Study Title:	OBSERVATIONAL, NON-RANDOMIZED, NOT CONTROLLED, MULTICENTRE POST MARKETING CLINICAL FOLLOW UP (PMCF) STUDY TO EVALUATE THE SAFETY AND PERFORMANCE OF THE MEDICAL DEVICE POLYBACTUM® IN REDUCING THE FREQUENCY OF RECURRENT BACTERIAL VAGINOSIS.
Clinical Investigation Plan Code:	OPEFF/0116FU/MD
Type:	Observational, Post Marketing Clinical Follow-Up
Medical Device/class:	Polybactum® / class IIa
Indication studied:	Recurrence of Bacterial Vaginosis
Design:	Allocation: Observational study (no allocation) Model: NA Masking: NA Primary Purpose: Follow-up
Name and address of Sponsor:	EFFIK Italia S.p.A Via dei Lavoratori, 54 Milano (Italy) Phone: +39 02 66 04 91 89 Fax: +39 02 61 77 03 58
Name and address of CRO:	Opera Contract Research Organization (Opera CRO) 10 Cozia Street, Building B, 300209 Timișoara (Romania)
date:	01.03.2018
Authors:	Dionisio Franco Barattini (Opera CRO) Dumitru-Emanuel Dogaru (Opera CRO) Georgeta Burov (Opera CRO) Elena Casolati (Private Practice and Consultant for Effik Italia) Luca Ardolino (Effik Italia) Filippo Murina (V Buzzi Hospital, University of Milan)
Statement of Compliance:	Clinical Investigation Protocol performed in accordance with International Standard ISO14155 (Second edition 2011-02-01)

.

EMERGENCY CONTACTS

SPONSOR	*Contact Name*	*Phone/fax/e-mail*
Effik Italia SpA	Dr.	Phone: e-mail:

DOCUMENT APPROVALS

The signatures listed below indicate approval of this document and its forms.

	Name	**Signature**	**Date**
Issue	*RA/QA*		
Review	*Clinical Director*		
Approval	*CEO*		

Signature on this page indicate the document has ben reviewed for clarity and completeness, and the document is approved for use.

Revision table		
REV	**DATE**	**DESCRIPTION**
1	01/03/2018	Following the Amendment n. 1 dated 22/12/2017 to Clinical Study OPEFF/0116/MD Protocol Version: 1.0 dated 04.05.2016

POST MARKET CLINICAL FOLLOW-UP STUDY APPROVAL - SPONSOR

I declare that the Post Market CLINICAL FOLLOW-UP STUDY protocol OPEFF/0116FU/MD (Rev: 01.00 - 01/03/2018) contains all necessary information required for the conduct of the study.

Name:

Date: _____/_____/_____

Signature:

Professional position:

Company: Effik Italia SpA

POST MARKET CLINICAL FOLLOW-UP STUDY APPROVAL - CENTRES

I have understood that all information pertaining to this study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. I agree to obtain the written Informed Consent and privacy statement from all participating patients.

Name: **Dr. Ciprian Crisan**

Date: _____/_____/_____

Signature:

Professional position: Investigator

Site: Clinica Medicala Dr. Crisan Ciprian, Timisoara (Romania)
(name & address)

Name: **Dr. Daniela Sirbu**

Date: _____/_____/_____

Signature:

Professional position: Investigator

Site: Clinica Medicala Dr. Sirbu Daniela, Timisoara (Romania)
(name & address)

Name: **Dr. Marius Biris**

Date: _____/_____/_____

Signature:

Professional position: Investigator

Site: Clinica Medicala Dr. Crisan Ciprian, Timisoara (Romania)
(name & address)

TABLE OF CONTENTS

Synopsis

SPONSOR NAME: **EFFIK Italia S.p.A.** **NAME OF FINISHED PRODUCT:** **Polybactum®**	Individual Study Table Referring to Part of the Dossier Volume: Page:	For National Authority Use only)
STUDY TITLE	Observational, non-randomized, not controlled, multicentre post marketing clinical follow up (PMCF) study to evaluate the safety and performance of the medical device POLYBACTUM® in reducing the frequency of recurrent bacterial vaginosis.	
PRINCIPAL INVESTIGATORS OF THE STUDY CENTERS	**Name: Dr. Ciprian Crisan** Clinica Medicala Dr. Crisan Ciprian, Timisoara (Romania) **Name: Dr. Daniela Sirbu** Clinica Medicala Dr. Sirbu Daniela, Timisoara (Romania) **Name: Dr. Marius Biris** Clinica Medicala Dr. Crisan Ciprian, Timisoara (Romania)	
PHASE OF DEVELOPMENT	Medical Device already CE Marked Class IIa	
STUDY OBJECTIVES	**The main objective** of this PMCF study will be to assess the safety, performance and long-term efficacy of Polybactum®. **Additional objectives:** • to detect potential emerging risks related to safety on the basis of eventual clinical evidence, through the extended follow up of patients who have used Polybactum® in the previous clinical trial, OPEFF/0116/MD (POLARIS).®	

METHODOLOGY	This is an observational, non-randomized, multicentre study. This design has been chosen to avoid that the protocol therapeutic strategy which could influence the Investigator. This follow-up study aimed to assess the safety, performance and long-term efficacy of Polybactum®. Furthermore, it should permit to detect potential emerging risks related to safety on the basis of eventual clinical evidence, through the extended follow up of patients who have used Polybactum® in the previously performed clinical trial OPEFF/0116/MD (POLARIS). Visits were planned to be conducted at: **A = Screening and Baseline visit.** • physical examination; • concomitant medications/treatments recordings; • confirmation of BV absence by a negative test with Amsel criteria. This means that only 0 or 1 variable is met; • ADE/ SADE/ USADE recordings. *This visit matched the final visit of OPEFF/0116/MD study* **B = Phone contact with interview**. • assessment of BV symptoms; • concomitant medications/treatments checking; • ADE/ SADE/ USADE recordings; *If the patient had any of the BV symptoms after baseline, the Investigator could decide to perform an unscheduled visit to verify the BV recurrence by Amsel criteria.* **C = Final visit (gynaecological examination and Amsel criteria check).** • physical examination; • concomitant medications/treatments recordings; • confirmation of BV absence by a negative test with Amsel criteria. This means that only 0 or 1 variable is met; • ADE/ SADE/ USADE recordings; *If was not possible to perform the visit, a phone call with interview should be done.*
METHODOLOGY	**Evaluations included:** • Amsel criteria (vaginal pH, whiff test, homogenous vaginal discharge, and clue cells at optical microscopy by phase-contrast) • Nugent Score (optional); • Vaginal swab (Lactobacillus determination);
NUMBER OF SUBJECTS	**Total number of subjects:** 35 enrolled subjects belonging to OPEFF/0116/MD study in Romania (no d.o. or patients with recurrence in this study were included in the follow up). The patients belonging from Italian sites of OPEFF/0116/MD study will not be included due to administrative reasons in the present follow up observational study.
INCLUSION CRITERIA	1. Women above 18 years. 2. Patients who have completed the study OPEFF/0116/MD (last visit performed) without any recurrence. 3. Subjects who voluntarily decide to participate in this observational study and sign the Informed Consent Form

EXCLUSION CRITERIA	1. Patients who do not want to visit for long-term follow-up observation. 2. Patients who need/want to receive other procedures or treatments for prevention of recurrence of BV after participating in the previous study Protocol No OPEFF/0116/MD. 3. Patients concomitantly included in different interventional clinical trials. 4. Patients who are or become ineligible to participate in the present observational study for other reasons, as assessed by Investigator.
INVESTIGATIONAL PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER CONTROL PRODUCT	**Dose of administration:** There is no IP administration during the follow-up study. **Mode of administration:** Not applicable **Batch number:** Not applicable **Control Product:** Not applicable. No control product was used.

CRITERIA FOR EFFICACY EVALUATION	***Primary Efficacy Assessments*** The primary efficacy endpoint will be the evaluation of BV recurrence identified by Amsel criteria. ***Secondary Efficacy Assessments*** The secondary efficacy endpoints will include: • Vaginal Lactobacillus microbiota. • Signs and symptoms of BV (vaginal discharge, burning, erythema, dyspareunia).
CRITERIA FOR SAFETY EVALUATION	• AE/SAE and ADE/SADE/USADE.
STATISTICAL METHODS	Statistical analyses will be conducted on all patients entered in the study. The quality and completeness of the collected data will be evaluated preliminarily compared to data analysis. If a patient will have missing information for one or more variables, the missing data will be not substituted. Quantitative variables (i.e. demographic) if normally distributed will be described through mean, standard deviation (SD); variables non-normally distributed will be described using median and range of interquartile. The Student's t-test and the Mann-Whitney U will be employed to perform comparative analysis in accordance to the distribution of these variables. In order to evaluate changes over time before and after the treatment, Paired t-test (if applicable) or Wilcoxon signed rank sum test will be planned to be performed for quantitative variables, while McNemar test will be planned to be used in order to evaluate changes for binary variables, while symmetry test will be performed in order to evaluate changes for qualitative (not binary) variables. Categorical variables will be described using frequencies and percentages and comparative analysis using a Chi^2 test. All statistical analyses will be performed using the R statistical software v 3.5. The final analysis (including performance and safety data) will be completed after all CRFs have been filled, and the database will be locked.

1. INTRODUCTION

While clinical evidence is an essential element of the premarket conformity assessment process to demonstrate conformity to Essential Principles, it is important to recognise that there may be limitations in the clinical data available in the pre-market phase. Such limitations may be due to, for example, the duration of pre-market clinical investigations, the number of subjects involved in an investigation, the relative homogeneity of subjects and investigators and the control of variables in the setting of a clinical investigation versus use in the full range of conditions encountered in general medical practice. It is appropriate to place a product on the market once conformity to the relevant Essential Principles, including a favourable risk/benefit ratio, has been demonstrated. Complete characterization of all risks may not always be possible or practicable in the pre-market phase. Therefore, there may be questions regarding residual risks that should be answered in the post market phase through the use of one or more systematic post-market clinical follow-up studies. In any case, Post-market clinical studies are not intended to replace the pre-market data necessary to make the decision as to approve the device. Post Market clinical follow-up (PMCF) studies are performed on a device within its intended use/purpose(s) according to the instructions for use. It is important to note that PMCF studies must be conducted according to applicable laws and regulations and should follow appropriate guidance and standards. The data and conclusions derived from the PMCF study are used to provide clinical evidence to support the post-market surveillance program and input into the clinical evaluation process.

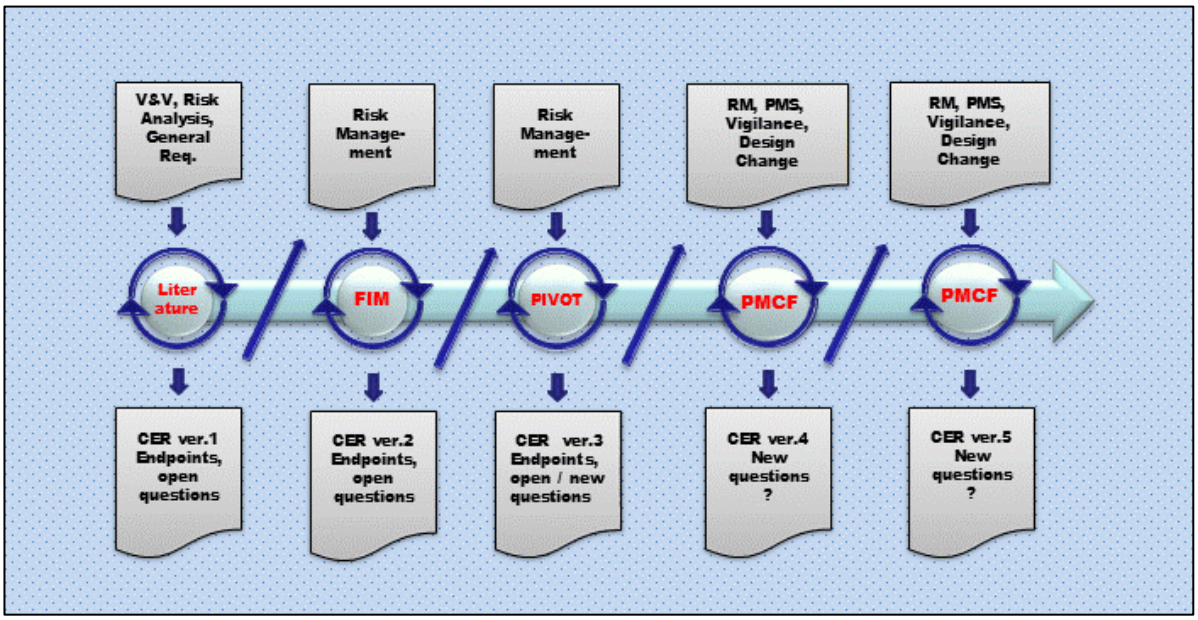

Figure 1: Updating a Clinical Development Plan include the PMCF iterations

This may result in the need to reassess whether the device continues to comply with the Essential Requirements. Such assessment may result in corrective or preventive actions, for example,

changes to the labelling/instructions for use, changes to manufacturing processes, changes to the device design, or public health notifications.

2. PMCF STUDY'S OBJECTIVE

2.1 Intended use of the Medical Device

Polybactum®, is a class IIa medical device with CE mark QPZ-1805-15 (issue date 30/03/2015) presented as vaginal ovules. Its components are.

As reported in its indication for use (IFU), Polybactum® is indicated for use in bacterial vaginosis, after the antibiotic therapy, to reduce the risk of relapses, protecting the vagina from infections (including Gardnerella vaginalis infections) through a barrier effect.

The medical device has to be administered directly by the patient, introducing each ovule deeply in the vagina. The treatment must be done after appropriate treatment of acute bacterial vaginosis (antibiotic therapy. Polybactum should be keep out of the reach of children.

This medical device is intended to be used only in accordance with the Instructions for use.

2.2 Preclinical considerations

Efficacy tests performed both on raw material (lauryl glucoside) and on Polybactum® were:

- *Mucoadhesivity* (*in vivo*): Mucoadhesivity of Polybactum® has been demonstrated *in vivo* for up to 72 hours study. This is the main property on which relies Polybactum® efficacy to impair adhesion of vaginal pathogen on the vaginal mucosa.
- *Film forming properties* of Polybactum® on a human reconstituted vaginal epithelium: it was evaluated through TEER and barrier permeability with a histological analysis. Polybactum® showed film forming properties with all the parameters evaluated.
- *Bacteriostatic activity*: a growth inhibitory activity has been found against, *Gardnerella vaginalis, Streptococcus agalactiae* and *Neisseria gonorrhea* with formulations at 0.15% of LG and with final Polybactum® formulation. No inhibitory effect has been found on *Candida albicans*. With Polybactum® at 50% w/v, a small inhibitory activity on *Lactobacillus crispatus* has been observed.

Safety tests performed both on raw material and on Polybactum® were:

- Barrier test, to evaluate the transepithelial passage of LG through the vaginal mucosa: barrier test on LG alone performed with aqueous solution of 0.01%, 0.1% and 1% of LG showed that the proportion of LG that crosses the vaginal epithelium is about 2% of the amount of LG applied. Barrier tests were also conducted with the final formulation containing various concentrations of LG (Polybactum® at 0.04%). In final formulation, the amount of LG that crosses the vaginal epithelium is negligible, less than 0.2%. Thus, although, when tested alone, a very small amount

of LG is able to pass through vaginal mucosa (i.e. 2%), Polybactum® exerts a strong barrier effect *vis-à-vis* LG as almost no LG crosses the vaginal epithelium.

- In vitro effect of LG alone on Lactobacilli showed that LG has no deleterious effect on vaginal flora.
- Cell viability and TEER with histology analysis, and sensitization tests were performed on LG alone, and LG alone up to 0.1% is not irritant and doesn't have a sensitization effect when tested in vitro.
- Biological safety of device was evaluated through biocompatibility tests: (cytotoxicity, irritation potential and sensitization). Test results showed that Polybactum® does not have cytotoxic effect, is minimally irritant for the vaginal mucosa membrane and is no sensitizing.

Polybactum® was also tested to verify product performance by the following tests:

- Mucoadhesivity: permanence time in vivo.
- Film forming properties of the ovules.
- Bacteriostatic ability of different formulations including the final formulation Polybactum® against vaginal pathogens and natural vaginal flora.

The results are detailed in the Investigator Brochure of the product (last version)

2.3 Risk Analysis Evaluation

In accordance with the requirements of the UNI EN ISO 14971:2012 on the application of risk management to medical devices were carried out:

- A preliminary identification of the characteristics of the devices through the use of the table in Appendix C of the UNI CEI EN ISO 14971;
- The risk analysis, which provides:
  - Identification of risks / hazards applicable to the device.
  - The definition of the risk management team.
  - Risk analysis in order to allow the definition of the estimate, reduction and control of any hazard / risk in accordance with the requirements of UNI CEI EN ISO 14971.
- The assessment of the residual risk.
- The review of the risk analysis based on the information / specifications emerged or changes during pre-production development of the product, and the results of the solutions adopted to control the risk.
- The assessment of compliance with the Essential Requirements described in Annex 1 of Directive 93/42/EEC updated by Directive 2007/47/EC.
- Management of post-production information.

The risk analysis performed on the medical device Polybactum® doesn't present risks that are not acceptable after suitable treatment and management [see table **Error! Reference source not found.**].

The greatest risks of the device Polybactum® are those related to biocompatibility, carcinogenicity and teratogenicity/fetal alterations; these risks were extensively investigated during the preclinical testing of raw material and final product which did not reveal any patent risk. [Report Hazard analysis table].

The analysis of the Essential Safety Requirements (Annex I of Dir 93/42/CEE updated to Directive 2007/47/EEC) has been processed and presented provided evidence of the satisfaction of each of the Essential Safety Requirement of Medical Device Directive (see Report Hazard analysis table).

According to technical specifications and performance features of Polybactum®, the following risks, contra-indications and warnings have been identified:

- Anticipated risks:
  - ✓ Local adverse reactions can be verified in case of hypersensitivity to one or more substances contained into the product.
  - ✓ The use of other vaginal treatment can compromise the efficacy of Polybactum®.
  - ✓ Polybactum® can be dangerous if it interacts with the fetus.
  - ✓ The interaction between Polybactum® and other medicinal products can cause adverse effects.
  - ✓ The product can be compromised in case of damaged box resulting in undesired effects.
  - ✓ The use of Polybactum® after the expiration date can cause adverse effects.
  - ✓ The product can be compromised if the storage conditions are not respected resulting in undesired effects.
  - ✓ The treatment with Polybactum® can adversely affect the effectiveness of contraceptives such us spermicides and diaphragm.
- Contraindications
  - ✓ Do not use in case of known hypersensitivity to one or more substances contained into the product.
  - ✓ Do not use in conjunction with other vaginal treatment.
  - ✓ Do not use in case of damaged box.
  - ✓ Do not use expired product.
- Warnings

- ✓ Polybactum® can be used during pregnancy but should be stopped after rupture of membranes to avoid interactions with the fetus.
- ✓ In order to avoid any interaction with other medicinal products, it is necessary to be informed of any other treatment on-going.
- ✓ The use of spermicidal substances and diaphragm are not recommended (to be tested by specific study) because of the risk to inactivate the contraceptive mean.
- ✓ Keep out of the sight and of the reach of children.
- ✓ Store in a dry place. Keep away from heat and light.

2.4 Clinical Evaluations

The clinical evaluation of POLYBACTUM resulted in a positive risk/benefit ratio for the application, after the assessment of the risks of the products and the benefit with regard to the intended use: a compound that adheres to the vaginal wall to help reducing vaginal infections and protecting the vagina from infections (including *Gardnerella vaginalis* infections) through a barrier effect.

The following considerations have been made:

- the clinical evidence demonstrates conformity with relevant Essential Requirements;
- the performance and safety of POLYBACTUM, for the healing of vaginal epithelium as an adjuvant for the treatment of vaginal bacterial and anaerobic infections and for the prevention of bacterial vaginosis recurrence as claimed have been established;
- the risks associated with the use of the medical device POLYBACTUM containing a solution of polycarbophil and lauryl glucoside are acceptable when weighed against the benefits to the patient;
- the conclusions are valid because the data reviewed can be considered sufficiently representative for disease management;
- the data, taken together with the available safety data, are sufficient to demonstrate compliance with the essential requirements covering safety and performance of the device under normal conditions of use;
- there are no gaps because there is sufficient data demonstrating the safe and effective use of the components;
- the claims made in the device labelling are substantiated by the clinical data.

All the considerations mentioned above validate the conclusion that the claims made in the labelling for the devices, when used in accordance with the label instructions, are substantiated by the clinical data. Although there are a number of study design issues which may introduce bias to the conclusions, these results together with the *in-vitro* and *in-vivo* performance studies supplied by the

manufacturer, firmly support the effectiveness of the Medical Devices for the management of vaginal infections, lesions and related conditions.

The Medical Device POLYBACTUM has been placed and has never been changed.

Since the data of its first commercialisation on the Italian market in 2015, there has been few reports of adverse events or complaints related to the use of the devices, none of which was severe or required hospitalisation and all resolved spontaneously; information available on the product were handled according to applicable procedures for complaint, feedback and surveillance/vigilance activities present in EFFIK quality management system. The PMS information available on the product are handled according to applicable procedures for complaint, feedback and surveillance/vigilance activities present in EFFIK quality management system.

2.5 Objective of the Study

The actual version of Polibactum® Clinical Evaluation Report (CER) demonstrates that this medical device reaches the prefixed scope regarding performance and safety during normal conditions of use, in accordance with Annex X of the Medical Device Directive (MDD) 93/42/EEC as amended by Directive 2007 /47 /EC, and that any claims made about the devices' performance, and safety (e.g. product labelling and instructions for use) are supported by suitable evidence.

In consideration of the indications reported in the current Instructions for Use (IFU) and in accordance with Annex X of the MDD, the current Polibactum® clinical evaluation needs to be kept up-to-date by clinical data obtained during the post market phase. Therefore, the objective of this PMCF study is to assess the safety, performance and long-term efficacy of Polibactum®. Furthermore it will permit us to detect potential emerging risks related to safety on the basis of eventual clinical evidence, through the extended follow up of patients who have used Polibactum® in the previously performed clinical trial OPEFF/0116/MD (POLARIS).

3 APPLICABLE LAWS, STANDARDS AND GUIDELINES

The activities planned in this PMCF study will be performed following the MEDDEV. 2.7.1: 2016, MEDDEV 2.12-1: 2013, MEDDEV 2.12-2: 2012, the International Standards: ISO 14155:2011(en), and EN ISO 14971:2012. In particular, the following ISO 14155:2011 key clauses will be considered in the present study:

- *Ethical Requirements.* That means to protect the rights, safety and well-being of the human subjects that are part of this clinical investigation. In particular, in this PMCF there will be no compensation for subjects participating and no enrolment of patients belonging to vulnerable populations; the study will be approved by the competent Ethical Committees (EC) and all patients will give their informed consent in writing prior to their involvement in the PMCF study.

- *Prior clinical investigation planning*. The following activities will be done in advance of starting the PMCF:
    - a risk analysis, with justification for the design of the clinical investigation based on the evaluation of pre-clinical data and the result of a clinical evaluation of the medical device;
    - the protocol of the study (the present document or clinical investigation plan following the f ISO 14971:2012 Annex A)
    - the monitoring plan;
    - The dissemination to Investigators of sufficient data on device safety and performance to justify human exposure during an investigation;
    - the rationale for selecting the sites.
- *Clinical Investigation conducting*. The following requirements will be performed:
    - Investigator site initiation visits, and monitoring activities;
    - documentation of any adverse events and/or deficiencies related to the medical device under investigation;
    - approval by EC of any amendment to the study;
    - maintained throughout the PMCF study of privacy and confidentiality of each subject enrolled;
    - data control and traceability and medical devices accountability;
    - recording documenting and accounting of enrolled patients and withdrawal;
    - conducting of audit of the PMCF study by the sponsor or its delegated (if requested).
- *Close out of the study.* The following requirements will be performed:
    - the study may be suspended or prematurely terminated by the Sponsor, the Principal Investigator, the EC or a regulatory authority (RA);
    - all the routine activities need to close-out the study will be performed by the Sponsor and/or its delegates;
    - upon the termination of the PMCF study a written final report (FR) will be prepared;
    - the FR and all relevant clinical investigation documents (as detailed in the ISO 14971:2012 Annex E) will be retained by the Sponsor and Principal Investigator as required under applicable regulatory requirements.
- *Responsibilities of the Sponsor.* These include:
    - the planning and conduct of the clinical investigation within prescribed quality assurance and quality control principles.
- *Responsibilities of the Principal Investigator.* These include:

- the management of the day-to-day activities of the study in accordance with the protocol, ensuring the integrity of investigation data, and safeguarding the rights, safety and well-being of the patients.

4 STUDY POPULATION

Corresponding to the CER scope, the patients selected for this PMCF study will be the patients who enrolled and completed the primary efficacy and safety assessment in the previous study *A Multicenter, open label, non-comparative, 3 months study to assess the performance and safety of the new medical device POLYBACTUM® in reducing the frequency of Recurrent Bacterial Vaginosis (Polaris – Polybactum to assess Recurrent Bacterial Vaginosis, Protocol No OPEFF/0116/MD)*.

It is planned to enrol at least 32 patients belonging to the 65-patient population of study OPEFF/0116/MD. These patients will be enrolled in the PMCF study and followed for 6 to 9 months after the end of the OPEFF/0116/MD (that means from 9 to 12 month after the end of the antibiotic therapy performed to cure the bacterial vaginosis).

5 INCLUSION/EXCLUSION CRITERIA

5.1 Inclusion criteria

Patients shall be eligible for the inclusion if all the following criteria are fulfilled:

1) Women above 18 years.
2) Patients who have completed the study OPEFF/0116/MD (last visit performed).
3) Subjects who voluntarily decide to participate in this observational study and sign the Informed Consent Form

5.2 Exclusion criteria

In the present study the criteria regarding the exclusion of pathology, or medical or personal condition, or concomitant treatment potentially interfering with the use of Polibactum®, will not be considered since the study is observational and no administration of Polibactum® is planned.

In addition, patients fulfilling ONE OR MORE of the following exclusion criteria will not be included in the study:

1) Patients who do not want to visit for long-term follow-up observation.
2) Patients who need/want to receive other procedures or treatments for prevention of recurrence of BV after participating in the previous study Protocol No OPEFF/0116/MD
3) Patients concomitantly included in different interventional clinical trials.
4) Patients who are or become ineligible to participate in the present observational study for other reasons by an investigator

5.3 Withdrawal or discontinuation criteria and procedures; patients lost to follow up

Subjects may withdraw from the study at any time at their own request. In this case, the Investigator will try to identify the reason for the withdrawal from the study, without forcing the subjects to provide it. A subject who is withdrawn cannot re-participate in the study. The reason should be documented in the CRF, completing the final visit in the date of discontinuation. The site must inform the CRO personnel when a subject withdraws from the study.

The investigator will complete and sign the appropriate CRF when a patient is lost to follow-up. The date and type of attempted communication with the patient will also be documented. Reason for withdrawals are the following:

1) Withdrawal of informed consent by the subject or his/her representative
2) 2) It is difficult for the subject to continue to be on study (including the case where continued study participation is difficult due to SAEs, ADEs, or the treatment of AEs).

   3) A violation of the inclusion/exclusion criteria is identified during the course of the study: i.e. all of the inclusion/exclusion criteria were met at the time of study enrolment, but subsequently, a violation was newly identified.

   4) Use or requirement of prohibited concomitant medication/treatment: the subject was treated with or is considered to be in need of treatment with the prohibited concomitant medication/treatment specified in section 10.6 of the protocol OPEFF/0116/MD (including other medical devices that may affect the study results).

   5) Lost to follow-up: it is not possible to verify the subject's health status despite attempting contacts by all possible methods.

   6) The subject does not follow the investigator's instructions or does not comply with instructions presented in the ICF which may affect the safety and efficacy assessments.

   7) The Investigator determines that study continuation is not desirable for other reasons

6 SELECTION OF SITES AND SETTING

The present study will carry out when Polibactum® is already on the-market in Italy and in Romania (the two countries where the OPEFF/0116/MD study was performed). The PMCF study will be performed in the 3 Romanian centres, because the two Italian sites cannot accept to perform the follow up period for administrative reasons. Therefore, private clinics in Romania are the setting where this PMCF study is performed.

The participating centres will be centres in which ethical committee has approved the participation in the PMCF study and entered in an agreement to participate.

The CVs and qualifications of clinicians willing to participate to the PMCF study will be reviewed and approved by the Sponsor before study initiation.

7 STUDY DESIGN

The present PMCF is an observational, non-randomized, multicentre study. This design has been chosen to avoid that the protocol therapeutic strategy which could influence the Investigator. In fact, the Investigator decision to treat the patient with Polibactum® administration happened at least 3 months before starting the present study and so the evaluations to find the bacterial vaginosis relapses will be performed by the Investigator in a period without treatment.

The study has been designed to assess the long-term safety, performance and efficacy of Polibactum® administration.

8 ENDPOINTS

8.1 Clinical Outcome and Performance

The primary endpoint will be the recurrence of BV identified by Amsel criteria.

A positive diagnosis for BV requires that three of the following four criteria must be met:

- a vaginal pH of greater than pH 4.5;
- proportion of clue cells ≥20% of total epithelial cells in the vaginal fluid;
- an off-white and thin vaginal discharge;
- fishy odor at whiff test.

Amsel is considered negative (no diagnosis of BV), if 0 or 1 variable are met.

If the two variables shall meet, the test will be considered doubt; therefore, adopting a conservative approach, the patient will be not included in the study if the test has been performed at the baseline visit. On the other hand, when two variables are met in the final visit, the case will be evaluated as a treatment failure.

The following three criteria will be checked at the Clinical Sites:

- Vaginal discharge colour (off-white or white-grey or milky) and type (thin and homogeneous); by direct evaluation of vaginal secretion.
- Vaginal pH; it can be determined directly with the use of pH paper (Merck, Darmstadt, Germany) with range 4.0-7.0. It is placed on the lateral vaginal walls to avoid contact with the alkaline secretion of the cervix. Alternatively, can be used a swab which is touched on pH paper.
- Whiff test: A sample of the vaginal discharge is placed on a glass slide or in a test tube; a drop of 10% potassium hydroxide (10%KOH) is added to evaluated presence of fishy odor caused by the release of amine.

The following criteria will be checked in the Local Laboratory:

- Clue cells: a vaginal smear is collected at the Clinical Center and sent to the Local Laboratory; two drops of vaginal discharge are covered with a coverslip and examined with an optical

microscopy by phase-contrast. Clue cells are identified as vaginal epithelial cells with such a heavy coating of bacteria that the peripheral borders are obscured. The test is positive if the number of clue cells is greater than 20%.

The primary outcome will be measured at the Baseline and at the Final Visit for all patients. The % of RBV will be calculated considering the change from baseline for primary outcome and adding the number of failures reported in the unscheduled visits.

During the 9 months follow up period the investigator will perform phone interviews (at 3, 6, and 9 month) to check the possible BV symptoms, (vaginal discharge, burning, erythema, dyspareunia. If the patient has any of the BV symptoms, the Investigator will perform a visit to verify the BV recurrence by the Amsel criteria. At the end of the PMCF study period the Investigator will perform (if possible) a final visit to confirm the data.

Previous study (Protocol No. OPEFF/0116/MD) duration was 3-month after the antibiotic therapy and with a short follow up after the last Polibactum® administration (about 30 days). The present PMCF will be focused on the long-term follow-up to verify performance and efficacy in a without treatment period.

8.2 Safety

Any adverse events (AE, SAE/ADE/SADE/ADE/DD) reported by patients or evaluated by the investigator PMCF study period will be recorded. The Polibactum® safety will be verified for long-term follow up (9 months) without treatment.

In this PMCF study is not planned the involvement of a DSMB. All safety data will be collected by reporting.

8.3 Procedures in case of Emergency and for SAE/SADE/ADE/DD

The Investigator of each Centre is responsible for ensuring the immediate and correct reporting of any potentially Serious Adverse Event (SAE), Serious Adverse Device Effect (SADE), Adverse Device Effect (ADE) and Device Deficiency (DD) discovered during the study. The Pharmacovigilance Dept. of the Sponsor will coordinate and control the process of AE Reporting and Reconciliation

The emergency contacts for this PMCF study are reported on page 3 of the present document.

8.4 Definitions and Procedure for Reporting AE, ADE, SAE, SADE AND DD

Safety will be assessed in all subjects treated with the investigational device, with frequency and severity of AEs recorded in the CRF taken into account. Any clinically significant abnormalities in vital signs will be recorded as AEs in the CRF. If an AE is observed, the following details will be collected and documented in the CRF: AE term, onset date, outcome, end date, severity, relationship to the investigational device, treatment status, etc.

Adverse Event

An adverse event is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device (IMD). This definition includes:

- events related to the IMD;
- events related to the procedures involved.

For users or other persons, this definition is restricted to events related to IMD.

Serious Adverse Event (SAE)

Any Adverse Event that:

a) led to death,

b) led to a serious deterioration in the health of the subject, users or other persons as defined by one or more of the following:

- a life-threatening illness or injury, or
- a permanent impairment of a body structure or a body function (including chronic diseases), or
- in-patient hospitalization or prolonged hospitalization or,
- medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function.

c) led to fetal distress, fetal death or a congenital abnormality or birth defect (including physical or mental impairment).

Planned hospitalization for a pre-existing condition, or a procedure required by the present CIP, without serious deterioration in health, is not considered a serious adverse event.

Adverse Device Event (ADE)

Adverse Event related to the use of the device under investigation.

This definition includes:

- adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, or operation, or any malfunction of the IMD;
- any event resulting from use error or from intentional misuse of the investigational medical device.

Serious Adverse Device Event (SADE)

Adverse Device Effect that has resulted in any of the consequences characteristic of a Serious Adverse Event.

Unanticipated Serious Adverse Device Event (USADE)

Serious Adverse Device Effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.

Anticipated Serious Adverse Device Event (SADE)

It is a Serious Adverse Device Effect which by its nature, incidence, severity or outcome has been identified in the risk analysis report

Device Deficiency (DD)

Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance.

- Device Deficiencies include malfunctions, use errors, and inadequate labelling.
- All Device Deficiencies that might have led to a Serious Adverse Device Effect shall be reported in accordance with Serious Adverse Event reporting procedures, as specified in this section.

Adverse Event classification

The Investigator is responsible for the AE classification, following Annex F of ISO14155 (reported in Annex 2)

Adverse Event Relatedness

The Investigator will be responsible for assessing the causal relationship of the AE. Specifically, the investigator will report whether the AE was related to the procedures or IMD administration.

The causal relationship for each adverse event will be rated as follows:

- Unrelated: The event is not related to the procedures, IMD, or progression of disease.
- Unlikely Related: The temporal sequence is such that the relationship is unlikely. It is unlikely there is any relation between the event and the procedure, IMD, or progression of disease.
- Possibly Related: The temporal sequence is such that the relationship is not unlikely or there is no contradicting evidence that can reasonably explain the subject's condition. There is a possibility of any relation between the event and the procedure, IMD, or progression of disease.
- Related: The temporal sequence is relevant, or the event abates upon completion of the procedure, IMD, or the event cannot be reasonably explained by the subject's condition or comorbidities. The event is related or most likely associated with the procedure, IMD, or progression of disease.

Adverse Event Severity

Each AE should be assessed for its severity, or the intensity of an event, experienced by the subject.

- Mild: Awareness of a sign or symptom that does not interfere with the subject's activity or is transient and is resolved without treatment or sequelae.
- Moderate: May interfere with the subject's activity and require additional intervention and/or treatment and may have additional sequelae.

- Severe: Significant discomfort to the subject and/or interferes with the subject's activity. Additional intervention and/or treatment are necessary. Additional sequelae occur. Severe is used to describe the intensity of an event experienced by the subject.

Adverse Event Reporting

All adverse events will be reported by the Investigator and reviewed by the Sponsor in compliance with applicable regulations. Adverse events may be volunteered by subjects, elicited by the investigator or designee, or collected via observation by the investigator.

All AEs will be assessed by the investigator who will determine whether or not the event is related to the procedure, related to the study device, and whether or not the event meets serious criteria. If it is determined that an AE has occurred, the investigator should obtain all the information required to complete the AE form on the CRF. Documents must be submitted to the sponsor within a timely manner to ensure timely assessment of the event as appropriate.

In addition, subjects will be instructed to contact the investigator or a member of their care team if any significant adverse events occur between study visits.

An adverse event assessment will be performed at each visit. Adverse events are reported starting from the day of study procedure until subject participation has ended (i.e., completion of study or withdrawal of consent). All adverse events must be followed until resolution, AE has stabilized, or the study has been completed.

Pre-existing medical conditions or symptoms observed prior to the study procedure date will not be recorded as an AE and should be collected in the subject's medical history. In the event there is a change (i.e., worsening) in the pre-existing medical condition or symptoms after the study procedure then an AE must be reported.

Serious Adverse Event Reporting

A completed SAE form must be entered in the clinical study database of the Sponsor within 24 hours of knowledge of the event according to national requirements.

Withdrawal from the study and all therapeutic measures will be at the discretion of the investigator. All SAEs will be followed until satisfactory resolution or until the investigator deems the event to be chronic or the subject to be stable.

The Sponsor will notify the regulatory agency of any unexpected, fatal, or life-threatening experience (expedited report) associated with the study as soon as possible but no later than ten working days after becoming aware of the event.

Copies of any reports to regulatory bodies regarding serious and unexpected AEs will be provided to the investigators for review and submission to the EC. The communication of any SAEs to the EC and Competent Authority if need, will be done according to national requirements in Romania by the Investigator). Copies of SAEs correspondence with the investigators, regulatory bodies, and

Sponsor must be retained with study records. In Annex 3 of the present PMCF protocol are reported the events considered reportable following the Annex 7, section 2.3.5 and Annex X, section 2.3.5 of Directives 90/385/EEC and 93/42/EEC respectively.

Treatment for adverse events

1) Drug treatment
2) Non-drug treatment
3) Drug and non-drug treatment
4) No treatment

Outcome

1) Recovered (resolved)
2) Recovering (resolving)
3) Not recovered (not resolved)
4) Recovered (resolved) with sequelae
5) Death
6) Unknown

Action taken for investigational device

1) Application discontinued
2) Dose decreased (duration of application decreased)
3) Dose increased (duration of application increased)
4) No dose (duration) change
5) Unknown
6) Not applicable

9 DEVICE UNDER INVESTIGATION

The Polybactum® medical device was used following the IFU during the previous trial OPEFF/0116/MD by all the patients enrolled in this PMCF study. In particular, in the OPEFF/0116/MD study, the patients have followed carefully the reported directions before device use, use, storage and maintenance (for additional information: Polybactum® IFU, revision May 2015). The patient population selection was done following the inclusion/exclusion of this PMCF study and of the previous OPEFF/0116/MD study (reported as contraindications in the IFU). Finally, the Investigator will keep in mind the precautions written in the IFU and the following warnings:

The device should be used only for its intended use.

do not use the device in case of damaged box;

do not use expired product.

In addition, the following warning were considered for OPEFF/0116/MD study only, because in the PMCF no Polybactum® administration is planned:

product for vaginal use only;

do not use in case of known hypersensitivity to one or more substances contained into the product;

do not use in conjunction with other vaginal treatment;

treatment should be stopped after rupture of membranes in case of pregnancy.

The use of spermicidal substances and diaphragm are not recommended because the risk to inactivate the contraceptive mean cannot be excluded.

10 CONCOMITANT TREATMENT

During all the PMCF study the following care and interventions are not allowed:

- Oral or vaginal antibiotic therapy or other vaginal therapies (like douching, spermicide);
- Oral or vaginal probiotics (e.g. vaginal lactobacilli);
- Other products or medication to treat or for prevention of BV.

In addition, the following concomitant treatments were not allowed during the OPEFF/0116/MD study only, because in the PMCF no Polybactum® administration is planned:

- Vaginal tampons.
- Use of an etonogestrel/ethinyl estradiol vaginal ring (Nuvaring®) or an intrauterine device.

11 DURATION OF PMCF STUDY AND ITS TERMINATION

The total duration of study (per subject) will be the following:

- 9±2 months (from Baseline Visit to Closure Visit)

The initial data collection will start during the final visit of OPEFF/0116/MD study (day 72 to day 84 from end of antibiotic therapy)

The final data collection (last study observation point) should be at the 9±2 months follow up (after the final visit of OPEFF/0116/MD study visit or at the end of the study, whichever is first.

The following timing for assessments and scheduled visits is given as a suggestion and is not to be used to define a specific diagnostic or therapeutic strategy for the patients. In fact, being an observational study, all decisions in this regard should be taken following the standard clinical practice of the centre and at the discretion of the Investigator.

Therefore, the following timing for assessments has presented only to assure that the collected data will be as homogeneous as possible:

	Screening Baseline Visit	**Telephone Call 1 Month 3***	**Telephone Call 2 Month 6****	**Final Visit (or Final Telephone Call) Month 9*****
Planned activities →	A	B	B	C
Output to the Sponsor →	D	D	D	D

Figure 2: timing for assessments
**= it will be accepted a ± 15 days window*
***= it will be accepted a ± 30 days window*
****= it will be accepted a ± 60 days window*

A = Screening and Baseline visit.

- physical examination;
- concomitant medications/treatments recordings;
- confirmation of BV absence by a negative test with Amsel criteria. This means that only 0 or 1 variable is met;
- ADE/ SADE/ USADE recordings.

This visit matches with the final visit of OPEFF/0116/MD study

B = Phone contact with interview.

- assessment of BV symptoms;
- concomitant medications/treatments checking;
- ADE/ SADE/ USADE recordings.

If the patient has any of the BV symptoms after baseline, the Investigator can decide to perform an unscheduled visit to verify the BV recurrence by the Amsel criteria.

C = Final visit with gynecological examination and check for Amsel criteria.

- physical examination;
- concomitant medications/treatments recordings;
- confirmation of BV absence by a negative test with Amsel criteria. This means that only 0 or 1 variable is met;
- ADE/ SADE/ USADE recordings.

If it is not possible perform the visit, a phone call with interview will be done.

D = Annex 4 provides the example of the format (e-CRF) which will be used to collect the evaluation data.

The Sponsor may decide the premature termination of the PMCF study in a Clinical Centre for any of the following reasons:

- the Centre cannot include an adequate number of subjects;
- serious and/or persistent non-compliance with the study;
- careless or premeditated false documentation in the CRFs;

- non-compliance with GCP, ISO 14155:2011 or competent authority /EC requirements;
- the Investigator asks to discontinue the study.

The timeline of the PMCF study is the following:

Activity/Step	Time to perform the activity
Enrolment period &Observation period First Patient In - Last Patient Out (FPI~LPOI)	9-12 months
Data collection period	2 months
Statistical Analysis (from Data Base Lock to Statistical Report)	2 months
Final Report (with performance and safety analysis)	2 months

Figure 4: timeline of the PMCF study

12 DATA COLLECTION AND MANAGEMENT

All data required by the protocol will be collected through electronic Case Report Forms (e-CRFs), and all information recorded in the e-CRFs should be consistent with source documents. Data collection through e-CRFs should be conducted in compliance with instructions for e-CRF preparation. By signing electronically, the investigator confirms that the e-CRF data entered are accurate, complete, legible, and timely. Data entered in the e-CRF will be transferred subsequently into a format that would allow statistical analysis to be performed as per the PMCF protocol requirements. According to each user's task, different profiles and permissions will be given to fill the e-CRF (i.e. to Investigators, to CRA, to Sponsor delegates). Each authorized user can update his or her own data and change the password securely.

The format provided will give the Investigators a simple and guided interface for data input. Most fields will be encoded through multiple choice selectors, thus minimizing free texts and facilitating input. All notified adverse events will be processed in real time and generate reports according to pharmacovigilance requirements. All clinical data will be transmitted through strong encryption according to HTTPs – SSL3 standard and through a web server secured by international certificates. The used platform will be fully compliant with FDA (CFR 21 Part 11 requirements for Electronic Clinical Data) and with CPMP/ICH135/95 indications. All user inputs and changes will be traced explicitly and recorded in Audit Trials (Tracking Table and Admin Tracking Table). The history of each single data will be available to Investigators, CRA and Sponsor delegates. Furthermore, each Investigator will have access to summary of all past input/changes for their own procedures. The investigators will not have access to inputs from other participating centres.

The database will be designed following the procedures reported in the Data Management Plan, enclosed in the Trial Master File, to be issued prior to study initiation. The database will be validated following a formal, documented validation plan, and the system will be tested against the specifications for the entry system. The database will be tested and validated to confirm the following characteristics: relational structure; simple standardized procedures for screen design; allowance for built-in quality assurance procedures; flexibility for system changes; large storage capacity; and compatibility and direct interface with analysis software.

Data cleaning will be performed according to the specifications outlined in the data management plan. All queries will be documented in data clarification forms which are recorded in the data base including the respective query resolutions.
The Sponsor will be responsible for all these activities. The confidentiality of records that could identify subjects will be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

At the end of the study, all study data and documents (signed consent forms, ethics committee approval letters, correspondence) will be archived securely in each centre by the Investigator responsible for a minimum of 15 years from the end of the study.

13 SAMPLE SIZE DETERMINATION AND STATISTICAL ANALYSIS

No sample size calculation was required for this PMCF observational study, since it was a follow-up study and only subjects belonging to the OPEFF/0116/MD study in Romania were enrolled. No d.o. or patients with recurrence in OPEFF/0116/MD study were included in the follow up). The patients belonging from Italian sites of OPEFF/0116/MD study will not be included due to administrative reasons in the present follow up observational study.

Statistical analyses will be conducted on all patients entered in the study. The quality and completeness of the collected data will be evaluated preliminarily compared to data analysis. If a patient is missing information for one or more variables, the missing data will not be replaced.
Quantitative variables (i.e. demographic) if normally distributed will be described through mean, standard deviation (SD); variables non-normally distributed will be described using median and range of interquartile. The Student's t-test and the Mann-Whitney U will be employed to perform comparative analysis in accordance to the distribution of these variables. In order to evaluate changes over time before and after the treatment, Paired t-test (if applicable) or Wilcoxon signed rank sum test will be performed for quantitative variables, while McNemar test will be used in order to evaluate changes for binary variables, while symmetry test will be performed in order to evaluate changes for qualitative (not binary) variables.

Categorical variables will be described using frequencies and percentages and comparative analysis using a Chi2 test.

All statistical analyses will be performed using the R statistical software v 3.5. The final analysis (including performance and safety data) will be completed after all eCRF have been filled, and the database has been locked.

14 QUALITY CONTROL

Sponsor Standard operating procedures (SOP) will guide the conduct of this PMCF study. These procedures include internal quality audits, rules for secure and confidential data storage, methods to maintain and archive project documents, quality-control procedures for programming, standards for writing analysis plans, and independent scientific review. The Sponsor can also delegate an independent Quality Assurance Consultant to perform audits that involve various aspects of the study. Such audits will be conducted according to the Sponsor Standard Operating Procedures (SOP) and other applicable procedures.

15 LIMITATION AND WEAKNESSES

A weakness of the present PMCF study is that several variables could affect the response of interest in a observational setting, and so it could be difficult to isolate their effects. As a result, interrelations among variables could be weaker than in classical randomized clinical trials (RCT). Another weakness could be the lack of precision in the measurement of several variables due to the use of telephone call to check the BV recurrence at month 3 and 6. We think these limitations and weaknesses could balance the strengths of this PMCF study, which has the heuristic quality of being close to real life and performed in a non-artificial setting.

16 COMPLIANCE TO ETHICS AND CONFIDENTIALITY OF COLLECTED DATA

The Sponsor of the present PMCF study is Effik Italia SpA (Italia).

Prior to the initiation of the study, the PMCF protocol, patient statement for privacy, patient consent form will be submitted to the EC of the Site for review and approval. The names of all subjects will be kept in confidence, and for the purpose of recording and assessment, the subjects will be identified by the number assigned in the study. The subjects will be informed that all clinical data will be stored in the computer and kept strictly confidential. The Investigator of each Centre is responsible for and will obtain written informed consent (ICF) from the patients involved in the PMCF study. In addition, they will be given an informational document on the protection of personal data. This document will explain who is responsible for the data resulting from the treatment/intervention of the patients in each centre, according to local legislation. By signing the protocol, the principal investigator agrees to appropriately obtain the IC from the patient, and to be inspected if requested.

Each subject enrolled will be identified by the subject number, a five digits code (i.e. 01.001), which will be the only identification element and will be used only for the purposes of this PMCF study. This code will consist of the Clinical Centre number (i.e. 01 corresponding to the Investigational Centre 01) and of the sequence number of the patient (i.e. .001 that means the 1st patient enrolled in this Centre). The Investigator will have a list of subject numbers and subject names in place to enable data retrieval in the future. The present study is observational and therefore, no insurance is requested.

17 PUBLICATION AND DATA SHARING POLICY

All information regarding this PMCF study, will be regarded as confidential. The Investigator of each Centre agree that scientific results from this study are to be considered the property of the Sponsor. Information regarding the operations and procedures of the Sponsor, obtained as a result of or in association with the conduct of this study, must be kept confidential.

Unpublished information contained herein, as well as any information received from the Sponsor for the purposes of this study, may not be disclosed to any third party without the prior written approval of the Sponsor.

The final version of this PMCF study will be registered on www.clinicaltrials.gov.

When reporting results of the study, the checklist entitled Strengthening the Reporting of Observational Studies in Epidemiology (STROBE, 2007) will be followed.

The Sponsor reserves the right to use the results obtained as documentary and scientific backing in proceedings regarding the regulatory authorities and/or for updating their own staff.

The Investigators and Sponsor agree that it needs to ensure the widest publication and dissemination of data in a coherent and responsible manner, allocating credit appropriately. The Sponsor is committed to publication of results after study conclusion through conference presentations, scientific journals, ministerial bulletins, direct communications to the IEC or other channels.

18. REFERENCES

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