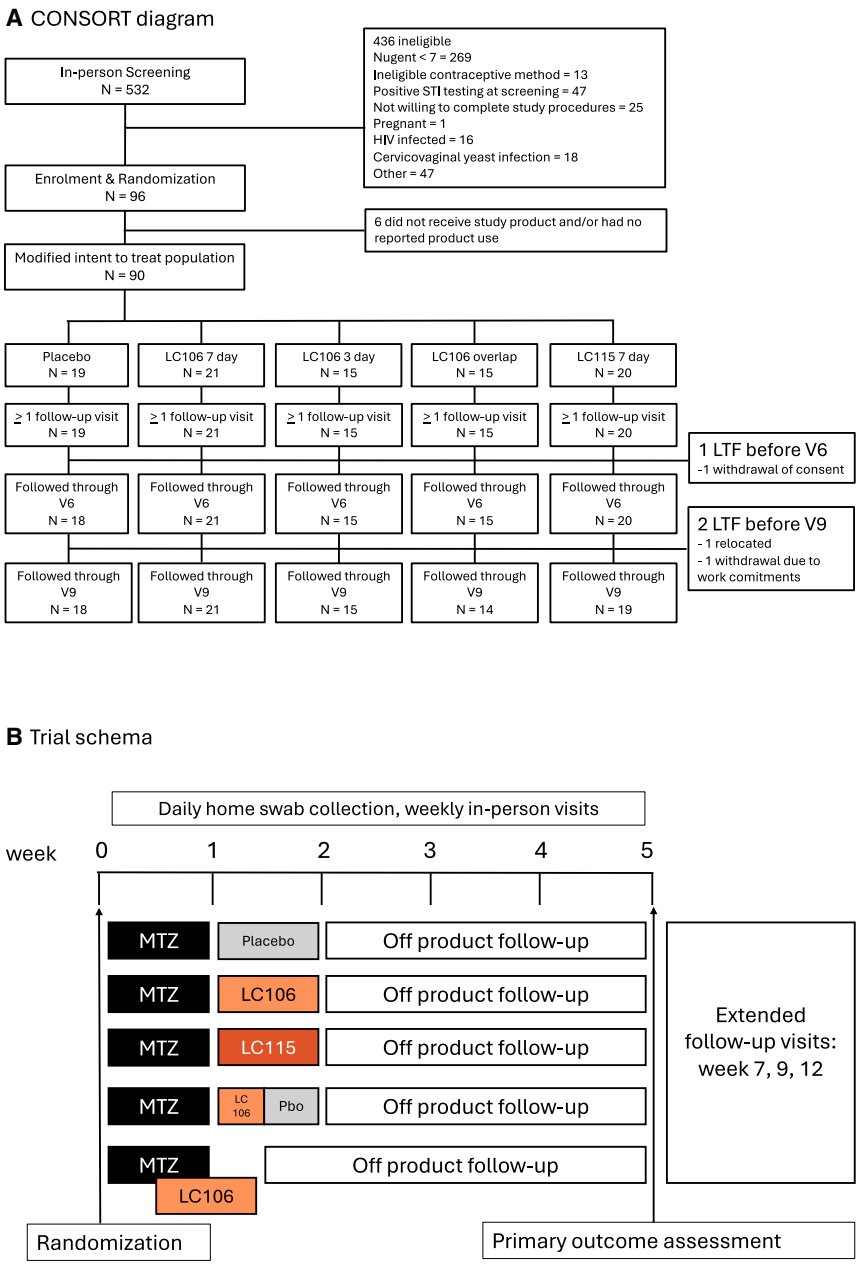

Figure 1. Phase 1 randomized placebo-controlled trial

(A) CONSORT diagram representing recruitment, enrollment, randomization, and follow-up.

(B) Schematic of study arms, visit schedule, and sample collection.

to 7 days of LBP treatment, the mean proportion of days with detection above the threshold was 67% (95% CI: 58%–75%). Participants with detection on at least half of dosing days were more likely to achieve colonization by week 5 than those without (75% [40/53] vs. 50% [6/12]).

In the two arms in which 7-day dosing began after metronidazole completion, 81% (17/21, LC106-7) and 90% (18/20, LC115) of participants had inserted a dose the same day or the day prior to the week 2 visit. Thus, detection at this visit may represent residual study product in the vagina rather than true colonization. A sensitivity analysis using only metagenomic sequencing data from weeks 3–5 reduced the proportion meeting the primary outcome (LC106-7: 57% vs. 71%; LC115: 50% vs. 80%), but colonization rates remained statistically significantly higher than placebo (Table S3). Across all visits remote from dosing, when LBP strains were detected by metagenomics, they comprised >50% of the microbiome in 87% (85/98) of visits in the active arms, suggesting that when retained post dosing, strains typically established dominance.

Three strains were most commonly detected across geographies

Each of the two LBPs contained multiple *L. crispatus* strains from both the US and South Africa (Table S4). Most detection events were attributable to three strains: C0022A1, C0059E1, and C0175A1 (Figure 3A). Detection rates were similar across sites (Figure 3B), except for South African strains UC101 and FF00051, which were more frequently detected in US participants immediately post-dosing. However, these differences did not persist at later visits, and the relative abundance of these strains never exceeded 20% at visits remote from dosing (Figures 3A, 3B, and S4).

Colonization with LBP was associated with lower BV recurrence

Of the 86 participants with a Nugent score available immediately post-metronidazole treatment, 21% (18/86) had persistent BV (score ≥7). Among these, 12 received an LBP, and five (42%) met the primary outcome. The relative risk of BV recurrence among participants receiving an LBP compared with placebo was 0.49 (95% CI: 0.27–0.89) at week 5 and 0.63 (0.38–1.03) at week 12 (Table 3). Among participants with a Nugent score <7 post-metronidazole who received an LBP (n = 57), those achieving the primary outcome by week 5 had a lower probability of BV recurrence over 12 weeks (33%, 13/40; 95% CI 19–49%) than those without colonization (71%, 12/17, 95% CI 44–90%).

The LBPs were safe and acceptable

Both LBP products were very safe and acceptable. Among the 91 participants included in the safety population, no serious adverse events (AEs) were observed (Tables 4 and S5). Grade 2 AEs were most frequent in the placebo arm, both overall and

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Table 1. Demographic and behavioral characteristics of participants at enrollment

		Placebo[a] *N* = 19	LC106-7[a] *N* = 21	LC106-3[a] *N* = 15	LC106-o[a] *N* = 15	LC115[a] *N* = 20
Site	South Africa	14 (74%)	14 (67%)	14 (93%)	14 (93%)	14 (70%)
	United States	5 (26%)	7 (33%)	1 (7%)	1 (7%)	6 (30%)
Age		29 (20–38)	29 (18–40)	26 (21–34)	25 (19–35)	30(20–40)
Race	Asian	1 (5.3%)	0 (0%)	1 (6.7%)	0 (0%)	0 (0%)
	Black	14 (74%)	18 (86%)	14 (93%)	15 (100%)	18 (90%)
	White	2 (11%)	3 (14%)	0 (0%)	0 (0%)	1(5%)
	other	1 (5.3%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)
	prefer no answer	1 (5.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Ethnicity[b]	not Hispanic	3 (60%)	6 (86%)	1 (100%)	1 (100%)	4 (67%)
	Hispanic	2 (40%)	1 (14%)	0 (0%)	0 (0%)	2 (33%)
Food insecurity	past 12 months	7 (37%)	7 (33%)	6 (40%)	5 (33%)	6 (30%)
Contraception[c]	COC	3 (16%)	5 (24%)	1 (6.7%)	1 (6.7%)	4 (20%)
	vaginal ring	0 (0%)	1 (4.8%)	0 (0%)	0 (0%)	0 (0%)
	DMPA	11 (58%)	10 (48%)	12 (80%)	11 (73%)	11 (55%)
	NET-EN	2 (11%)	2 (9.5%)	2 (13%)	3 (20%)	2 (10%)
	implant	1 (5.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Lng-IUD	2 (11%)	2 (9.5%)	0 (0%)	0 (0%)	2 (10%)
	other	0 (0%)	1 (4.8%)	0 (0%)	0 (0%)	1 (5%)
Number of partners past month		1 (1–2)	1 (0–2)	1 (0–2)	1 (0–3)	1 (0–2)
Number of lifetime partners		5 (1–10)	5 (1–90)	4 (1–10)	4 (1–20)	5 (1–10)
Gender partners	No sex	1 (5%)	3 (14%)	1 (6.7%)	1 (6.7%)	3 (15%)
	Male only	18 (95%)	18 (86%)	14 (93%)	14 (93%)	17 (85%)

[a]Median (min-max); *n* (%)
[b]Ethnicity percentages only calculated for American participants
[c]COC, combined estrogen/progesterone oral contraceptives; DMPA, depo medroxyprogesterone acetate; NET-EN, norethisterone enanthate; implant, etonorgestrel contraceptive implant; Lng-IUD, levonorgestrel-containing intrauterine device

for local/genitourinary AEs. Most participants reported willingness to use the product again: 17/19 (89%) placebo, 16/21 (76%) LC106-7, 15/15 (100%) LC106-3, 14/15 (93%) LC106-o, 18/20 (90%) LC115.

DISCUSSION

We hypothesized that the establishment of an *L. crispatus*-dominant vaginal microbiome is an essential step in reducing BV recurrence after antibiotic treatment. This randomized, first-in-human, multi-site trial provides evidence that a multi-strain exogenous *L. crispatus* LBP can durably engraft—and often persist to 12 weeks—following a short 3–7-day dosing regimen. Our target product profile is a product administered for a short duration that does not require maintenance dosing. Over 60% of participants who received LBP demonstrated metagenomic detection of LBP strains, and persistence for up to 12 weeks was observed after as few as three doses. Importantly, among women cured post-metronidazole, colonization by week 5 was associated with substantially lower BV recurrence over

Table 2. Proportion of participants at each site who achieved the primary outcome of detection of a live biotherapeutic product strain of *L. crispatus* at >5% relative abundance at any time during the first 5 weeks

	Placebo	LC106-7	LC106-3	LC106-o	LC115
South Africa	*N* = 14	*N* = 14	*N* = 14	*N* = 14	*N* = 14
n (%) with outcome	0 (0%)	9 (64%)	7 (50%)	7 (50%)	10 (71%)
95% CI		39%–84%	27%–73%	27%–73%	45%–88%
United States	*N* = 5	*N* = 7	*N* = 1	*N* = 1	*N* = 6
n (%) with outcome	1 (20%)	6 (86%)	1 (100%)	1 (100%)	6 (100%)
95% CI		49%–97%	21%–100%	21%–100%	61%–100%
RR (95% CI)	Ref	13.57(1.72 – inf)	10.13(1.37 – inf)	10.13(1.37 – inf)	15.2(2.1 – inf)
Adjusted *p* value		<0.001	<0.001	<0.001	<0.001

The p values are computed using unconditional exact tests on the data from the two sites combined, comparing rates in each active arm vs. placebo and adjusted for multiple comparisons by controlling for the false discovery rate.

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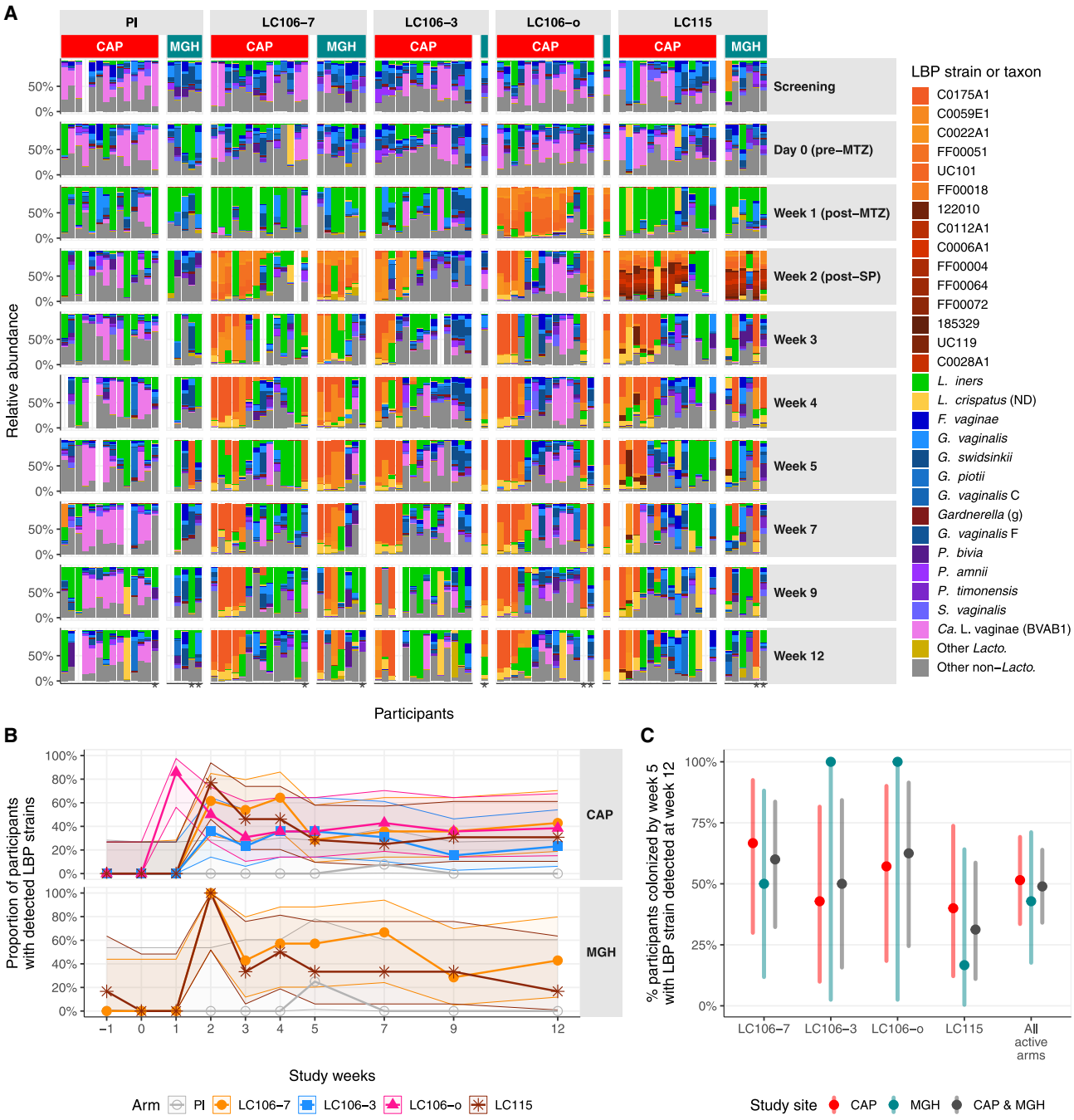

Figure 2. When present, live biotherapeutic strains were abundant

(A) Vaginal bacteria and live biotherapeutic product (LBP) strain relative abundances estimated by metagenomic sequencing of samples from all clinic visits. Participants are grouped by study arm (placebo [PI], 7 days of LC106 [LC106-7], 3 days of LC106 [LC106-3], 7 days of LC106 overlapping with metronidazole [LC106-o], and 7 days of LC115 [LC115]) and site (CAP, South African site; MGH, United States site), with each participant's samples aligned in a single column. Participants marked by an asterisk at the bottom of their column are included in the modified intent-to-treat population but not the per-protocol population. A white space indicates a missed visit or a sample that failed sequencing. *L. crispatus* strains contained in the LBP are represented in hues of orange, while *L. crispatus* not classified as an LBP strain is shown as yellow. LC106 is the 6-strain product, and LC115 is the 15-strain product.

(B and C) Colonization appeared to happen early, as seen in (B) the proportion of all mITT participants with detection of an LBP strain at ≥5% relative abundance or a total LBP strain relative abundance ≥10% by metagenomic sequencing at each visit, stratified by site (CAP, South African site; MGH, United States site; discontinued arms at MGH, each with a single participant, were excluded; shaded area indicates the 95% confidence interval), and (C) the proportion of participants with an LBP strain detected according to the metagenomic definition during the first 5 weeks who continue to have an LBP strain detected at week 12 (proportion and 95% confidence interval represented).

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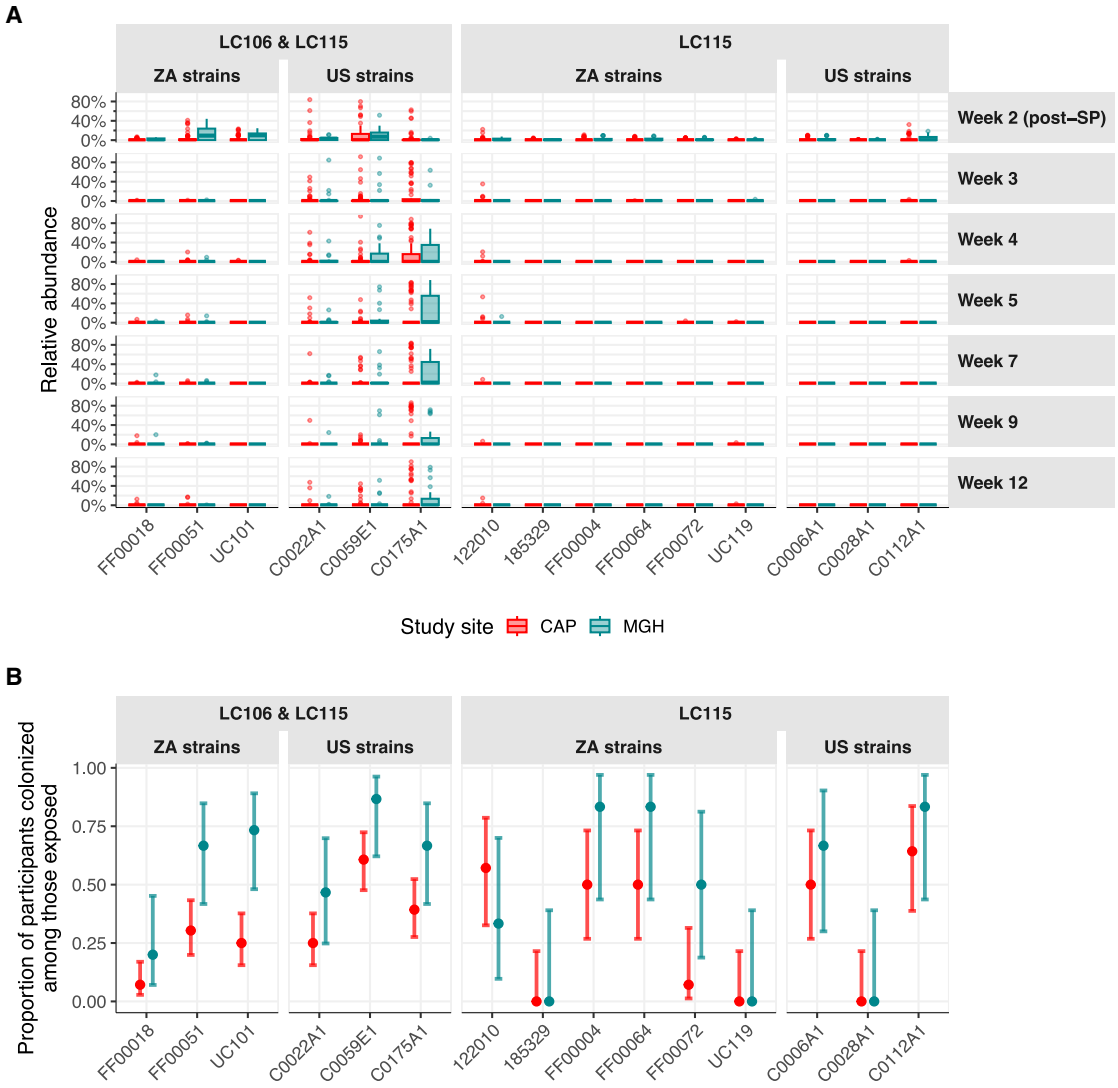

Figure 3. Three strains were commonly detected across geographies

(A) Relative abundance of each strain contained in the LBPs at each visit as estimated by metagenomic sequencing. Strains are organized according to geographical origin (ZA, South Africa; US, United States) and the product(s) in which they are included. Colonization rates are reported separately for each site (CAP, South African site; MGH, United States site; SP, study product). The boxes represent median and interquartile range.

(B) Colonization success varied by strain, as seen in (B) the proportion of women exposed to a specific LBP strain who had that strain detected by metagenomic sequencing at any point during follow up, stratified by site. Strains are organized by geographical origin (ZA, South Africa; US, United States) and product(s) in which they are included. Colonization rates are reported separately for each site (CAP, South African site; MGH, United States site) as proportion and 95% confidence interval.

12 weeks. Findings were consistent across geographically distinct cohorts, suggesting the potential for a single formulation to be effective across populations. Both safety and acceptability were high.

The goal of this LBP is to establish a durable, *L. crispatus*-dominant microbiome, thereby reducing the rates of adverse health outcomes associated with diverse, non-*Lactobacillus*-dominant microbiomes. In this trial, early engraftment was common, and when LBP strains were detected, they typically dominated the community, suggesting that once established, they thrived. Moreover, participants who met our primary outcome of LBP colonization had a lower risk of recurrent BV compared with those who did not, supporting the biological plausibility of a multi-strain *L. crispatus* LBP as an adjunct to antibiotic treatment. However, durability remains the key challenge: by week 12, strains persisted in only 49% of early colonizers, underscoring the need to optimize dose, schedule, and formulation.

The scientific rationale for a multi-strain LBP stems from evidence that in individuals with stable *L. crispatus*-dominated vaginal microbiomes, the metagenome contains more *L. crispatus* genes than can be explained by a single isolate.[26] We hypothesized that including multiple strains of *L. crispatus* in the LBPs would enhance colonization success by increasing

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Table 3. Proportion of people with Nugent score BV (7–10) after metronidazole treatment, by 5 and by 12 weeks

	Placebo*N* = 19	LC106-7*N* = 21	LC106-3*N* = 15	LC106-o*N* = 15	LC115*N* = 20	Active arms combined
Post metronidazole						
Persistence, *n* (%)	6 (35%)	3 (14%)	6 (40%)	1 (7%)	2 (11%)	12 (17%)
BV outcome missing	2	0	0	1	1	2
By week 5						
Recurrence, *n* (%)[a]	6 (67%)	4 (24%)	4 (44%)	4 (33%)	6 (35%)	18 (33%)
95% CI	35%–88%	10%–47%	19%–73%	14%–61%	17%–59%	22%–46%
BV outcome missing	2	1	0	1	0	2
RR (95% CI)	Ref	0.35 (0.13–0.93)	0.67 (0.28–1.58)	0.50 (0.20–1.26)	0.53 (0.24–1.17)	0.49 (0.27–0.89)
By Week 12						
Recurrence, *n* (%)[a]	7 (70%)	8 (44%)	5 (56%)	6 (46%)	6 (35%)	25 (44%)
95% CI	40%–89%	25%–66%	27%–81%	23%–71%	17%–59%	32%–57%
BV outcome missing	1	0	0	0	0	0
RR (95% CI)	Ref	0.63 (0.33–1.22)	0.79 (0.39–1.62)	0.66 (0.32–1.35)	0.5 (0.24–1.08)	0.63 (0.38–1.03)

[a]Recurrence rate calculated as the number of people with a known outcome at that visit divided by the number of people known to have cleared BV after treatment AND who had a known outcome at the visit.

community functional capacity and allowing for host-specific selection, where different women might preferentially support different strains, yielding heterogeneous strain colonization patterns. Contrary to that expectation, three strains consistently emerged as the most frequent colonizers across arms (LC106, LC115) and sites, suggesting broad host compatibility. To extend benefits to a larger population, optimization is still needed—for example, by adding complementary strains or adjusting supporting chemistry/excipients to enhance engraftment and durability.

Our results align with previous studies of the single-strain vaginal *L. crispatus* LBP Lactin-V, which used daily dosing for 5 days followed by twice-weekly maintenance for 4–11 weeks. In those trials, the Lactin-V strain was detectable by qPCR in 69%–67% of women 1 week post-dosing and in 44%–47% 4–12 weeks post-dosing.[22,24] Similarly, we observed LBP strain detection in over half of participants in the week immediately following treatment using metagenomics, with this dropping to a third of participants after an additional 7 weeks off product. The risk ratio for BV recurrence in the US Lactin-V phase 2b study was 0.66 (0.44, 0.87) in the week post-dosing. Notably, our trial demonstrated a relative risk of 0.49 3 weeks after dosing and 0.63 at the 3-month follow-up visit. These rates were achieved without maintenance dosing, suggesting that durable colonization may occur early or not at all and that a short dosing course may be sufficient.

Limitations of the study

Several limitations warrant consideration. Due to the modest sample size, the study was underpowered to detect small differences between active study arms, limiting conclusions about the optimal dosing strategy or LBP composition. The lower enrollment at the US site means we had insufficient power to perform within- or between-site analyses of the data. Thus, results from this trial should be considered preliminary proof of concept, which needs additional verification in larger studies. Adherence

Table 4. AEs reported for the safety population during the VIBRANT trial

		Number of events (% of participants with events)				
		Placebo (*N*=20)	LC106-7 (*N*=21)	LC106-3 (*N*=15)	LC106-o (*N*=15)	LC115 (*N*=20)
Adverse events	total	63(80%)	53(76%)	34(73%)	32(73%)	46(90%)
	grade 1	33(60%)	36(57%)	17(53%)	13(53%)	27(65%)
	grade 2	30(65%)	17(48%)	17(60%)	19(60%)	19(45%)
	grade 3+	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Related AEs	total	11(30%)	5(19%)	10(40%)	4(20%)	8(25%)
	grade 1	7(25%)	4(14%)	8(33%)	3(20%)	8(25%)
	grade 2	4(5%)	1(5%)	2(13%)	1(7%)	0(0%)
	grade 3+	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Local/genitourinary AEs	total	39(60%)	33(52%)	8(20%)	9(40%)	28(55%)
	grade 1	23(35%)	25(48%)	6(13%)	9(40%)	18(40%)
	grade 2	16(45%)	8(24%)	2(13%)	0(0%)	10(20%)
	grade 3+	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Severe AEs	total	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

was measured by participant report and examination of returned tablet packages and applicators, which may not fully capture actual use. However, post hoc analysis of strain-specific qPCR on daily swabs collected during dosing showed good agreement between self-reported adherence and observed exposure to the LBP strains. Site differences also influenced study conduct: in the US, telephone pre-screening was used, whereas in South Africa, screening was conducted exclusively in person. Stringent inclusion criteria also affected enrollment differently at the two sites: in the US, there was a higher-than-expected preference for non-hormonal contraceptive methods, while in South Africa, a high rate of Amsel-negative but Nugent-positive BV slowed accrual, necessitating study protocol amendments.

Conclusions

In this phase 1 study of multi-strain *L. crispatus* LBPs, we demonstrated that these products were safe, acceptable, and capable of establishing durable colonization after a short dosing regimen in geographically diverse populations. These findings lay the foundation for the development of transformational interventions to optimize the vaginal microbiome.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Caroline Mitchell (caroline.mitchell@mgh.harvard.edu).

Materials availability

This study did not generate any new, unique reagents.

Data and code availability

- The data used for the analysis have been deposited on Zenodo and are publicly available as of the date of publication at https://zenodo.org/records/18201511.
- The metagenomic sequences generated for this analysis have been deposited in the Sequence Read Archive, BioProject PRJNA1337104 and can be accessed at https://dataview.ncbi.nlm.nih.gov/object/PRJNA1337104?reviewer=l6cftrvp147h7nd52ibifnv8nn.
- All original code has been deposited at GitHub and is publicly available at https://zenodo.org/records/18201310 (analysis) and https://zenodo.org/records/18044747 (data preparation and QC) as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization: D.P., J.-A.S.P., D.S.K., J.R., and C.M.M.; funding acquisition: D.P. and C.M.M.; developed the study protocol: D.P., L.S., S.N., L.L., C.C., N.A.M., A.M.P., J.-A.S.P., A.-U.H., S.H., D.S.K., J.R., and C.M.M.; provided isolates for the LBP: S.N., J.-A.S.P., B.K., H.B.J., A.-U.H., F.H., D.A.R., D.S.K., and J.R.; conducted the clinical trial: D.P., C.C., N.A.M., A.M.P., A.K., B.C.D., and C.M.M.; processed and evaluated trial samples: S.N., A.M., A.K., N. Magini, N. Mitchev, G.M., and M.G.; generated laboratory data for trial samples: S.N., A.M.P., A.M., A.K., N. Magini, N. Mitchev, G.M., A.K., B.C.D., M.G., J.X., L.R., B.S., S.C., and L.L.; performed analysis: L.S., L.L., M.F., L.V., J.E., and A.K.; writing of initial draft: D.P., L.S., L.L., L.V., and C.M.M.; review of final draft: all authors

DECLARATION OF INTERESTS

C.M.M. has been a consultant for Freya Biosciences and DIVA Inc. and serves on the scientific advisory board of Ancilia Biosciences and Concerto Biosciences. C.M.M. has a financial interest in Ancilia Biosciences & Company developing a new class of live biotherapeutics and other bacterial products. Dr. Mitchell's interests were reviewed and are managed by MGH and Mass General Brigham in accordance with their conflict-of-interest policies. J.R. is co-founder of LUCA Biologics, a biotechnology company focusing on translating microbiome research into live biotherapeutic drugs for women's health. J.R. has a financial interest in Ancilia Biosciences as a member of its scientific advisory board. D.S.K., J.R., F.H., C.M.M., M.F., J.-A. S. P., A.-U.H., and S.N. are all named inventors on patent no. US 2025/0195589 A1 for the LBP tested in the trial.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.chom.2026.02.016.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
Streptococcus agalactiae (in Mock Community 2)	ATCC	ATCC-12403
Lactobacillus jensenii (in Mock Community 2)	ATCC	ATCC-25258
Gardnerella vaginalis (in Mock Community 2)	ATCC	ATCC-14018
Lactobacillus gasseri (in Mock Community 2)	ATCC	ATCC-9857
Lactobacillus crispatus (in Mock Community 2)	ATCC	ATCC-33820
Atopobium vaginae (aka Fannyhessea vaginae) (in Mock Community 2)	ATCC	ATCC-BAA-55
Prevotella bivia (in Mock Community 2)	ATCC	ATCC-29303
Lactobacillus iners (in Mock Community 2)	ATCC	ATCC-55195
Biological samples		
Vaginal swabs	This study	N/A
Chemicals, peptides, and recombinant proteins		
Nucleic acid stabilizing buffer	Qiagen	Buffer C2.1
PicoGreen BR assay	ThermoFisher	Cat#Q32853
Nuclease free water	Life technologies	Cat#AM9937
PrimeTime qPCR master mix	Integrated DNA Technologies	Cat#055771
Critical commercial assays		
MagAttract PowerMicrobiome DNA/RNA EP Kit	Qiagen	Cat#27500-4-EP
NEBNext Ultra II DNA Library Prep Kit for Illumina	NEB	Cat#E7645L
Novaseq S4 Reagent Kit, 300 cycles	Illumina	Cat#20028312
Gram stain kit	Fischer Scientific	23-001-823
DNA 1K Reagent Kit	Revvity	Cat#CLS760673
Deposited data		
Metagenomic sequencing data	This study	https://dataview.ncbi.nlm.nih.gov/object/PRJNA1337104?reviewer=l6cftrvp147h7nd52ibifnv8nn
Metadata	This study	https://zenodo.org/records/18201511
Oligonucleotides		
Primers and probes for qPCR assays	This paper	See Table 6
Recombinant DNA		
gBlock Gene Fragment (standard for qPCR assays)	This paper	Document S2
Software and algorithms		
R	The Comprehensive R Archive Network	https://cran.r-project.org/; RRID: SCR_001905
BMTagger (v3.1.0)	Rotmistrovsky et al.[27]	https://ftp.ncbi.nlm.nih.gov/pub/agarwala/bmtagger/; RRID: SCR_014619
Fastp v0.21.0	Chen et al.[28]	https://github.com/OpenGene/fastp; RRID: SCR_016962
VIRGO2 (V1.0)	France et al.[29]	https://github.com/ravel-lab/VIRGO2/
kSanity (v.1.0)	France et al.[30]	https://github.com/ravel-lab/kSanity
R packages	N/A	exact2x2 v1.7.0; gtsummary v2.4.0; brms v2.23.0; binom v1.1; tidyverse v2.0.0; gt v1.1.0; patchwork v1.3.2; ggpubr v0.6.2; ggh4x v0.3.1; ggtext v0.1.2; ggbeeswarm v0.7.2; labelled v2.16.0; scales v1.4.0

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bioconductor packages	N/A	SummarizedExperiment v1.36.0; tidySummarizedExperiment v1.16.0; RRID: SCR_006442
Custom code (analysis)	This paper	https://zenodo.org/records/18201310
Custom code (QC and data preparation)	This paper	https://zenodo.org/records/18044747
Other		
Puritan PurFlock Ultra® Flocked Swabs	Avantor	75788–570
Micro Essential Lab Hydrion™ Single Roll pH Test Paper Dispensers	Fisher Scientific	14-850-121
Microlab STAR robotic platform	Hamilton	N/A
Firefly	SPT Labtech	N/A
NovaSeq 6000	Illumina	Cat#20012850; RRID: SCR_016387

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study design and participants

From February 9, 2024 through February 19, 2025 we conducted a two-site randomized, controlled Phase 1 trial of two multi-strain *L. crispatus* vaginal live biotherapeutic products in Vulindlela, KwaZulu Natal, South Africa and Boston, Massachusetts, United States. The protocol was reviewed and approved by SAHPRA (20230615) and FDA (IND 029629), as well as ethics committees at both sites (BREC/00005620/2023; MGB IRB 2023P001035). The study was registered on clinicaltrials.gov (NCT06135974). This study enrolled cis-gender women age 18–40. Details about the study participants' race, ethnicity, socioeconomic status, and geographic origin are provided in Table 1.

METHOD DETAILS

Recruitment and enrollment

Participants were recruited through in-person community outreach teams (South Africa), online and physical advertisements (US), direct recruitment through the electronic health record (US), and local clinics (both sites). The CAPRISA team of recruiters introduced the study widely at different community meetings and forums within the Vulindlela area. This allowed women to receive essential information about the study and to register their interest by leaving their contact details with site staff for further information and/or subsequent scheduling of a screening visit. During information sessions following the initial introduction of the study, women aged 18 to 40 years who were currently experiencing or had previously experienced vaginal symptoms, were invited to screen for the study. At the US site, telephone pre-screening was conducted to reduce the number of in-person screening visits. Inclusion criteria included a diagnosis of bacterial vaginosis (BV), and negative testing for yeast (by microscopy), *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* (by NAAT), as well as negative serology for syphilis and HIV at the screening visit. Exclusion criteria included pregnancy, significant medical history, and use of antibiotics or probiotics in the past 30 days. Complete inclusion and exclusion criteria are detailed in the study protocol.[31]

Initially, BV was diagnosed at both sites using both Amsel criteria ($\geq$ 3/4)[32] and Nugent score 7–10.[33] However, at the South African site many participants met Nugent criteria but not Amsel criteria. A review of the literature indicated that this discrepancy is common in microbiome studies in African populations,[34] and so use of the Amsel criteria was stopped at the South African site in July 2024.

Initially, all participants were required to use either continuous combined oral contraceptives or injectable progestin-only contraceptives to suppress menses. However, reluctance to use hormonal contraception or current use of an IUD were two of the primary reasons for exclusion during pre-screening in the US. In August 2024 the protocol in the US was amended to allow participants to use any form of contraception.

Interventions

All participants received oral metronidazole, taken twice daily for 7 days. The dosage differed between the sites according to local standards of care: 500mg twice daily in US, and 400mg twice daily in South Africa. Participants were randomized to one of five arms: 7 days of vaginal placebo tablet following metronidazole, 7 days of LC106, a six-strain *L. crispatus* live biotherapeutic vaginal tablet, following metronidazole (LC106-7), 3 days of LC106 + 4 days of placebo following metronidazole (LC106-3), 7 days of LC106 starting on day 3 of metronidazole (overlap arm, LC106-o), and 7 days of a fifteen-strain *L. crispatus* live biotherapeutic vaginal tablet following metronidazole (LC115). The study product was administered using a vaginal applicator. In the pharmacy, study product was maintained at 2–8°C, but participants were asked to keep it at room temperature at home during the week of use. Due to slow accrual at the US site, enrollment in the LC106-3 and the LC106-o arms was stopped in May 2024, and planned enrollment for the South African site was increased accordingly.

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Randomization and blinding

An unblinded statistician created randomization lists stratified by site. Randomization was performed at the time of enrollment (*i.e.,* once eligibility was confirmed) using sequentially numbered, opaque envelopes. The "overlap" arm was unblinded, but all other arms received blinded study product, dispensed in identical-appearing boxes. When two arms were dropped at the US site, the unblinded statistician informed the study team which randomization envelopes to remove.

Study procedures

Participants meeting eligibility criteria after screening returned for an enrollment visit, were dispensed oral metronidazole and were randomized. At that visit, participants were also asked to practice administration of a placebo tablet to ensure that they felt comfortable with administering study product. Study product was dispensed at week 1 visit (after metronidazole completion) or at enrollment for those in the overlap arm. Participants in all but the overlap arm initiated study product within 24 hours of completing metronidazole. Follow-up included weekly in-person visits until week 5, and additional visits at week 7, 9 and 12. At each in-person visit participants self-collected vaginal swabs. Blood was drawn for testing for syphilis and HIV serology testing at screening, week 5 and week 12. Additionally, vaginal swabs were collected for *N. gonorrhoeae*, *C. trachomatis and T. vaginalis* testing at the same visits. Between enrollment and the week 5 visits, participants completed daily diaries documenting product use, bleeding and sexual activity and self-collected a single vaginal swab which was placed in a nucleic acid stabilizing buffer stored at room temperature and returned to the study team at weekly visits.

Outcomes

The primary outcomes for the study were 1) vaginal detection of any single strains included in the LBP at $\geq$ 5% relative abundance, or any combination of LBP strains at $\geq$ 10% relative abundance using metagenomic sequencing at any point prior to the 5-week study visit and 2) safety, as measured by reported adverse events. Adverse events were graded according to the DAIDS Female Genital Grading Table.[35] If participants missed a visit or if metagenomic sequencing could not be performed for a sample, we assumed absence of colonization at that time point (worst-case assumption). Secondary outcomes included detection of the LBP strains between week 5 and week 12, recurrence of BV by Nugent score, the proportion of people with *L. crispatus*-dominance (> 50% relative abundance), and acceptability of the study product.

Sample processing

Vaginal flocked swabs were placed in cryovials with 1 mL nucleic acid stabilizing buffer (Buffer C2.1, Qiagen, Germantown, MD, USA) and were stored at -80°C until use. Polyester swabs were collected for Amsel and Nugent assessment. Nugent scoring was performed by at least two independent readers, and if scores differed by more than 2 points or spanned diagnosis categories a third reader was used to adjudicate the score. Each site cross-read slides from the other site to ensure that scoring consistency.

Measurement of adherence to metronidazole and LBP

Adherence to both metronidazole and study product was assessed using: 1) participant daily diary, 2) self-report at the weekly in-person visits, and 3) vaginal applicator staining with 0.05% methylene blue solution for study product.[36,37]

DNA extraction

Vaginal bacterial DNA was extracted using a high-throughput automated workflow optimized for cervicovaginal microbiome profiling. Vaginal swab specimens collected in C2.1 solution (Qiagen) were transferred into 96-well plates and processed using the MagAttract PowerMicrobiome DNA/RNA EP Kit (Qiagen, Germantown, MD, USA) according to a custom protocol automated on a Microlab STAR robotic liquid-handling platform (Hamilton, Reno, NV, USA). Mechanical lysis was performed using a TissueLyser II (Qiagen) at 20 Hz for 20 min to ensure efficient disruption of both Gram-positive and Gram-negative bacterial taxa. Each extraction run included negative controls (nuclease-free water and unused swabs placed in C2.1 solution) and positive controls comprising two defined mock communities: Mock1, comprised a standardized vaginal microbial community supplemented with all 15 *Lactobacillus crispatus* strains included in the live biotherapeutic product (LBP), and Mock2, consisting of the same 15 LBP-derived *L. crispatus* strains combined with selected bacterial isolates. DNA was purified by magnetic bead–based separation, eluted in nuclease-free water, and quantified using a Qubit fluorometer (Thermo Fisher Scientific, USA) to confirm adequate yield and quality for downstream 16S rRNA gene amplicon sequencing, qPCR, and metagenomic analyses. Automated extraction was used to minimize operator-dependent variability and ensure reproducibility across large-sample sets.

Metagenomic sequencing & data processing

The shotgun metagenomic sequence libraries were constructed from the extracted DNA using NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB, Massachusetts, USA), according to manufacturer's instructions. The resulting libraries were sequenced on an Illumina NovaSeq 6000 (150 bp paired end mode, targeting 45 million read pairs per sample) at Maryland Genomics (https://marylandgenomics.org/). Human reads were detected and removed from all metagenomic sequencing data using BMTagger and the GRCh38 reference genome,[27] and remaining reads were quality filtered using fastp[28] (v0.22.0, sliding window: size 4 bp, Q12, minimum read length:55 bp). The reads were then mapped to the VIRGO2 non-redundant gene catalog to establish taxonomic composition[29] (v1, default settings). The relative abundances of each taxon were computed as the proportion of the

gene-length-adjusted number of reads assigned to that taxon over the sum of all gene-length-adjusted reads assigned to bacterial content (*i.e.,* excluding reads assigned to the human host genome or to the genomes of non-bacterial microbiota organisms). We next used kSanity to detect and quantify the abundance of the *L. crispatus* strains that comprise LC106 and LC115[30] (v1, k=55). The output of kSanity reports the abundance of each LBP strain, relative to the abundance of all *L. crispatus*. The LBP strain abundances were then multiplied by the overall relative abundance of *L. crispatus*, as estimated by VIRGO2, to establish their relative abundance in the overall community.

Strain-specific qPCR assays

Detection and quantification of LBP strains was also performed using strain-specific qPCR assays. A total of 15 assays were developed, each targeting a unique gene region of an individual *L. crispatus* strain present in the LBPs (Table S5). The 15 assays were multiplexed in groups of three using three different fluorophores (Cy5, HEX or FAM). The qPCR reactions included the following components: 0.5μL of each primer mix (18μM forward primer, 18μM reverse primer, 5μM probe), 5μL of PrimeTime qPCR master mix (IDT, New Jersey, USA), 2μL of DNA template, and 1.5μL of water (10μL total reaction volume). New Jersey, USA), 2μL of DNA template, and 1.5μL of water (10μL total reaction volume). A universal gDNA standard was generated by designing a 2.7-kb synthetic gene fragment containing all 15 amplicon regions (Document S2). A standard curve was made by serial dilution of the gene fragment with 1 mg/ml poly A (Sigma, Missouri, USA) in water spanning from 10^1 to 10^7 copies per μL. Reactions were performed on a CFX-384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Pennsylvania, USA), in triplicate with a gDNA standard curve spanning from 10^1 to 10^7 copies per μL. Thermocycling included an initial 10min of denaturing at 95°C followed by 40 cycles of 95°C for 15s and 62°C for 1 minute.

QUANTIFICATION AND STATISTICAL ANALYSIS

Sample size calculation

We assumed that at most 10% of participants in the placebo arm would have detectable LBP strains within the first 5 weeks. We defined a clinically significant difference between arms as a $\geq$ 50% absolute difference in LBP strain detection between any active arm and placebo. With 10 participants per arm, the trial had 82.3% power to detect a difference of 60% in the colonization proportion between an active arm and placebo using a one-sided Fisher's exact test with a significance threshold of 0.05 (PASS 2020). Our initial goal was to recruit sufficient participants at each site to allow within-site analyses, however due to slow accrual, the US site did not achieve 10 people per arm.

Analysis populations

For the assessment of product safety we included data from all randomized participants who met inclusion/ exclusion criteria and received at least one LBP dose and who had any post-LBP data available. Participants were analyzed according to treatment received. For analyses of colonization we used a modified intent-to-treat (mITT) population that included all randomized participants who met inclusion/exclusion criteria, used at least one dose of study product, and returned for at least one follow-up visit. The per protocol (PP) analyses included all randomized people who met inclusion/ exclusion criteria, did not meet replacement criteria, completed at least 80% of study product doses (by self-report and/or applicator staining), and had at least one follow-up visit prior to week 7. Participants were analyzed in the groups to which they were assigned. Four participants met replacement criteria, of whom two were replaced. All four were included in the mITT population, but only the final two were included in the PP population.

Primary outcome testing

One-sided unconditional exact test (Fisher-adjusted method)[38] was used to test whether the colonization proportion in each of the active arms was greater than in the placebo arm (data from both sites were considered simultaneously). Multiple testing was accounted for by adjusting the *p*-value to control for the false-discovery rate using the Benjamini-Hochberg procedure.[39] Confidence intervals for proportions (primary outcomes and all other proportions reported here) were computed using the "score" method.[40]

Secondary outcome analyses

For the analysis of BV recurrence, risk ratios and associated 95% Wald confidence intervals were calculated. To avoid inflating Type I error rates or power loss due to multiple testing adjustment, statistical tests were not performed for any of our secondary outcomes, as specified *a priori* in our statistical analysis plan. Confidence intervals should therefore not be used to infer definitive effects

Post-hoc analyses

Our daily qPCR data were generated after unblinding, however the technicians and bioinformaticians processing the samples and data were blinded to participant/sample assignment. For the mean proportion of days exposed, confidence intervals were calculated using Student's method.

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Statistical tests

Continuous variables summarized in Table 1 by median and range were compared across study arms using the Kruskal-Wallis rank sum test. Categorical variables were analyzed using either Fisher's exact test (for small cell counts) or Pearson's chi-squared test (when expected frequencies were sufficient), depending on the distribution of the data.

ADDITIONAL RESOURCES

The study was registered on clinicaltrials.gov (NCT06135974).