IPRAD PHARMA - 174 quai de Jemmapes – 75010 PARIS

CLINICAL STUDY REPORT

1. TITLE

<u>Study:</u> eVaFlore

STUDY OF THE EFFICACY AND SAFETY OF A TREATMENT WITH TOTAL FREEZE-DRIED CULTURES OF LACTOBACILLUS CRISPATUS – *IP 174178* ADMINISTERED INTRAVAGINALLY IN THE PREVENTION OF BACTERIAL VAGINOSIS RECURRENCE

A national, randomised, phase III, multicentre, 2-arm, parallel-group, superiority study versus placebo

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STUDY OF THE EFFICACY AND SAFETY OF *LACTOBACILLUS CRISPATUS* – IP 174178

Study of the efficacy and safety of a treatment with total freeze-dried cultures of *Lactobacillus crispatus – IP 174178* administered intravaginally in the prevention of bacterial vaginosis recurrence.

A national, randomised, phase III, multicentre, 2-arm, parallel-group, double-blind, superiority study versus placebo.

Sponsor No. IPR EVAFLORE 12
EudraCT: 2012-002975-33
Investigational medicinal product: Total freeze-dried cultures of *Lactobacillus crispatus* – IP 174178
Indication: Bacterial vaginosis
Phase: III
Trial start date: 26 April 2013
Trial end date: 08 April 2015
Name and address of sponsor: Laboratoires IPRAD PHARMA 174 Quai de Jemmapes – 75010 PARIS – France
ICH / GCP declaration: This study was conducted in accordance with the ICH-GCP guidelines, in particular in terms of study document archiving

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ETUDE SUR L'EFFICACITE ET LA TOLERANCE D'UN TRAITEMENT PAR CULTURES TOTALES LYOPHILISEES DE *LACTOBACILLUS CRISPATUS - IP 174178* ADMINISTREES PAR VOIE INTRA VAGINALE DANS LA PREVENTION DE LA RECIDIVE DES VAGINOSES BACTERIENNES

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2. SYNOPSIS

Sponsor:	IPRAD PHARMA, 174 Quai de Jemmapes – 75010 PARIS - France
Name of Product and/or Code:	*Lactobacillus crispatus* – IP 174178
Study title:	Study of the efficacy and safety of a treatment with total freeze-dried cultures of *Lactobacillus crispatus* – IP 174178 administered intravaginally in the prevention of bacterial vaginosis recurrence. A national, randomised, phase III, multicentre, 2-arm, parallel-group, double-blind, superiority study versus placebo.
Scientific Committee:	Prof. Jean-François Bergmann Dr Jean-Marc Bohbot Prof. Florence Bretelle Prof. Jean-Michel Cardot Prof. Emile Darai Dr Christel Neut
Study context:	Bacterial vaginosis is a common and recurring infection that can cause obstetric/gynaecological complications and a deterioration in quality of life. One of the main causes of this recurrence is an imbalance in the vaginal flora due to a lack of commensal Lactobacillus flora. The use of probiotics, lactobacilli specific to the targeted environment, can help to re-establish this balance and reduce the frequency of recurrence.
Number of centres involved in this study:	This study was carried out in 29 centres in France. Gynaecologists and general practitioners were chosen with a high level of competence in the fields of clinical trials and vaginosis, and with a sufficient number of potential patients. The coordinating center was the Institut Fournier (Paris).
Study schedule:	Duration of the study: April 2013 to April 2015 • Enrolment and follow-up period: - 1st enrolment: 26 April 2013 - Last enrolment: April 2015 - Last patient, last visit: 26 October 2015 • Final report: 22 December 2016
Monitoring and Logistics:	ITEC Services, an ISO 9001 certified contract research organisation, was responsible for the monitoring and the logistical organisation of the project.
Submissions	Ile de France III CPP (ethics committee) and ANSM (French National Agency for Medicines and Health Products Safety).

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Methodology:	Phase III Clinical Study - Prospective - Randomised - Multicenter - Double-blind - Two parallel groups - Versus placebo Study in 3 parts: • V1 → V2: Treatment of an acute infection with metronidazole • V2 → V4: Treatment with the study product or the placebo (Primary endpoint) • V4 → V5: Monitoring after treatment (assessment of post-treatment recurrence)
Study objectives:	Primary objective: The primary objective of this study was to assess the efficacy of *Lactobacillus crispatus* IP 174178 by comparing the percentage of patients presenting at least one bacteriologically confirmed clinical recurrence at V4 (end of treatment) among patients presenting bacterial vaginosis treated with *Lactobacillus crispatus* IP 174178 in accordance with a 4-cycle therapeutic regimen versus placebo. The efficacy of 4-cycle *Lactobacillus crispatus* IP 174178 was compared to the efficacy of the Placebo. The recurrences had to be confirmed with a Nugent score ≥ 7. Secondary objectives: **The secondary objectives were to assess, during the active treatment period:** *The time to onset of the first bacteriologically confirmed clinical RECURRENCE[1].* *The number of monthly bacteriologically confirmed clinical recurrences per patient up to visit V4* All of the objectives relating to the bacteriologically confirmed clinical recurrences were also studied for: ✓ Clinical recurrences (Amsel criteria solely) ✓ Asymptomatic bacterial vaginosis episodes

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	The number of days on which the patient had clinical signs (malodorous discharge) as a proportion of the treatment duration (assessment by the patient using a patient diary) at V4 *Clinical tolerability of the treatment* *Overall tolerability in the opinion of the investigator* **The secondary objectives were to assess, <u>during the post-active treatment period</u>:** The efficacy of *Lactobacillus crispatus* IP 174178 by comparing the time to onset of the first post-treatment recurrence[2] among patients presenting bacterial vaginosis treated with *Lactobacillus crispatus* IP 174178 or placebo. The time to onset of the first recurrence was calculated from visit V4. The follow-up end date corresponded to the end-of-follow-up visit, V5. The efficacy of 4-cycle *Lactobacillus crispatus* IP 174178 was compared to the efficacy of the Placebo. Three types of recurrence were considered: ✓ Bacteriologically confirmed clinical recurrences ✓ Clinical recurrences ✓ Asymptomatic bacterial vaginosis episodes

[1] <u>RECURRENCE DURING TREATMENT</u>: Clinical episode, bacteriologically confirmed, occurring <u>during</u> the study treatment period [4 cycles]. The treatment period is defined as the V2-V4 interval.

[2] <u>RECURRENCE POST-TREATMENT</u>: Clinical episode, bacteriologically confirmed, occurring after the treatment period and during the monitoring period [3 cycles].

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Inclusion criteria:	**Criteria associated with the disease:** Patients with a history of bacterial vaginosis: at least 2 documented episodes of bacterial vaginosis in the previous 12 months. - Patients with symptomatic bacterial vaginosis, characterised by the presence of the 3 following clinical criteria (from among the 4 criteria) at V1: • Homogenous white/grey vaginal discharge, • Characteristic fishy odour caused by the spontaneous release of amine or during the potassium hydroxide test or “whiff test", • Vaginal pH greater than 4.5, Patients with a Nugent score ≥ 7 (use of the sample collected at V1). Patients cured clinically (none of the 3 Amsel criteria as specified above) after a 7-day treatment with metronidazole (Flagyl®). **General criteria:** - Women. - Patients over 18 years of age. - For women of childbearing age: • Negative urine pregnancy test, • Use of a contraceptive method considered to be effective by the investigator (excluding spermicides). - Patients who have received all the information about the study and who have given their written consent. - Patients registered with Social Security or other social protection.
Exclusion criteria:	**Criteria associated with the disease or gynaecological in nature:** - Presence of a vaginal yeast, bacterial or virus infection (other than bacterial vaginosis), presumed or proven to be gynaecological in nature, treated or untreated within one month prior to enrolment or present at enrolment. - Presence of an existing gynaecological infection that could affect the assessment of the trial treatment (severe cervical dysplasia or carcinoma in situ, invasive carcinoma, intra-epithelial cervical neoplasia, squamous intra-epithelial lesions, etc.) **Criteria associated with the treatment:** - Antibiotics or antifungal treatments administered systemically during the month prior to the screening visit, excluding treatments for a bacterial vaginosis episode. - Use of local probiotic treatment in the month prior to the screening visit. - Use of antiseptics in the month prior to the screening visit, excluding treatments for a bacterial vaginosis episode. - Use of prebiotics (acidifiers) during the two weeks prior to the screening visit. - Use of products containing oestrogens by local administration in the month prior to the screening visit. - Allergy to one of the active ingredients or one of the excipients contained in the study products.

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	General criteria: - Patients who will not be able to comply with the constraints of the protocol. - Patients who are pregnant or breastfeeding. - Postmenopausal patients. - Patients whose menstrual bleeding lasts more than 12 days per month. - Patients who have participated in a clinical study in the 3 months prior to enrolment in this protocol. - Patients with a severe acute or a chronic disease considered by the investigator to be incompatible with participating in this study, or with a serious infection which could be life-threatening in the short term. - Patients with a depressed immune system. - Patients with a prior disease that, in the view of the investigator, is likely to compromise the results of the study or expose the patient to additional risk. - Patients with psychological or linguistic factors that limit their ability to understand and sign the informed consent form. - Patients deprived of their liberty by court decision or subject to guardianship authority. - Patients likely not to follow the treatment (problem with compliance). - Patients it is not possible to contact in the event of an emergency.
Specific conditions:	For the objectives of this study: - Patients screened in accordance with the clinical criteria and with a Nugent score <7 (use of the sample collected at V1), were treated with metronidazole (Flagyl®) but were not randomised. - Patients screened in accordance with the clinical criteria and with a Nugent score ≥7 (use of the sample collected at V1) who, after they were treated with metronidazole (Flagyl®) (in accordance with the protocol recommendations), were cured clinically, were randomised. - Randomised patients who had a clinical recurrence during the study, had to be treated again with metronidazole (Flagyl®). The recurrence had to be confirmed with a bacteriological test.

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Study products:	• **1st arm** **Name of product:** – *Lactobacillus crispatus* IP 174178 containing between 10^9 CFU per gram at release and 10^7 CFU per gram at expiry (2 years). Dosage: 1 vaginal capsule (with hard shell) per day Route of administration: intravaginal Treatment duration: 4 x 14-day cycles • **2nd arm** **Placebo**: suppocire AS2 semisynthetic glycerides made up of glycerides of saturated fatty acids from C8 to C18 Dosage: 1 vaginal capsule per day Route of administration: intravaginal Treatment duration: 4 x 14-day cycles
Treatment of initial and recurring infections:	Oral administration of metronidazole (Flagyl®): 500 mg morning and evening for 7 days. In the event of clinical failure (presence of at least one of the three Amsel criteria as specified above) of the treatment of the current episode: the patient was not randomised into the study.
Endpoints: Efficacy:	**Primary endpoint** Manifestation or non-manifestation of recurrence during treatment (clinical, proven with vaginal cytobacteriological exam) at 4 months (V4). **Secondary efficacy endpoints:** 1. Time to first recurrence (clinical, proven with vaginal cytobacteriological exam). 2. Manifestation or non-manifestation of recurrence post-treatment (after treatment period), 3 months of monitoring (V5). 3. Manifestation or non-manifestation of isolated clinical recurrences (3 Amsel criteria as specified above) at 4 months. 4. Number of recurrences (clinical, proven with vaginal cytobacteriological exam) per year and per patient. 5. Total number of days with symptoms (malodorous discharge) reported by the patient (patient diary). 6. Presence or absence of asymptomatic bacterial vaginosis at end of follow-up (at V4 and V5 or before in cases of early withdrawal from the trial).

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Tolerability:	**Tolerability criteria:** 1. Assessment of overall tolerability by the investigator, after interviewing the patient. This assessment was performed at visits V3 and V4 in accordance with the following classification system: 1 = Very good tolerability: no symptom of discomfort and no sign revealed during the examination. 2 = Good tolerability: several minimal and short-lasting symptoms of discomfort not leading to the temporary discontinuation of the application of the study product. No objective signs revealed during the examination. 3 = Moderate tolerability: symptoms of discomfort and distinct or persistent objective signs revealed during the examination, not leading to the temporary discontinuation of the application of the study product. 4 = Poor tolerability: symptoms and/or objective signs forcing the patient to stop taking the study treatment. 2. Frequency of adverse events The adverse events occurring during the study, noted by the patient in her patient diary or collected by the investigator during the patient visits, were recorded in the CRF.
Other assessments:	Clinical symptoms of vaginosis assessed by the patient and entered in the patient diary, quality-of-life scale.
Conduct of the study:	The study was conducted in three phases: - a treatment phase for the initial bacterial vaginosis episode (visit 1 corresponds to this phase) - a treatment phase for the prevention of recurrences (visits 2, 3 and 4 are included in this phase) - a monitoring phase AFTER treatment (3 months) to investigate any recurrences occurring post-treatment. **D-7 (7 to 10 days before V2): screening visit: V1** - Explanation of the Protocol and its constraints - Signature of the information leaflet and informed consent form - Clinical examination (assessment of the 3 Amsel criteria as specified above) - Bacteriological sample for the Nugent test 1 (Diagnostic). - Pregnancy test - Supply of the treatment for the bacterial vaginosis episode - Metronidazole (Flagyl®) - Scheduling of appointment for visit V2 at D0, by the investigator. **D0: screening visit: V2** - Results of Nugent 1 test (≥ 7 confirmation of bacterial vaginosis) - Clinical examination (assessment of the 3 Amsel criteria as specified above) - Collection of bacteriological sample for the Nugent test 2 - Verification of Protocol compliance - Recovery of the FLAGYL® treatment box and blister pack, verification of compliance - Verification of inclusion and exclusion criteria - Randomisation - List of adverse events and concomitant treatments

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	- Supply of study treatment (2 months of treatment) - Scheduling of appointment for visit V3 (D56+/-7) ***D28 : Contact by telephone No. 1: V2 + 28 days** - Reminder on taking treatment - Reminder to fill in patient diary **D56 +/-7: V3** - Clinical examination (assessment of the 3 Amsel criteria as specified above) (+ Collection of bacteriological sample for Nugent test, only if 3 Amsel criteria are present) - Verification of Protocol compliance - List of clinical assessment criteria - List of V2 bacteriological assessment criteria - List of adverse events and concomitant treatments - Recovery of treatment boxes and unused capsules - Supply of study treatment (2 months of treatment) - Scheduling of appointment for visit V4 (D112+/-7) ***D84 +/-7: Contact by telephone No. 2: V3 + 28 days** - Reminder on taking treatment - Reminder to fill in patient diary **D112 +/-7: V4** - Physical examination (assessment of the 3 Amsel criteria as specified above) (+ Collection of bacteriological sample for Nugent test) - Verification of Protocol compliance - List of clinical assessment criteria - List of V3 bacteriological assessment criteria if applicable - Assessment of tolerability by the investigator and the patient - Recovery of treatment boxes and unused capsules - List of adverse events and concomitant treatments **Visits for recurring infections (recurrences while taking treatment between V4 and V5):** - Clinical examination (assessment of the 3 Amsel criteria as specified above) - Collection of bacteriological sample for Nugent test (only if 3 Amsel criteria are present) - Treatment of bacterial vaginosis episode with metronidazole (Flagyl®) (only if 3 Amsel criteria are present) - List of adverse events and concomitant treatments ***D140 +/-15: Contact by telephone No. 3: V4 + 28 days** - Reminder on taking treatment - Reminder to fill in patient diary **D(112 + 84) 196 +/- 7d: V5** - Physical examination (assessment of the 3 Amsel criteria as specified above) (+ Collection of bacteriological sample for Nugent test) - Bacterial vaginosis episodes

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	- List of clinical assessment criteria - List of V4 bacteriological assessment criteria if applicable - List of adverse events and concomitant treatments In the event of recurrence: the study treatment is administered without any changes in accordance with the initial schedule. **: phone contacts were performed by an independent company.*
Statistical methods:	**Comparative superiority study.** **<u>Analysis population:</u>** - Tolerability (SAF): All randomised patients who have used at least one capsule of the study treatment. - Intention to treat (FAS): All randomised patients who have taken at least one capsule of the study treatment. - Per protocol (PP): All patients who are assessable for the FAS, who have completed the study without any major deviation from the Protocol. Efficacy analysis: FAS population (primary population), PP population. Tolerability analysis: tolerability population (= SAF population) **<u>Number of patients</u>:** The calculation of the number of subjects necessary was based on the comparison of survival without any event at the end of the active treatment between the two groups using the two-tailed formulation of the chi-squared test. An event was understood to be a bacteriologically confirmed clinical recurrence. Thus, using the hypothesis: Percentage of patients not having a recurrence in the placebo group: 50% Expected difference: 20% (4 cycles of active treatment versus placebo) Power: 80% Type I error alpha 5%, two-sided. One had to plan for a minimum of 98 assessable patients per group. Assuming a percentage of patients lost to follow-up during the course of the study of approximately 10%, it would have been necessary to have 110 patients for the primary endpoint. Furthermore, assuming that the percentage of patients screened with a Nugent score <7 at V1 was approximately 15% (false positives) and that the percentage of patients screened with a Nugent score ≥7 at V1, but not clinically cured at the end of the curative treatment (secondary exclusion criterion), was approximately 25%, it would have been necessary to screen 174 patients in each of the treatment groups in order to have 98 ITT (assessable) patients in each of the treatment groups.

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	In theory, 348 patients had to be screened. In total, 167 patients were screened, but only 100 were enrolled and randomised. Among these 100 patients enrolled and randomised between 26 April 2013 and 08 April 2015 by 29 doctors who agreed to participate in the study, 2 patients were excluded from the FAS analysis due to not having taken the treatment, resulting in a study population (FAS) of 98 patients. **<u>Main results:</u>** Our population (FAS) of 98 patients had a mean age at screening of 35.7 years ± 8.9, a mean BMI of 23 ± 4.7 (kg/m²) and smoking status divided into three categories: current smoker (43.9%) / former smoker (13.3%) / never smoked (42.9%); With a predominance of current smokers (52.1%) in the placebo group versus 36% in the *Lactobacillus crispatus* group. For this criterion, additional analyses were performed in order to compare the 2 treatment groups, which did not reveal any significant difference with respect to smoking status (p=*0.2592)*. The mean number of vaginal showers > 2/month was 8.2% in our population, varying from 4% in the *Lactobacillus crispatus* group to 12.5% in the placebo group, with no significant difference between the 2 treatment groups (p=0.1552). 69.5% of women had children, with a mean of 2 children. Our study population (FAS) demonstrated a real benefit, based on the comparison of survival without an event at 4 months in each group, with an increase in the number of patients without recurrence at 4 months (59% in the placebo group versus 79.5% in the *Lactobacillus crispatus* IP 174178 group). There was a significant difference (p=0.0298) in the time to onset of the first bacteriologically confirmed clinical recurrence between V2 and V4 inclusive, in favour of the *Lactobacillus crispatus* IP 174178 group. Thus, compared to the placebo arm, the *Lactobacillus crispatus* arm had: - Fewer patients who presented at least one bacteriologically confirmed clinical recurrence between V2 and V4 (20.5% versus 41%), and a <u>longer</u> time to onset of the first recurrence (3.8 months versus 2.9 months), - Fewer patients who presented at least one clinical recurrence between V2 and V4 (25.0% versus 48%) and a longer time to onset of the first recurrence (3.8 months versus 2.8 months). Finally, the tolerability assessed by the investigator was overall very good, since only 3 adverse events considered to be related to the treatment were reported, of which only 1 in the Lactobacillus crispatus IP 174178 group. (This latter event corresponded to an error in the route administration).