CLINICAL STUDY PROTOCOL

IND NUMBER 104785/EUDRACT NUMBER 2015-005787-42

A Phase 2, Dose-ranging, 12-week Randomized, Double-blind, Placebo-controlled, Parallel-group Study Evaluating the Efficacy and Safety of Three Formulations of Ultra-low Dose Estriol Vaginal Gel (0.005% Estriol Vaginal Gel, 0.002% Estriol Vaginal Gel, 0.0008% Estriol Vaginal Gel) for the Treatment of Vaginal Dryness in Postmenopausal Women with Vulvovaginal Atrophy

Protocol No. ITFE-2092-C1

Sponsor:	ITF Research Pharma SLU c/ San Rafael 3 28108 Madrid, Spain
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Version of Protocol:	Final 5.0
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CONFIDENTIAL

The study will be conducted according to the International Conference on Harmonisation Harmonised Tripartite Guideline E6 (R1/R2): Good Clinical Practice.

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Protocol Synopsis

Protocol Number:	ITFE-2092-C1
Title:	A Phase 2, Dose-ranging, 12-week, Randomized, Double-blind, Placebo-controlled, Parallel-group Study Evaluating the Efficacy and Safety of Three Formulations of Ultra-low Dose Estriol Vaginal Gel (0.005% Estriol Vaginal Gel, 0.002% Estriol Vaginal Gel, 0.0008% Estriol Vaginal Gel) for the Treatment of Vaginal Dryness in Postmenopausal Women with Vulvovaginal Atrophy
Sponsor:	ITF Research Pharma SLU San Rafael 3 28108 Madrid, Spain
Study Phase:	Phase 2
Study Sites:	Approximately 25, in 5 European countries (Spain, Italy, Sweden, Hungary, Czech Republic).
Indication:	Treatment of vaginal dryness in postmenopausal women with vulvovaginal atrophy
Rationale:	Local estrogen therapy is the recommended approach to treat symptoms related to postmenopausal vulvovaginal atrophy. An estriol-based formulation allowing a lower dose per application provides reduced estrogen exposure while maintaining efficacy, resulting in fewer systemic effects and a more favorable risk-benefit ratio for the patient.
Objectives:	Primary objective: Evaluate the efficacy of 0.005%, 0.002%, and 0.0008% estriol vaginal gel and determine the minimal effective dose for the treatment of postmenopausal vaginal atrophy in women who report moderate to severe vaginal dryness as the most bothersome symptom. Secondary objectives: Evaluate the efficacy of the three formulations of estriol vaginal gel in the improvement of other symptoms and signs of vulvovaginal atrophy. Evaluate the safety and tolerability of the three formulations of estriol vaginal gel. Exploratory objectives: Evaluate the final subjective global perception of efficacy of the three formulations of estriol vaginal gel. Evaluate the subject`s acceptability of the three formulations.

Subject Population:

Inclusion criteria:

1. Capable of understanding the written informed consent, provides signed and witnessed written informed consent, and agrees to comply with the protocol procedures and assessments
2. Age ≥40 and ≤80 years
3. Postmenopausal (≥12 months since last spontaneous menstrual period, or having 6 months of spontaneous amenorrhea with serum FSH levels >40 IU/L, or ≥6 weeks since bilateral oophorectomy with or without hysterectomy)
4. BMI ≤36 kg/m^2
5. Vaginal Maturation Index ≤ 5% superficial cells on a vaginal smear
6. Vaginal pH >5
7. Moderate to severe vaginal dryness currently reported as the most bothersome symptom of vaginal atrophy.
8. Negative mammogram at screening or documented negative mammogram within 9 months prior to randomization, with normal breast examination at screening.
9. Negative Papanicolau test at screening (in women with cervix).

Exclusion criteria:

1. Subjects with contraindications for hormone therapy with estrogens such as those diagnosed or history of: malignant and premalignant lesions of the breast and/or endometrium, malignancy of the colon, malignant melanoma, hepatic tumor, venous thromboembolic conditions (including deep vein thrombosis or pulmonary embolism), arterial thromboembolic conditions (including angina pectoris, myocardial infarction, or cerebrovascular accident), coagulopathies, vaginal bleeding of unknown etiology, acute liver disease or a history of liver disease as long as liver function tests have failed to return to normal, or porphyria.
2. Subjects who have abnormal laboratory values at screening that the investigator considers clinically relevant for the purposes of the study.
3. Subjects with any medical-surgical pathology which is not controlled at the time of inclusion in the study.

Subject Population:

Exclusion criteria (continued):

4. Subjects with any acute or chronic condition whose management or progression may interfere with the subject´s participation in the study.
5. Subject with uncontrolled hypertension (≥140 mmHg systolic blood pressure and/or ≥90 mmHg diastolic blood pressure).
6. Subjects with Grade II or higher utero-vaginal prolapse.
7. Subjects with uterine polyps.
8. Subjects with symptomatic and/or large uterine fibroids (>3 cm) and/or palpable fibroids at gynecological examination.
9. Subjects who have had urogenital surgery within 3 months of baseline visit.
10. Subjects with signs and symptoms suggestive of infection of the genital or urinary tract requiring treatment at the start of the study.
11. In women who have a uterus, evidence of hyperplasia, cancer or other endometrial pathology in endometrial biopsy.
12. Subjects who have received the following treatments within the specified time periods prior to screening procedures: any type of non-hormonal vulvovaginal treatment in the 7 days (including cosmetics expected to have an impact on vaginal pH such as special feminine wash gels); phytoestrogens by any route within 1 month; vaginal hormone therapy within 1 month; hormone therapy (estrogen alone, progestin alone or estrogen/progestin combination) by oral, intrauterine or transdermal route within 2 months; progestational implants, estrogen, or estrogen/progestational injectable within 3 months; estrogen pellet therapy or progestin injectable drug therapy within 6 months; percutaneous estrogen lotions or gels within 1 month; testosterone or testosterone derivatives, DHEA, tibolone, or SERMs by any route within 2 months;
13. Subjects receiving antiepileptic drugs (barbiturates, hydantoins, carbamazepine), certain antibiotics and other antiinfective medicinal products; phenylbutazone; preparations based on medicinal plants that contain St. John’s Wort.

Subject Population:

Exclusion criteria (continued):

14. Subjects who are allergic to any of the components of the medication under study.
15. Subjects who are currently participating or have participated in the experimental evaluation of any product within 8 weeks of the start of the study.

Study Design:

Dose-ranging, 12-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group study.

Estimated Study Duration:

Study duration will be approximately 20 weeks from the Screening Visit to End-of-Study phone call. Subject exposure to study drug will last 12 weeks, with a total of up to 6 study visits (including one telephone call visit).

Efficacy Assessments:

The co-primary efficacy variables in this study are:

- Mean change from Baseline to Week 12 in the proportion of superficial and parabasal cells of the vaginal epithelium on vaginal smear;
- Mean change from Baseline to Week 12 in vaginal pH;
- Mean change from Baseline to Week 12 in the severity of vaginal dryness

The secondary efficacy variables:

- Mean change from Baseline to Week 3 in the proportion of superficial and parabasal cells of the vaginal epithelium;
- Mean change from Baseline to Week 3 in vaginal pH;
- Mean change from Baseline to Week 3 in the severity of vaginal dryness
- Mean change of other individual vaginal symptoms (dyspareunia, pruritus, burning and dysuria) and signs (pallor, friability, thinning or flattening of folds, petechiae, and dry mucosa) of vaginal atrophy from Baseline to Week 3 and from Baseline to Week 12
- Mean change in the Global Symptom Score from Baseline to Week 3 and from Baseline to Week 12

The exploratory efficacy variables:

- Evaluation of the final subjective global perception of efficacy by subjects at Week 12.
- Evaluation of the acceptability of the therapy at Week 12.

Safety Assessments:	The safety variables in this study are: • Evaluation of adverse events • Histological evaluation of the endometrium by endometrial biopsy • Evaluation of the endometrium by ultrasonography • Change in hormone levels • Change in clinical safety laboratory parameters • Safety assessments of lipids and of carbohydrate and coagulation parameters (antithrombin III, factor V Leiden, protein-C and protein-S)
Study Drug, Dosage, and Route of Administration:	0.005% estriol vaginal gel, 1 g (50 µg estriol/intravaginal application dose), 0.002% estriol vaginal gel, 1 g (20 µg estriol/intravaginal application dose), 0.0008% estriol vaginal gel, 1 g (8 µg estriol/intravaginal application dose), placebo vaginal gel, 1 g (intravaginal administration)
Sample Size:	A total of 280 subjects randomly assigned equally to 0.005% estriol vaginal gel, 0.002% estriol vaginal gel, 0.0008% estriol vaginal gel, or placebo.

Statistical Methods:

The Intent-to-Treat (ITT) Population, the Per Protocol (PP) Population, and the Safety Population will be used to present the results.

Changes from Baseline to Week 12 in vaginal dryness will be analyzed using a Wilcoxon Rank Sum test, controlled for country, to obtain the p-values. The changes from Baseline to Week 12 in vaginal pH and the changes in proportions of parabasal and superficial cells will be analyzed using a parametric ANCOVA. The model will include fixed effects of treatment and country, and the baseline value will be used as a covariate. Model assumptions will be examined and, if required, a Wilcoxon Rank Sum test as described for vaginal dryness will be used. Missing values will be estimated using last observation carried forward (LOCF). Only post-Baseline values will be carried forward.

For the co-primary endpoints (change from Baseline to Week 12 in proportion of superficial and parabasal cells of the vaginal epithelium, pH, vaginal dryness), a gatekeeping procedure will be employed. 0.005% dose will be compared to placebo, and if this is significant at a one-sided alpha level of 0.025 then the other doses versus placebo can be considered in order of strength (strongest first); if all four co-primary endpoints are significant at the one-sided 2.5% level, then the next lowest dose can be presented; this approach will avoid any issues of multiple testing with four co-primary endpoints and three comparisons.

The change from Baseline to Week 12 in the severity of individual vaginal symptoms (dyspareunia, dryness, pruritus, burning and dysuria) and signs (pallor, friability, thinning, petechiae, flattening of folds and dry mucosa) will be assessed using the same Wilcoxon Rank Sum test as for the co-primary endpoint of vaginal dryness at Week 12. The change from Baseline to Week 12 in the global symptom score will be assessed using the same parametric ANCOVA as for the co-primary endpoints of vaginal pH and proportions of parabasal and superficial cells at Week 12. The Week 3 efficacy endpoints will be assessed using similar methodology as Week 12.

Endpoints of acceptability of therapy, final subjective global perception, and safety analyses will be summarized by treatment group.

Date of Protocol:

07 June 2017

List of Abbreviations

Abbreviation	Definition
AE	adverse event
ANCOVA	analysis of covariance
BMI	body mass index
CFR	Code of Federal Regulations
CRO	contract research organization
CSA	clinical study agreement
E1	estrone
E2	17β-estradiol
E3	estriol
ECG	electrocardiogram
eCRF	electronic case report form
ER	estrogen receptor
FDA	US Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GI	Gastrointestinal
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	independent ethics committee
IRB	institutional review board
ITT	intention to treat
IWRS	Interactive Web Response System
LH	luteinizing hormone
LOCF	Last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
OTC	over-the-counter
PP	per protocol
PV	pharmacovigilance
SAE	serious adverse event
SAP	statistical analysis plan
SERM	selective estrogen receptor modulator
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TVU	transvaginal ultrasound

1 Introduction

Vulvovaginal atrophy is a natural consequence of the progressive estrogen deficiency that occurs in menopause. Epidemiological data have indicated that about 50% of otherwise healthy women over 60 years of age experience symptoms related to urogenital atrophy such as vaginal dryness, dyspareunia, burning, itching, as well as urinary complaints or infections of the lower urinary tract. As these alterations frequently affect the quality of life of postmenopausal women, it is important for doctors to detect their presence and offer treatment options.

Estrogen therapy is the most effective treatment of moderate to severe symptoms of vulvar and vaginal atrophy. Scientific societies agree that when estrogen therapy is considered for treatment of vaginal atrophy, local treatment is advised (The 2007 Hormone Therapy Position Statement of the North American Menopause Society, 2007). One advantage of local treatment with estrogen is avoidance of first-pass liver metabolism, making it possible to use lower doses of estrogen compared with oral therapy; the local route also minimize systemic adverse effects.

The primary risks of taking an estrogen product after menopause is that it can cause or exacerbate certain types of tumors, such as breast or endometrium. Taking low dose estrogens vaginally decreases the risk of the occurrence of these tumors, as a consequence of the very low doses employed that permit a local vaginal effect while the potential of absorption is minimized. It has been shown that lower doses of vaginal estrogen therapy than previously used, even with less frequent administration, often yield satisfactory results. The lowest effective dose of estrogen consistent with treatment goals, benefits, and risks for the individual woman should be the therapeutic goal (The 2012 Hormone Therapy Position Statement of the North American Menopause Society, 2012).

Estriol (E3) is one of the most important estrogens produced in the human body, along with 17β-estradiol (E2) and estrone (E1). Estriol is normally produced in the liver by irreversible transformation of estradiol and estrone. This process is considered to be a metabolic detoxification mechanism, by which potent estrogens are transformed into less active metabolites such as estriol. Also, estriol is produced in significant amounts during pregnancy as it is synthesized by the placenta. In all instances, there is no conversion from estriol to estradiol or estrone.

The physiological effect of estrogens is complex: they exert their action through the activation of specific nuclear receptors, and different estrogens bind with different affinities to their receptors. Two subtypes of estrogen receptors (ERs) have been identified; these subtypes are ERα and ERß, with different tissue distributions, although there is some overlap in their expression, as ERα is most abundantly expressed in the endometrium, breast, and ovarian stroma. The relative binding affinities of different estrogens for their receptors has been investigated in different models against 17ß-estradiol, the most potent natural estrogen. In this sense, the binding affinities of estriol to ERα and ERß have been shown to be 14% and 21% of those of estradiol, respectively (Gruber CJ, 2002). Also, the differential expression of receptors in tissues results in topological (tissue) differences in the affinities of the various estrogens. Compared to estradiol, estriol shows a relatively high affinity for the receptors in bladder and vaginal tissue, but relatively low affinity for receptors in the uterine and breast tissue (Bergink EW, 1984). The lower affinity in the uterus results in minimal or no uterotropic influence with estriol exposure (Takahi K, 2000) (Raz R, 1993) (Padwick ML, 1986) (Reynolds TM, 1993). The non-uterotrophic effect is also attributed, in part, to its relatively short duration of nuclear receptor binding and rapid plasma clearance (Cardozo L, 1998) (van Haaften M, 1997).

Other claimed advantages of estriol over other natural estrogens include a protective effect against development of breast cancer, minimal mitogenic activity, less effect on plasma lipids and coagulation factors/platelets, and an ability to reduce/block the uterotrophic activity of other estrogens (competition with receptor binding) (Takahi K, 2000) (Padwick ML, 1986) (Hayashi T, 2000) (Nakayama H, 1999).

Estriol formulations have been marketed since 1958 for the treatment of vulvovaginal atrophy in more than 50 countries worldwide, including all European countries, but not the United States. Topical (vaginal) formulations are currently available as creams (containing 1 mg estriol/1 g cream) or as ovules (containing 0.5 mg estriol). Local vaginal estrogen therapy can also be administered in the form of estradiol vaginal tablets or vaginal creams, estradiol-releasing vaginal rings and conjugated estrogen-based vaginal creams.

The search for therapeutic alternatives which may present improvements in relation to the current products has been encouraged. I

[redacted]

Clinical research to develop this product was performed in European populations under European guidelines. [redacted]

2 Study Objectives

2.1 Primary Objective

The primary objective of this study is to evaluate the efficacy of 0.005%, 0.002%, and 0.0008% estriol vaginal gel and to determine the minimal effective dose for the treatment of postmenopausal vaginal atrophy in women that refer moderate to severe vaginal dryness as the most bothersome symptom.

2.2 Secondary Objectives

The secondary objectives of this study are to:

- Evaluate the efficacy of the three formulations of estriol vaginal gel in the improvement of other symptoms and signs of vulvovaginal atrophy.
- Evaluate the safety and tolerability of the three formulations of estriol vaginal gel.

2.3 Exploratory Objectives

The exploratory objectives of this study are to:

- Evaluate the final subjective global perception of efficacy of the three formulations of estriol vaginal gel.
- Evaluate the subject`s acceptability of the three formulations of estriol vaginal gel.

3 Investigational Plan

3.1 Study Design

This study is a double-blind, randomized, dose-ranging, placebo-controlled multicenter study with four parallel groups. Approximately 25 sites in Europe will be included in the study. The study setting will include academic hospitals and gynecological clinics. The study population is postmenopausal women who report moderate to severe vaginal dryness as their most bothersome symptom in addition to other symptoms and/or signs of postmenopausal vulvovaginal atrophy.

After providing informed consent, potential subjects will be evaluated during the screening period to determine if they are eligible to participate in the study. A screening identification number will be assigned to each eligible subject according to their order of arrival into the study at screening visit. Subjects who meet all of the inclusion criteria (see Section 4.1.1) and none of the exclusion criteria (see Section 4.1.2) will be randomized into the study. A total of 280 subjects will be enrolled and randomly allocated to one of the four treatment groups (0.005%, 0.002%, or 0.0008% estriol vaginal gel or placebo vaginal gel); the allocation to treatment group will be 1:1:1:1. A blocked randomization across the entire study (i.e. all sites as a whole) will be used. Subjects will be assigned a randomization code according to their order of arrival into the study at the Baseline Visit. Initial treatment consists of daily vaginal administration of 1 gram of randomly-assigned estriol gel or placebo gel using the supplied applicator. After 21 days of daily administration, treatment will continue with twice-weekly administration up to Week 12 (i.e., Mondays and Thursdays).

The duration of trial participation for each woman is expected to be approximately 20 weeks (from Screening to End-of-Study phone call) although the time on study drug will be approximately 12 weeks. Subject completion is defined as the completion of all phases of the study, including the End-of-Study phone call to be performed for safety purposes at Week 16.

An overview of the study visit procedures is given in the Schedule of Events (Table 1). Details of specific procedures and testing are provided in Section 6.

Table 1: Schedule of Events

Procedure	Screening Visit	Baseline	Week 3	Week 8	Week 12/ Early Termination	Week 16/ End of Study Phone Call
Visit Window (days)	**–30 to -1**	**1**	**21 ±3**	**56 ± 5**	**84 ± 5**	**112 ± 5**
Informed consent	X					
Randomization		X				
Medical history, medication history, and demographics	X					
Physical examination [a]	X				X	
12 lead electrocardiogram [b]	X				X	
Gynecological examination [c]	X				X	
Transvaginal ultrasound (women who have a uterus)	X				X	
Mammogram (if no negative mammogram documented within 9 months prior to randomization)[d]	X					
Clinical laboratory tests [e]	X				X	
Endometrial biopsy (women who have a uterus)	X				X	
Adverse event reporting [f]	X	X	X	X	X	X
Concomitant medication reporting	X	X	X	X	X	X
Evaluation of vaginal dryness [g]	X		X		X	
Evaluation of symptoms and signs of vulvovaginal atrophy [g, h]		X	X		X	
Vaginal pH	X		X		X	
Vaginal smear for evaluation of vaginal cytology	X		X		X	
Evaluation of global efficacy [i]					X	
Evaluation of acceptability [j]					X	
Administration of the first dose of the study medication [k]		X				
Study drug and supply dispensing [l]		X	X	X		
Reconciliation of returned study drug			X	X	X	

Table 1: Schedule of Events

Procedure	Screening Visit	Baseline	Week 3	Week 8	Week 12/ Early Termination	Week 16/ End of Study Phone Call
a	Physical examination to include vital sign measurements (height [Screening visit only], weight, blood pressure, heart rate).					
b	Two ECG exams will be performed in each of these two visits, about 5 minutes apart one from another.					
c	Gynecological examination to include visual inspection of external genitalia, speculum examination of vagina and cervix (if present), bimanual palpation of uterus and ovaries (if present), breast palpation. Papanicolau exam will be performed only at screening visit.					
d	Neither mammograms performed as study specific procedure nor negative mammogram documented within 9 months prior to randomization will be recorded in the eCRF but archived in subject's clinical records					
e	Laboratory testing performed under fasting conditions at Screening and Week 12 includes: standard hematology panel plus prothrombin time, activated thromboplastin partial time, fibrinogen, antithrombin III/protein C/protein S; standard chemistry panel (glucose, cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides albumin, alanine aminotransferase, aspartate aminotransferase, creatine phosphokinase, bilirubin, creatinine, total protein, uric acid, blood urea nitrogen); hormones (estradiol, estriol, estrone, follicle-stimulating hormone, luteinizing hormone); and standard urinalysis with microbiology. Factor V Leiden testing will be performed only at screening visit (or Week 12/ET visit if not obtained at screening)					
f	AEs and SAEs will be recorded from screening (post ICF signature) and subsequently at every study visit and up to 30 days post last study drug administration. Tolerability of study drug will be evaluated at weeks 3, 8, 12, and 16.					
g	For the evaluation of symptoms and signs, each symptom and each sign will be graded as: 0=absent; 1=mild; 2=moderate; 3=severe					
h	Signs and symptoms other than vaginal dryness to be evaluated are the following: Symptoms: dyspareunia, pruritus or itching, burning, dysuria. Signs: pallor, friability, thinning or flattening of folds, presence of petechiae, and dry mucosa					
i	Global efficacy will be assessed as: highly efficacious, efficacious, non-efficacious, detrimental					
j	Global acceptability will be assessed as: excellent, good, acceptable, unacceptable					
k	The investigator will administer the first study medication or will supervise the patient doing the administration					
l	Medication kits will be dispensed via IWRS					

3.1.1 Rationale of Study Design

[redacted]

The study design is concordant with a dose response approach (three doses of estrogens will be tested and compared to placebo), as well as the indication and the estrogenic nature of the product, and thus follows the recommendations of the FDA in Draft Jan 2003 Recommendations for Clinical Evaluation of Estrogen/Progestins to Treat Vulvovaginal Symptoms.

A placebo arm has been included in the study as per the above referenced FDA recommendations, in which it is indicated to demonstrate a statistically significant improvement versus placebo in the primary efficacy analyses. By selecting placebo as comparator, all potential influences on the actual or apparent course of the disease other than those arising from the pharmacologic action of the test drug are controlled. Placebo-controlled trials measure the total pharmacologically mediated effect of treatment, and provide the maximum ability to distinguish adverse effects caused by a drug from those resulting from underlying disease or intercurrent illness. (ICH Guideline E10)

4 Subject Selection and Withdrawal Criteria

4.1 Selection of Study Population

Approximately 280 subjects will be enrolled at approximately 25 sites in Europe. Subjects will be assigned to study drug only if they meet all of the inclusion criteria and none of the exclusion criteria.

4.1.1 Inclusion Criteria

Each subject must meet all of the following criteria to be enrolled in this study:

1. Is capable of understanding the written informed consent, provides signed and witnessed written informed consent, and agrees to comply with protocol procedures and assessments.
2. Is ≥40 and ≤80 years old.
3. Is postmenopausal, defined as ≥12 months since last spontaneous menstrual period, or having 6 months of spontaneous amenorrhea with serum follicle-stimulating hormone (FSH) level >40 IU/L, or ≥6 weeks since bilateral oophorectomy with or without hysterectomy.
4. Has a body mass index (BMI) ≤36 kg/m^2.
5. Vaginal Maturation Index showed ≤5% superficial cells on a vaginal smear.
6. Vaginal pH is >5.
7. Moderate to severe vaginal dryness is currently reported as the most bothersome symptom of vaginal atrophy.
8. Negative mammogram at screening or documented negative mammogram within 9 months prior to randomization and normal breast examination at screening.
9. Negative Papanicolau test at screening (in women with a cervix).

4.1.2 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

1. Subjects with contraindication to hormone therapy with estrogens, such as those diagnosed with or having a history of:
    - Malignant and premalignant lesions of the breasts or endometrium

- Malignancy of the colon
- Malignant melanoma
- Hepatic tumor
- Venous thromboembolic conditions (including deep vein thrombosis or pulmonary embolism)
- arterial thromboembolic conditions (including angina pectoris, myocardial infarction, or cerebrovascular accident)
- Coagulopathies
- Vaginal bleeding of unknown etiology
- Acute liver disease or a history of liver disease as long as liver function tests have failed to return to normal.
- Porphyria

2. Subjects with abnormal laboratory values at screening that the investigator considers clinically relevant for the purposes of the study.
3. Subjects with any medical-surgical pathology which is not controlled at the time of study inclusion.
4. Subjects with any acute or chronic condition whose management or progression may interfere with the subject's participation in the study.
5. Subjects with uncontrolled hypertension (≥140 mmHg systolic blood pressure and/or ≥90 mmHg diastolic blood pressure).
6. Subjects with Grade II or higher uterovaginal prolapse.
7. Subjects with uterine polyps.
8. Subjects with symptomatic and/or large (>3 cm) uterine fibroids and/or palpable fibroids at gynecological examination.
9. Subjects who have had urogenital surgery within 3 months of the Baseline Visit.
10. Subjects with signs and symptoms suggestive of infection of the genital or urinary tract requiring treatment at the start of the study.

11. In women who have a uterus, evidence of hyperplasia, cancer, or other endometrial pathology at endometrial biopsy.
12. Subjects who have received the following treatments within the specified time period prior to screening:
    - Any type of non-hormonal vulvovaginal treatment within 7 days prior to screening (including cosmetics expected to have an impact on vaginal pH such as special feminine wash gels)
    - Phytoestrogens by any route in the period of 1 month prior to screening
    - Vaginal hormone therapy within 1 month prior to screening
    - Hormone therapy (estrogen alone, progestin alone, or estrogen/progestin combination) by oral, intrauterine, or transdermal route within 2 months prior to screening.
    - Progestational implants, estrogen, or estrogen/progestational injectable within 3 months prior to screening
    - Estrogen pellet therapy or progestin injectable drug therapy within 6 months prior to screening
    - Percutaneous estrogen lotions/gels within 1 month prior to screening
    - Testosterone or testosterone derivatives, dehydroepiandrosterone, tibolone, or selective estrogen receptor modulators (SERMs) by any route within 2 months prior to screening.
13. Subjects in treatment with antiepileptic drugs (e.g., barbiturates, hydantoins, carbamazepine), certain antibiotics and other anti-infective medicinal products (e.g. rifampicin, rifabutin, tetracyclines, ketoconazole, evirapine, efavirenz, ritonavir, nelfinavir); phenyobutazone, preparations based on medicinal plants that contain St. John's Wort (*Hypericum perforatum*).
14. Subjects who are allergic to any of the ingredients in the investigational medicinal product.
15. Subjects who are currently participating or have participated in the experimental evaluation of any product within 8 weeks of the start of study.

4.2 Withdrawal of Subjects from the Study

The duration of the study is defined for each subject as the date signed written informed consent is provided through the last follow-up phone call at Week 16.

Participation in this study is strictly voluntary. A subject has the right to withdraw from the study at any time and for any reason. If she chooses to withdraw, the investigator must be informed immediately.

4.2.1 Reasons for Withdrawal/Discontinuation

Subjects may withdraw from the study at any time and for any reason without prejudice to their future medical care by the investigator or at the study site. Every effort should be made to keep subjects in the study. The reasons for subjects not completing the study will be recorded. A subject may be withdrawn from the study for any of the following reasons:

1. Does not meet the protocol inclusion or exclusion criteria.
2. Noncompliance with the protocol and/or treatment schedule.
3. An adverse event (AE) that in the investigator's opinion may be possibly related to the study drug or examinations requires withdrawal from the study.
4. The subject presents with jaundice or deterioration in liver function
5. The subject presents with significant increase in blood pressure
6. The subject presents with new onset of migraine-type headache
7. The subject withdraws consent or the investigator or Sponsor decides to discontinue the subject's participation in the study.
8. The investigator may also terminate a subject`s study participation if considered it is in her best interest.

The investigator will also withdraw a subject if ITF Research Pharma SLU terminates the study. Upon occurrence of a serious or intolerable AE, the investigator will confer with the Sponsor. If a subject is discontinued because of an AE, the event will be followed until it is resolved. Any subject may withdraw his or her consent at any time.

4.2.2 Handling of Withdrawals

Subjects are free to withdraw from the study or study drug at any time upon request. Subject participation in the study may be stopped at any time at the discretion of the investigator or at the request of the Sponsor.

Subjects who discontinue study drug or active participation in the study will no longer receive study drug. When a subject withdraws from the study, the reason(s) for withdrawal shall be recorded by the investigator on the relevant page of the electronic case report form (eCRF).

Any subject who prematurely terminates participation and who has received at least one dose of the test or reference product will undergo a final examination (see Section 6.1.5 for details).

If study participation is terminated due to an AE possibly related to the study drug or study examinations, the subject must be followed-up by additional examinations according to the medical judgment of the investigator until the abnormal condition is resolved or the investigator deems further observations or examinations to be no longer medically indicated. Subjects who fail to return for final assessments will be contacted by the site (via two telephone calls and then a registered letter) in an attempt to have them comply with the protocol.

It is vital to obtain follow-up data on any subject withdrawn because of an AE or serious AE (SAE). In every case, efforts must be made to undertake protocol-specified, safety, follow-up procedures. All subjects withdrawn from the study should be registered accordingly on the Interactive Web Response System (IWRS).

4.2.3 Re-screenings and Replacements

Screen failures (subjects not randomized into the study) may be re-screened in cases where the unmet selection criteria have been resolved. A new Informed Consent will be needed for re-screened subjects. Timelines permitted for re-screening will vary at the discretion of the investigator depending on the unmet criterium and the time needed to have it clinically resolved. Only invasive procedures biopsy and Factor V Leiden will not be repeated if subjects are rescreened within 1 month of previous failure. Data obtained from screening visit will be used again for the re-screened subject in her new screening visit under her new

rescreening number. Randomized subjects prematurely withdrawn from the study will not be replaced.

5 Study Treatments

5.1 Method of Assigning Subjects to Treatment Groups

Subjects will be randomly assigned to receive 0.005% estriol vaginal gel, 0.002% estriol vaginal gel, 0.0008% estriol vaginal gel, or placebo vaginal gel using a 1:1:1:1 allocation ratio. Subjects will be assigned a randomization code according to their order of arrival into the study at the Baseline Visit.

The randomization schedule is prepared by the designated unblinded statistical programmer and reviewed and distributed by the designated unblinded statistician of [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

All subjects will start treatment with a single daily application of 1 g of gel during the first 3 weeks of study participation, and then twice weekly from Weeks 4 to 12. Study drug will be administered at bedtime, except the first dose, which will be administered by the investigator or by the subject in the presence of the investigator at the office at baseline visit. Twice weekly dosing during Weeks 4 through 12 is recommended to be preferably carried out on, for example, a Monday and Thursday schedule.

5.3 Identity of Investigational Product

Estriol is chemically described as 3,16α, 17β-trihydroxy-1,3,5(10)-estratriene. It has an empirical formula of $C_{18}H_{24}O_3$, and the structural formula is shown in Figure 1.

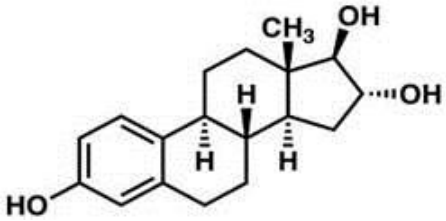

Figure 1: Estriol Structural Formula

The molecular weight of estriol is 288.38.

The drug product is a homogeneous colorless, odorless clear-to-slightly-translucent gel containing 0.05 mg/g of estriol. It contains, as excipients, AA-1 polycarbophil [redacted] Carbopol® 971P [redacted] glycerol, sodium methyl parahydroxybenzoate, sodium propyl parahydroxybenzoate, hydrochloric acid, sodium hydroxide, and water.

Formulations of 0.005%, 0.002%, and 0.0008% estriol vaginal gel are to be used in this study. The estriol vaginal gel formulations will be manufactured and released by [redacted] under the supervision of ITF Research Pharma SLU.

The composition of the placebo gel is the same as the active with the exception of estriol. The placebo vaginal gel will be identical in appearance, texture, and odor to the estriol vaginal gel products. Placebo vaginal gel will be manufactured by [redacted] under the supervision of ITF Research Pharma SLU.

ITF Research Pharma SLU will provide the Storage Site [redacted] with adequate supplies of estriol gel to be sent to the study sites. The following drug supplies will be used in the study: estriol vaginal gel and placebo vaginal gel, both supplied as 10 g packaged in aluminum tubes with polyethylene screw caps.

5.4 Management of Clinical Supplies

5.4.1 Study Drug Packaging and Storage

Estriol vaginal gel and matching placebo will be prepared in aluminum tubes (with internal lacquer) closed with a polyethylene screw cap, containing 10 g of drug product, packaged and shipped by [redacted] to the designated packaging/labeling vendor ([redacted] The designated packaging/labeling vendor ([redacted] will then ship the experimental product to the study sites. The investigational product is packaged as a kit containing a tube of vaginal gel and ten 1-g capacity single-use vaginal applicators.

The studies of stability with the investigational product packaged in 10-g tube indicate that the product should be stored under conditions not to exceed +25ºC.

Packaging and labelling of the medicinal products will be performed in accordance with GMP and national regulatory requirements.

Each study participant will be provided with an adequate number of 10-g tubes and 1-g capacity vaginal applicators to fulfill the dosing regimen requirements. Study drug provided to each subject at the Baseline Visit will consist of three kits, each containing one 10-g tube and 10 applicators (for 21 expected applications). At the Week 3 Visit, each subject will receive two kits of the study drug/applicators (for 10 expected applications), and at the Week 8 Visit, two additional kits of study drug/applicators (for 8 expected applications) will be provided.

The Principal investigator or sub-investigator must explain to the subject how to administer the vaginal gel. The first application should be performed at site at baseline visit by the investigator or by the subject in the presence of the investigator. Subjects will be instructed to administer the rest of the treatment applications at bedtime to allow for maximum retention of the gel.

5.4.2 Test Article Accountability

The investigator will maintain accurate records of receipt of all medication kits, including dates of receipt. Reasons for departure from the expected dispensing regimen must also be recorded.

Subjects must bring used and unused study drug containers as well as unused applicators with them to the Week 3, Week 8, and Week 12 study visits. Used applicators will be discarded by the subjects as normal household waste. All empty and partially empty containers of study drug will be stored at the site until the study closure visit has been performed.

Drug accountability will be performed by the investigator on an ongoing basis. Final reconciliation of drug shipped, drug consumed and drug remaining will be performed at study closure. This reconciliation will be logged on the applicable forms, signed and dated. Any discrepancies noted will be investigated, resolved and documented prior to return or destruction.

All unused and used supplies will be returned to the designated packaging/labeling vendor [redacted] for destruction.

The Sponsor will be permitted access to the study supplies at any time within usual business hours and with appropriate notice during or after completion of the study to perform drug accountability reconciliation.

5.5 Overdose Management

Toxicity for estriol is very low. Overdose of 0.005%, 0.002%, and 0.0008% estriol vaginal gel with vaginal application is very unlikely. Symptoms that may occur in the case of a high dose being accidently ingested include nausea, vomiting, and vaginal bleeding (in women).

An overdose of placebo vaginal gel is very unlikely. However, symptoms that may occur with accidental ingestion include nausea and vomiting.

An overdose is any dose of study drug given to a subject or taken by a subject that exceeds the dose described in the protocol. Any overdose, with or without associated AEs, must be

promptly documented in the eCRF. Overdoses without signs or symptoms need to be recorded as AEs; in case of any AEs associated with the overdose, these should be also reported on relevant AE/SAE sections in the eCRF.

5.5.1 Treatment of Overdose

There is no known specific antidote for an overdose of estriol gel or placebo gel. If necessary, symptomatic treatment should be instituted.

5.5.2 Medication Errors

Medication errors may result in this study from administration of study product outside the scheduled posology or use of incorrect amount of study product. Such medication errors occurring to a study subject are to be captured on the eCRF.

Medication errors are reportable irrespective of the presence of an associated AE or SAE; these include medication errors involving subject exposure to the drug and potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the participating study subject.

5.6 Misuse for Illegal Purposes

Though the active ingredient of the estriol vaginal gel under investigation is a hormone, this is not likely to be an issue of concern in the study.

If misuse of the study materials is identified by the investigator, immediate notification to the Sponsor is indicated.

5.7 Blinding

The study drug, 0.005% estriol vaginal gel, 0.002% estriol vaginal gel, 0.0008% estriol vaginal gel and the placebo vaginal gel will be of identical appearance, aroma, and texture in order to maintain the study blind. Treatments will be labelled undistinguishably. The Sponsor, the investigators, study site staff, and study subjects will be blinded to the identity of the study drugs used.

5.7.1 Breaking the Blind

Throughout the study, the medication with which the subject is being treated will be kept strictly confidential and only authorized persons will have access to this information, unless it is necessary to break the blind due to safety reasons. The unblinding must be done only by the site's principal investigator or delegated authorized site staff, and only when the knowledge of the treatment assignment is deemed essential for the subject´s care.

For emergency situations, an unblinding function will be available via the Interactive Web Response System.

The investigator should immediately inform the study monitor that a treatment code has been opened.

The blinding may be broken for expedited reporting purposes in the case of a Suspected Unexpected Serious Adverse Reaction (SUSAR). Expectedness will be determined by establishing whether the AE is among those identified in the ITFE-2092 Investigator's Brochure

The randomization codes will be unblinded and made available for the project statistician to perform the data analysis when the study has been finalized, the database has been verified, and all protocol violations have been determined. All personnel involved directly in the study shall be unaware of the treatment assigned to the subjects until the database has been closed.

5.8 Treatment Compliance

Subjects must bring unused study drug and applicators with them to the Week 3, Week 8, and Week 12 study visits. Used applicators will be discarded by the subjects as normal household waste.

Treatment compliance will be evaluated by the investigator's team at Weeks 3, 8, and 12 by reviewing study drug and returned supplies (non-used applicators), supported by subject reporting.

5.9 Prior and Concomitant Therapy

Use of all concomitant medications will be recorded in the subject's eCRF. The minimum requirement is that the drug name and the dates of administration are to be recorded. This will include all prescription drugs, herbal products, and over-the-counter (OTC) medications from 28 days prior to the start of study drug and up to 30 days (±5 days) following the last dose of investigational product; the reason for the drug administration must be recorded in the eCRF. Any changes in concomitant medications also will be recorded in the subject's eCRF.

Record the name, start date (if known), indication for use, and whether ongoing or discontinued for all medications/treatments.

The concomitant use of another drug with estrogens, progestins, androgen derivatives, SERMs, as well as phytoestrogen-based and cosmetics products expected to have an impact on vaginal pH such as special feminine wash gels is prohibited during the study.

In vitro and in vivo studies have shown that estrogens are metabolized partially by cytochrome P450 3A4 (CYP3A4). Therefore, inducers or inhibitors of CYP3A4 may affect estrogen drug metabolism. Inducers of CYP3A4 such as St. John's Wort preparations (*Hypericum perforatum*), phenobarbital, carbamazepine, and rifampin may reduce plasma concentrations of estrogens, possibly resulting in a decrease in therapeutic effects and/or changes in the uterine bleeding profile. Inhibitors of CYP3A4 such as erythromycin, clarithromycin, ketoconazole, itraconazole, and ritonavir may increase plasma concentrations of estrogens and may result in side effects. As noted in Section 4.1.2, concomitant use of these medications is prohibited.

Any concomitant medication deemed necessary for the welfare of the subject during the study may be given at the discretion of the investigator. However, it is the responsibility of the investigator to ensure that details regarding the medication are recorded in full in the eCRF.

6 Study Assessments and Procedures

Before performing any study procedures, all potential subjects will sign an informed consent form (ICF). Subjects will have the opportunity to have any questions answered before signing the ICF. The investigator must address all questions raised by the subject. The investigator will also sign the ICF. A subject number will be assigned to each subject.

The following assessments and measurements will be carried out at the times specified in the study flow chart. A summary of the schedule of events is provided in Table 1.

The time at which study procedures are conducted should follow the protocol timelines as closely as possible. However, it is recognized in a clinical setting that protocol-specified timings may not occur exactly as specified in the protocol. Provided that activities are carried out within the time windows outlined in the Schedule of Events (Table 1), it will not be necessary to file a protocol deviation for activities outside the nominal time defined in the protocol.

6.1 Study Visits

6.1.1 Screening Visit

Screening is defined as the period prior to the administration of study drug. In this study, screening will cover a maximum period of 30 days before the baseline visit on the day of randomization.

A subject will enter the screening period if basic study criteria are matched with the potential participant's medical condition and profile. If the subject appears to meet these basic study criteria, they will be offered the opportunity to participate in the study. In case of agreement to participate, the subject will be requested to sign an ICF. A subject who agrees to participate and signs the ICF is to be given a copy of the fully signed consent signed by herself and the investigator or designee.

During the Screening Visit, the investigator or designee will obtain/perform the following:

- Investigator to register subject in IWRS and obtain the subject number.
- Record demographic details (date of birth, race);

- Record recent (within the past year) and relevant gynecological, medical, and surgical history and any chronic or ongoing medical conditions for which the subject received or is receiving medical treatment;
- Record use of prohibited medications during the periods defined in the exclusion criteria (see Appendix 12.3 and Appendix 12.4).
- Record concomitant medications taken, including the 4 weeks prior to study entry.
- Perform a general physical examination (height, weight) including seated for at least 5 minutes blood pressure, heart rate. Note any abnormal physical examination findings;
- Perform 12-lead electrocardiogram (ECG). Two ECG exams will be performed, approximately 5 minutes apart one from the other.
- Perform gynecological examination including a visual inspection of the external genitalia, speculum examination of the vagina and cervix, collection of a Papanicolau smear, as well as bimanual palpation of the uterus and ovaries (if present), and breast palpation. Any abnormalities that could interfere with study procedures or assessments should be recorded. At all post-screening visits, the investigator should report any clinically significant changes from baseline.
- Perform mammogram if documented negative mammogram within 9 months prior to randomization is not available.
- Record presence and intensity of the symptom vaginal dryness; obtain vaginal smear for evaluation of vaginal cytology; test vaginal pH;
- If the woman has a uterus, perform transvaginal ultrasound to determine endometrial thickness and identify any gynecological abnormalities that would interfere with administration of the study drug, study procedures or results;
- If the woman has an intact uterus, an endometrial biopsy will be performed;
- Safety laboratory parameters (biochemistry and hematology, including coagulation and lipids, urinalysis) and serum hormone levels. These analysis should be performed under fasting conditions
- Report adverse events

The investigator or other qualified designee is to review information obtained to ensure subject meets all of the inclusion and none of the exclusion criteria for all measurements studied in the Screening Visit.

Before the Baseline Visit, the investigator will confirm that the subject meets all the inclusion and exclusion criteria, including the endometrial histological assessment.

Subjects with signs and symptoms suggestive of infection of the genital or urinary tract requiring treatment at the start of the study will be excluded. However, once treated and symptoms have resolved (or there are negative microbiology findings), subjects can be re-screened into the study. Refer to section 4.2.3 for more details.

6.1.2 Baseline Visit

This visit will be performed on Day 1. The following procedures will be performed:

- Randomization: the investigator should randomize the subject through IWRS in which a randomization code and study drug kits will be allocated
- Gynecological examination including evaluation of symptoms and signs of vulvovaginal atrophy other than vaginal dryness
- Recording of adverse events since Screening Visit
- Recording of concomitant medications
- Administration of first dose of study drug by the investigator or by the subject in presence of the investigator Dispensing of dosing instructions and adequate medication supplies for 3 weeks (three medication kits)

6.1.3 Week 3 Visit

This visit will be scheduled on the day after having completed 21 days of treatment. For organizational reasons, a deviation of 3 days before or after the scheduled date is allowed, with the investigator attempting to schedule the visit as close as possible to the estimated ideal date.

The following procedures will be performed:

- Evaluation of symptoms and signs of vaginal atrophy, including vaginal dryness.
- Determination of vaginal pH
- Vaginal smear for evaluation of vaginal cytology
- Recording of adverse events
- Recording of concomitant medications

- Reconciliation of returned medication
- Dispensing adequate medication supplies via IWRS through the Week 8 Visit (two medication kits)

6.1.4 Week 8 Visit

This visit will be scheduled on the day after having completed the end of 8 weeks of study participation. For organizational reasons, a deviation of 5 days before or after the scheduled visit date is allowed, with the investigator attempting to schedule the visit as close as possible to the estimated ideal date.

The following procedures will be performed:

- Recording of adverse events
- Recording of concomitant medications
- Reconciliation of returned medication
- Dispensing adequate medication supplies via IWRS through the Week 12 Visit (two medication kits)

6.1.5 Week 12 Visit/Early Termination Visit

This visit will be scheduled on the day after having completed Week 12 of the study or if the subject terminates study participation before Week 12. For organizational reasons, a deviation of 5 days before or after the estimated ideal date is allowed, with the investigator attempting to schedule the visit as close as possible to the estimated ideal date.

If a subject is discontinued/withdrawn the investigator should register the subject status as such in IWRS.

The following procedures will be performed:

- Evaluation of symptoms and signs of vaginal atrophy, including vaginal dryness
- Determination of vaginal pH
- Vaginal smear for evaluation of vaginal cytology
- Evaluation of global efficacy
- Evaluation of acceptability
- Transvaginal ultrasound in women with a uterus

- Perform an endometrial biopsy in women with a uterus
- Safety laboratory parameters (biochemistry and hematology, including coagulation and lipids, urinalysis) and serum hormone levels. These analyses should be performed under fasting conditions.
- Recording of adverse events
- Measurement of vital signs (blood pressure, heart rate, weight)
- Perform physical examination
- Perform 12-lead electrocardiogram. Two ECG exams will be performed, approximately 5 minutes apart one from the other.
- Perform gynecological examination including a visual inspection of the external genitalia, speculum examination of the vagina and cervix, bimanual palpation of the uterus and ovaries (if present), and breast palpation.
- Recording of concomitant medications
- Reconciliation of returned medication

6.1.6 Week 16 – End-of-Study Phone Call

All subjects who completed the full 12 weeks of study drug will have a phone call scheduled for 4 weeks after last medication dose to record any AEs that may have appeared after treatment discontinuation, and to document changes in concomitant medication use. For organizational reasons, a deviation of 5 days before or after the estimated date will be allowed.

When a subject has completed the study the investigator should register the subject completed in IWRS.

6.2 Efficacy Assessments

The main efficacy variables in this study are: change in the proportion of superficial and parabasal cells of the vaginal epithelium; change in vaginal pH; and change in the severity of vaginal dryness.

Secondary efficacy variables are: change in the severity of individual vaginal symptoms and signs of vaginal atrophy (other than vaginal dryness); and change in the Global Symptom Score.

The exploratory efficacy variables include evaluation of the final subjective global perception of efficacy by subjects at the end of study and evaluation of acceptability of the therapy at Week 12.

The minimally effective dose of estriol vaginal gel will be determined based on using the gatekeeping procedure outlined in Section 7.8.1.1, the minimal effective dose will therefore be the lowest dose at which a significant difference is seen when compared with placebo.

The gynecological procedures and evaluations to be performed for the assessment of the efficacy variables include the vaginal cytology, determination of the vaginal pH, evaluation of the different vaginal signs and symptoms, determination of the Global Symptom Score, and the evaluation of the global impression of efficacy.

6.2.1. Vaginal Cytology

Vaginal smears will be performed to evaluate the maturation index of the vaginal mucosa at Screening Visit and at the Week 3 and Week 12/Early Termination visits.

Two vaginal smears should be collected per subject, from the bottom of the right and left vaginal walls respectively. The vaginal cytological samples will be fixed whilst they are still moist with a water-soluble fixation spray for cytodiagnosis.

The vaginal cytological samples will be sent to the centralized laboratory for analysis. One sample is to be sent to the central laboratory for analysis, with the second sample retained at the site with no requirement of specific environmental storage conditions. Samples will be evaluated by two different cytopathologists who will be blinded as to subject treatment assignment. The back-up sample will be evaluated only in the event that the initial samples are invalid or have suffered any type of incident such as breakage or loss.

For the cytologic evaluation, the number of parabasal, intermediate, and superficial cells will be calculated. The percentages of each type of cells will be calculated.

6.2.2 Vaginal pH Determination

For pH determination, a reactive pH strip will be used. On each occasion, the investigator will insert the strip into the vagina and moisten it as much as possible with the vaginal wall

secretions. Once adequate wetting has been achieved, the colors on the strip will be checked against a standard pH scale (provided by the manufacturer) to determine the pH value. The reading should be made while the strip is still moist. The strips will be discarded appropriately once the pH has been recorded.

Vaginal pH determination will be performed at Screening Visit, at the Week 3 Visit, and at the Week 12/Early Termination Visit.

6.2.3 Evaluation of Vaginal Symptoms and Signs

6.2.3.1 Evaluation of Symptoms

At study visits the investigators will interview the subjects on the presence and intensity of the symptoms of vulvovaginal atrophy they have. Study subjects will self-assess their symptoms and report their assessments to the investigators to be recorded. .

The symptoms evaluated in this study include vaginal dryness, pruritus or itching, burning, dyspareunia, and dysuria.

Each symptom will be scored by the subject using a numeric scale from 0 to 3, as shown below:

0 - Absent: The symptom is not present.

1 - Mild: The symptom is of mild intensity,

2 - Moderate: The symptom is of moderate intensity

3 - Severe: The symptom is severe in intensity.

The presence and intensity of vaginal symptoms of vulvovaginal atrophy will be assessed at the following visits: Screening (vaginal dryness only); Baseline (pruritus or itching, burning, dyspareunia, and dysuria); Week 3 (all 5 symptoms: vaginal dryness, pruritus or itching, burning, dyspareunia, and dysuria); and Week 12/Early Termination (all 5 symptoms: vaginal dryness, pruritus or itching, burning, dyspareunia, and dysuria).

Specific to the evaluation of dyspareunia, women will be also asked if they have had sexual activity with penetration during the study period.

6.2.3.2 Evaluation of Signs

The presence and severity of most typical signs of vulvovaginal atrophy will be recorded according to the visual inspection of the vagina as performed by the investigator.

The signs evaluated in this study include vaginal mucosa with flattening of folds or thinning, dryness of the mucosa, pallor of the mucosa, friability of the mucosa, and presence of petechiae.

Each one of these signs will be scored by the investigator on a numerical Liekert scale as follows:

0 – Absent: The sign is not present.

1 – Mild: The sign is present and is considered a mild alteration.

2 – Moderate: The sign is present and is considered a moderate alteration.

3 – Severe: The sign is present and is considered a severe alteration.

The presence and intensity of all these 5 signs of vulvovaginal atrophy will be assessed at the Baseline Visit, the Week 3 Visit, and at the Week 12/Early Termination Visit.

6.2.3.3 Evaluation of Global Symptom Score

A Global Symptom Score will be obtained by summing the numerical scores of all symptoms at different time points: Screening/Baseline, Week 3 and Week 12/Early termination visit. Thus, the Global Symptom Score will range at Screening/Baseline between 2 (at least moderate vaginal dryness as the only symptom) to 15 (all five studied symptoms severe in intensity). At Weeks 3 and Week 12/Early Termination visits the Global Symptom Score can range between 0 (no symptoms) and 15 (all symptoms severe in intensity). The Global Symptom Score will be evaluated when all 5 individual symptoms scores are available at a given time point.

6.2.4 Evaluation of Global Efficacy

At the Week 12/Early Withdrawal Visit, the subject will be interviewed by the investigator on her perception of the global efficacy of the therapy received over the completed study period. Global efficacy is defined as the subject's perception of the effect of the treatment on her vaginal-related symptomatology.

The subject will be asked to rate the global efficacy of the treatment as: highly efficacious, efficacious, non-efficacious, or detrimental.

6.3 Safety Assessments

6.3.1 Clinical Laboratory Assessments

At the Screening and Week 12/Early Termination Visits, blood and urine specimens will be collected under fasting conditions for the following measurements:

- Hematology: Standard hematology panel plus prothrombin time, activated thromboplastin partial time, fibrinogen, and antithrombin III/protein C/protein S. Factor V Leiden will be performed at either screening visit or week 12/ET visit if not previously obtained at screening.

- Blood chemistry: Standard chemistry panel including albumin, alanine aminotransferase, aspartate aminotransferase, creatine phosphokinase, bilirubin, creatinine, total protein, uric acid, blood urea nitrogen, glucose, and lipids (cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides)

- Hormones: Estradiol, estriol, estrone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH)

- Urinalysis: Standard urinalysis including testing for presence of blood, glucose, ketones, and protein, with microbiological analysis

A laboratory manual will be provided to each site, detailing procedures for sample collection, storage, and shipment. Samples will be destroyed at the end of the study.

6.3.2 Transvaginal Ultrasound

Transvaginal ultrasound (TVU) will be performed by the investigator, sub-investigator or designated trained member of the staff at the Screening Visit to determine whether there are any abnormalities of the ovaries, whether the uterus and cervix are present, and whether there are any physical issues that would exclude the subject from participation in the study. In addition, TVU will be performed to measure endometrial thickness and to investigate the presence of any abnormal morphological findings. Follow-up TVU will be

conducted during the Week 12/Early Termination Visit, and any clinically significant changes from baseline will be noted.

6.3.3 Endometrial Biopsy

Endometrial biopsy will be performed per the provided standard collection method. Samples are to be fixed and stored using directions supplied by the centralized laboratory. The investigator shall do his/her best to obtain endometrial tissue for analysis. The Investigator must record whether tissue was obtained, and send the tissue obtained to the centralized laboratory to be processed.

At the centralized laboratory, a single pathologist will assess the slides from the endometrial biopsies obtained at Screening (per the standardized criteria for determining hyperplasia, as detailed in ***Blaustein's Pathology of the Female Genital Tract*** (Kurman RJ, 2011) as well as any biopsies obtained if a subject experiences vaginal bleeding while on study drug. Three independent pathologists (from different institutions with independent fiduciary and organizational reporting), blinded to subject's treatment group and to each other's readings, will assess the endometrial biopsies obtained at the Week 12/Early Termination Visit. The concurrence of two of the three pathologists will be accepted as the final diagnosis. If there is no agreement among the three pathologists, the most severe pathologic diagnosis (i.e., atypical hyperplasia > complex hyperplasia > simple hyperplasia > benign endometrium) would be used as the final diagnosis. If hyperplasia is diagnosed by the single safety reader for a subject who has bled while on study drug, this diagnosis be maintained for the efficacy evaluation and the slides become part of the slide set given to the two other pathologists for reading.

Per the draft FDA guidance on estrogen and estrogen/progestin drug products to treat vasomotor symptoms and vulvar and vaginal atrophy symptoms (US Dept. Health and Human Services, 2003), the set of slides distributed to each of these pathologists for the end-of-study pathology review will incorporate control slides representing a randomly selected 10% of the screening normal slides and all slides from subjects excluded for the diagnosis of hyperplasia or cancer to insure quality control.

6.3.4 Adverse Events

6.3.4.1 Definitions of Adverse Events

The investigator is responsible for reporting all adverse events (AEs) that are observed or reported during the study, regardless of their relationship to study drug or their clinical significance.

An AE is defined as any untoward medical occurrence in a subject enrolled into this study regardless of its causal relationship to study drug. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporarily associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. Subjects will be instructed to contact the investigator at any time, after the subject gives informed consent until the end of study, if any symptoms develop.

Note that, for the purpose of this protocol, the following circumstances – with or without associated adverse event/reaction - should be included the eCRF:

- Overdose, abuse, misuse
- Off-label use
- Medication error

A treatment-emergent AE (TEAE) is defined as any event not present before exposure to study drug or any event already present that worsens in either intensity or frequency after exposure to study drug.

A serious AE (SAE) is defined as any event that:

- Results in death;
- Is immediately life-threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity; or
- Is a congenital anomaly/birth defect

- Is a medically significant event

Medically significant events are defined as important medical events that may not result in death, be life-threatening, or require hospitalization but may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.

A **life-threatening adverse event** is one that places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred. It does not include an event that, had it occurred in a more severe form, might have caused death.

An **unexpected adverse event** is any adverse drug event, the nature, specificity or severity of which is not consistent with the applicable product information (e.g. Investigator's brochure for an unauthorized investigational product or summary of product characteristics for an authorized product).

Enter only one event as the reason for the SAE on the SAE form. Other non-serious events which did not directly result in the SAE should be detailed in the description section on the SAE form and listed separately as AEs if necessary.

ANY SERIOUS ADVERSE EVENT MUST BE REPORTED IMMEDIATELY (WITHIN 24 HOURS) BY THE STUDY SITE VIA EMAIL OR FAX TO [redacted] PHARMACOVIGILANCE GROUP USING THE SAE FORM PROVIDED. ENTER THE SUBJECT'S AVAILABLE INFORMATION INTO THE eCRF WITHIN 48 HOURS.

A SAE occurring during the study must be reported to the [redacted] Pharmacovigilance Group. Any such SAE due to any cause, whether or not related to the study drug, must be reported within 24 hours of occurrence or when the Investigator becomes aware of the event. SAEs occurring more than 4 weeks of stopping the treatment

and considered related to the study drug will be communicated. Notification can be made with the SAE reporting form by using the dedicated fax line or by scanning and emailing to the safety mailbox for the [redacted] Pharmacovigilance Group:

[redacted] **Pharmacovigilance Fax Number:** [redacted]

[redacted] **Pharmacovigilance Email:** [redacted]

The event must also be recorded on the standard AE eCRF. Preliminary reports of SAEs must be followed by detailed descriptions later on, including clear and anonymized photocopies of hospital case reports, consultant reports, autopsy reports, and other documents when requested and applicable. SAE reports must be made whether or not the Investigator considers the event to be related to the investigational drug.

Appropriate remedial measures should be taken to treat the SAE, and the response should be recorded. Clinical, laboratory, and diagnostic measures should be employed as needed in order to determine the etiology of the problem. The Investigator must report all additional follow-up SAE information to the [redacted] Pharmacovigilance Group within 24 hours of awareness of the information. All SAEs will be followed until the Investigator and Sponsor agree the event is satisfactorily resolved.

Any SAE that is not resolved by the end of the study or upon discontinuation of the subject's participation in the study is to be followed until it either resolves, stabilizes, returns to baseline values (if a baseline value is available), or is shown to not be attributable to the study drug or procedures.

Reports relating to the subject's subsequent medical course following a SAE must be submitted to [redacted] until the event has subsided or, in case of permanent impairment, it stabilizes and the overall clinical outcome has been ascertained.

All SAEs will be reported to the institutional review board (IRB)/independent ethics committee (IEC) periodically for SAEs that meet reporting guidelines or at a frequency determined by the IRB/IEC, at the end of the study, or per local institutional guidelines.

All suspected unexpected serious adverse reactions (SUSARs) will be reported in an expedited manner to [redacted] per the ICH Guideline for GCP and IRB/IEC

by the investigators and to all relevant regulatory authorities [redacted]. A SUSAR is a SAE that is both related to the study drug (Relationship = Related) **and** unexpected (not previously described in the Investigator's Brochure). Expectedness will be judged by the Sponsor's medical monitor. It is therefore essential that all SAEs are reported within the 24-hour guideline so that the expectedness determination can be made. All SUSARs require immediate attention and expedited reporting to relevant regulatory authorities.

[redacted]is to ensure that all relevant information about **fatal or life threatening** SUSARs are appropriately recorded and reported to the concerned Regulatory Authority and to the concerned IRB(s)/IEC(s) within 7 calendar days of the date of first report [redacted] Follow-up information is to be subsequently communicated to the relevant Regulatory Authority within an additional 8 calendar days.

All other SUSARs are to be reported to the Regulatory Authority and to the IRB(s)/IEC(s) concerned as soon as possible within a maximum of 15 calendar days of first knowledge by [redacted].

The medical monitor/Sponsor or designee must also inform all investigators studying the investigational medicinal product about SUSARs and ensure that IRBs/IECs have been notified.

For reported death of a subject, the investigator shall supply the Sponsor, [redacted]nd/or the IRB(s)/IEC(s) with additional information requested on an expedited basis.

The following events are **not** considered SAEs:

- A visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event).
- Elective surgery planned before signing consent.
- Hospitalization which is due solely to a planned study visit and without prolongation.
- Routine health assessment requiring admission for baseline/trending health status (e.g., routine colonoscopy).

- Medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reasons).
- An overnight stay in the hospital that is only due to transportation, organization, or accommodation problems and without medical background.

These events will be reported in the eCRF and in the subject record.

6.3.4.2 Eliciting and Documenting Adverse Events

Adverse events will be assessed from the time the subject signs the informed consent form until exit from the study.

Events that occur prior to signing the informed consent form will be entered as medical history; AEs that have an onset after signing the informed consent will be entered on the AE form.

Subjects will be questioned and observed for evidence of AEs, whether or not related to study drug. For any pre-existing condition or abnormal laboratory finding that changes in severity after dosing, the change should be recorded as an AE. New abnormal laboratory findings that are considered clinically significant by the investigator are considered AEs.

All AEs will be collected through the Follow-up Visit (i.e. the Week 16 phone call, 4 weeks after discontinuation of study drug). Adverse event details are to be documented, including start and stop dates, whether continuous or intermittent, severity, any intervention utilized to correct the event, outcome of the event, and the relationship to the study drug.

All AEs determined by the investigator to be related to study drug must be followed until resolution, until they are considered stable, or for at least 30 days after discontinuation of study drug, whichever occurs first.

Adverse events should be classified following the Medical Dictionary for Regulatory Activities (MedDRA). Safety/PV will use the MedDRA medical dictionary to map the AE/SAE verbatim terms to specific system organ classes (SOC) and preferred terms.

Note that, for the purpose of this protocol, the following circumstances (with or without associated adverse event/reaction) should be included in the data base and will be submitted to the Sponsor, being managed in the same way and time as adverse events/reactions: any overdose (with or without associated AEs), abuse, misuse, off-label use, or medication error.

The causal relation between the investigational product and the AE will be assessed by the investigator using the following assessment criteria:

UNRELATED: This category applies to those AEs that are clearly and incontrovertibly due to extraneous causes (disease, environment, etc).

UNLIKELY: This category applies to those AEs that are judged to be unrelated to the study drug, but for which no extraneous cause may be found. An AE may be considered unlikely to be related to study drug if or when it meets 2 of the following criteria: (1) it does not follow a reasonable temporal sequence from administration of the study drug; (2) it could readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (3) it does not follow a known pattern of response to the study drug; or (4) it does not reappear or worsen when the drug is re-administered.

POSSIBLY: This category applies to those AEs for which a connection with the study drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possibly related if or when it meets 2 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; or (3) it follows a known pattern of response to the study drug.

PROBABLY: This category applies to those AEs that the investigator feels with a high degree of certainty are related to the study drug. An AE may be considered probably related if or when it meets 3 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (3) it disappears or decreases on cessation or reduction in dose (note that there are exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; for example, as in bone marrow depression, fixed drug eruptions, or tardive dyskinesia); or (4) it follows a known pattern of response to the study drug.

DEFINITELY: This category applies to those AEs that the investigator feels are incontrovertibly related to study drug. An AE may be assigned an attribution of definitely related if or when it meets all of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient; (3) it disappears or decreases on cessation or reduction in dose and recurs with re-exposure to drug (if rechallenge occurs); and (4) it follows a known pattern of response to the study drug.

Possibly, probably and definitely will fit the characterization of "related AE." Unlikely and unrelated will fit the characterization of "not related AE."

6.3.4.3 Reporting Adverse Events

All AEs reported or observed during the study will be recorded on the AE page in the eCRF. Information to be collected includes subject code, age, site, drug treatment and dose (if available), event term, time of onset, investigator-specified assessment of severity and relationship to study drug, time of resolution of the event, seriousness, any required treatment or evaluations, outcome, improvement of the AE after suspension/withdrawal of study drug, time of study (at screening, during treatment, or at follow-up) and action taken with the study drug. Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed to adequate resolution. MedDRA will be used to code all AEs.

Any medical condition that is present at the time that the subject is screened but does not deteriorate should not be reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

Any AE that meets SAE criteria (Section 6.3.4.1) must be reported to [redacted] immediately (i.e., within 24 hours) after the time site personnel first learn about the event. The contact information in Section 6.3.4.1 is to be used for SAE reporting.

6.3.4.4 Assessment of Severity

The severity, or intensity, of an AE refers to the extent to which an AE affects the subject's daily activities. The intensity of the AE will be rated as mild, moderate, or severe using the following criteria:

MILD: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.
MODERATE: An event that is sufficiently discomforting to interfere with normal everyday activities.
SEVERE: An event that considerably restricts or prevents normal everyday activities.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events

characterized as intermittent do not require documentation of onset and duration of each episode.

6.3.4.5 Assessment of Causality

The investigator's assessment of an AE's relationship to study drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship or association of the test article in causing or contributing to the AE will be characterized using the following classification and criteria:

Not Related AEs = those with no relationship with the study drug; other factor(s) (including subject's clinical state or other therapy administered) may be causative.

Related AEs = those with a reasonable possibility that the event was caused by the study drug (i.e. a time relationship exists, the AE is a known effect of the study drug or class of drugs, or the AE resolves/abates upon discontinuation of study drug), and other possible causative factor(s) may exist.

6.3.4.6 Follow-Up of Subjects Reporting Adverse Events

All AEs must be reported in detail on the appropriate page in the eCRF and followed to satisfactory resolution, until the investigator deems the event to be chronic or not clinically significant, or until the subject is considered to be stable.

6.4 Evaluation of Acceptability

At the Week 12/Early Termination Visit, the subject will be asked to rate the acceptability of the therapy received over the completed study period. The acceptability is defined as the subject`s perceptions of the ease in the method of administration and cleanliness of the product. The subject will be asked to rate the acceptability of the therapy as: excellent, good, acceptable, or unacceptable.

6.5 Laboratory Analyses

Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis, as described in Section 6.3.1), including those that worsen from baseline, felt to be clinically

significant in the medical and scientific judgment of the investigator are to be recorded as AEs or SAEs.

However, any clinically significant safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition, are **not** to be reported as AEs or SAEs.

6.6 Sample Collections

Procedures for sample collection, storage, and shipping for analysis are provided in the study site laboratory manuals.

Samples obtained for clinical laboratory assessments will be destroyed at the end of the study. The remaining biopsy and smear samples will be stored [redacted]

7 Statistical and Analytical Plan

A detailed statistical analysis plan will be prepared prior to completing the database and unblinding the study. Summaries for quantitative variables will include the mean, median, quartiles, standard deviation or standard error, minimum, and maximum. For qualitative variables, the summaries will include the number and percentage of subjects for each outcome. Statistical significance will be declared if the one-sided p-value is ≤ 0.025. All computations will be performed using SAS® [redacted]

The analysis of the 12-week double-blind study will be conducted when all data for all subjects have been entered, cleaned and locked.

7.1 Primary Efficacy Endpoints

The 4 co-primary efficacy endpoints in this study are:

- change from Baseline to Week 12 in the severity of vaginal dryness;
- change from Baseline to Week 12 in vaginal pH;
- change from Baseline to Week 12 in the proportion of parabasal cells of the vaginal epithelium;
- and change from Baseline to Week 12 in the proportion of superficial cells of the vaginal epithelium.

7.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints in this study are:

- change from Baseline to Week 12 in the severity of individual vaginal symptoms including dyspareunia, pruritus, burning and dysuria;
- change from Baseline to Week 12 in the Global Symptom Score
- change from Baseline to Week 12 in the severity of individual vaginal signs including pallor, friability, thinning or flattening of folds, petechiae and dry mucosa;

- change from Baseline to Week 3 in the proportion of superficial cells of the vaginal epithelium;
- change from Baseline to Week 3 in the proportion of parabasal cells of the vaginal epithelium;
- change from Baseline to Week 3 in the severity of vaginal dryness;
- change from Baseline to Week 3 in vaginal pH;
- change from Baseline to Week 3 in the severity of individual vaginal symptoms including dyspareunia, pruritus, burning and dysuria;
- change from Baseline to Week 3 in the severity of individual vaginal signs including pallor, friability, thinning or flattening of folds, petechiae and dry mucosa;
- change from Baseline to Week 3 in the Global Symptom Score.

7.3 Exploratory Efficacy Endpoints

The following exploratory endpoints will be analyzed:

- evaluation of the final subjective global perception of efficacy at Week 12;
- evaluation of the acceptability of the therapy at Week 12.

7.4 Safety Endpoints

The following safety variables will be analyzed:

- laboratory parameters
    - biochemistry, hematology and urinalysis at Week 12;
    - serum lipids, and coagulation parameters at Week 12;
    - change from Baseline in hormone levels (estradiol, estrone, estriol, FSH, LH);

- change from Baseline in uterine evaluation by transvaginal ultrasound at Week 12 for women with an intact uterus;
- change from Baseline in endometrial histology at Week 12 for women with an intact uterus;
- frequency and severity of AEs;
- change from Baseline in ECG QTc

7.5 Sample Size Calculations

A total of 280 female subjects will be enrolled in the study.

The estimates of treatment differences and variability were gained from the results of Study ITFE-2026-C2.

A sample size of 57 in each group will have 90% power to detect a treatment difference for change in severity of vaginal dryness, assuming 5 categories with proportions in each treatment group as shown in the table below, using a Wilcoxon rank sum test with a 0.025 one-sided significance level.

Table 2 Assumed Proportions for Wilcoxon Rank Sum Test of Vaginal Dryness

Change in Vaginal Dryness Severity	Proportion in Estriol Vaginal Gel Group	Proportion in Placebo Group
-3	0.189	0.118
-2	0.423	0.196
-1	0.270	0.353
0	0.118	0.333
1	0	0

A sample size of 48 in each group will have 90% power to detect a treatment difference of -0.89 for change in vaginal pH, assuming a common standard deviation of 1.33 using a two-group t-test with a 0.025 one-sided significance level.

A sample size of 24 in each group will have 90% power to detect a treatment difference of 21 for change in proportion of superficial cells, assuming a standard deviation of 26.3 for 0.005% estriol vaginal gel and 14.8 for placebo vaginal gel, using a two-group Satterthwaite t-test with a 0.025 one-sided significance level.

A sample size of 9 in each group will have 90% power to detect a treatment difference of -53 for change in proportion of parabasal cells, assuming a standard deviation of 37.9 for 0.005% estriol vaginal gel and 19.6 for placebo vaginal gel, using a two-group Satterthwaite t-test with a 0.025 one-sided significance level.

The largest sample size calculated for a single co-primary endpoint has been considered the sample size required for the whole study, thus, 57 subjects per group are estimated. Accounting for an 18% drop-out rate, and rounding numbers, approximately 70 subjects/group have been finally estimated.

7.6 Analysis Sets

The following analysis sets will be used in the statistical analyses:

- The Intent-to-Treat (ITT) population is defined as all randomized subjects. The ITT population will be used to present all efficacy analyses, including the 4 co-primary efficacy endpoints. Subjects will be summarized according to the treatment to which they were randomized.

- Per Protocol (PP) population is defined as all randomized subjects who completed the study to Week 12 with no major protocol deviations impacting the co-primary efficacy analyses. The major deviations impacting the analysis will be defined in more detail in the statistical analysis plan (SAP). The PP population will be used for confirmation of efficacy analyses. Subjects will be summarized according to the treatment to which they were randomized.

- The Safety population includes all randomized subjects who administered at least one dose of study treatment. The Safety population will be used to present all safety analyses. Subjects will be summarized according to the actual treatment received.

7.7 Description of Subgroups to be analyzed

No subgroup analyses are planned.

7.8 Statistical Analysis Methodology

All computations will be performed using SAS® (Version 9.3 or later). Continuous variables will be summarized using the mean, the standard deviation, median, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages. Data will be listed in data listings.

Details of the statistical analyses, methods, and data conventions are described in the SAP.

7.8.1 Analysis of Primary Efficacy Endpoints

The changes from baseline to Week 12 in vaginal dryness will be analyzed using a Wilcoxon Rank Sum test, controlled for country, to obtain the p-values. The Hodges-Lehmann estimate of median difference between treatment groups (0.005% versus placebo, 0.002% versus placebo and 0.0008% versus placebo) will also be presented with 95% confidence intervals. It should be noted that, since the statistical tests are one-sided, only one limit of each confidence interval is of particular interest for analysis. Details of statistics to be presented will be described in the Statistical Analysis Plan. Missing values at Week 12 will be estimated using the last observation carried forward (LOCF). Only post-Baseline values will be carried forward.

The changes from baseline to Week 12 in vaginal pH, and the changes in proportions of parabasal and superficial cells will be analyzed using a parametric ANCOVA model. The model will include fixed effects of treatment and country, and the baseline value will be used as a covariate. The adjusted (least-square) mean change from baseline to Week 12 will be presented for each treatment, along with the corresponding standard error. The difference in adjusted means between treatment groups (0.005% versus placebo, 0.002% versus placebo and 0.0008% versus placebo) will also be presented with 95% confidence intervals and p-values. It should be noted that, since the statistical tests are one-sided, only one limit of each confidence interval is of particular interest for analysis. Model assumptions will be examined and, if required, a Wilcoxon Rank Sum test as described for vaginal dryness will be used. Missing values will be estimated using LOCF. Only post-Baseline values will be carried forward.

No adjustment for multiplicity will be made, since statistical significance in all four co-primary endpoints is required to declare efficacy.

The robustness of the LOCF approach for missing data will be addressed by completing a sensitivity analysis. This will be an observed case analysis and will use no imputation methods in order to assess the robustness of the results.

7.8.1.1 Gatekeeping Procedure

For the co-primary endpoints a gatekeeping procedure will be employed. The 0.005% dose will be compared to placebo, and if this is significant at a one-sided alpha level of 0.025 for all 4 co-primary endpoints, then the 0.002% dose versus placebo can be considered. If this is also significant at a one-sided alpha level of 0.025 for all 4 co-primary endpoints, then the 0.0008% dose versus placebo can be considered. The following table (Table 3) outlines the approach to be taken.

Table 3: Gatekeeping Procedure for Efficacy Analyses

Test	Endpoints	All endpoints significant	Result	Next step
0.005% versus placebo	Vaginal dryness Vaginal pH Parabasal cells Superficial cells	Yes	If treatment difference exists at 0.005% estriol gel, move to next dose	Continue to test 0.002% estriol gel versus placebo
	Vaginal dryness Vaginal pH Parabasal cells Superficial cells	No	Not enough evidence to conclude a difference exists with 0.005% estriol gel.	Stop testing
0.002% versus placebo	Vaginal dryness Vaginal pH Parabasal cells Superficial cells	Yes	If treatment difference exists at 0.002% estriol gel, move to next dose	Continue to test 0.0008% estriol gel versus placebo
	Vaginal dryness Vaginal pH Parabasal cells Superficial cells	No	Not enough evidence to conclude a difference exists at 0.002% estriol gel	Stop testing
0.0008% versus placebo	Vaginal dryness Vaginal pH Parabasal cells Superficial cells	Yes	Treatment difference exists at 0.0008% estriol gel	Stop testing

Test	Endpoints	All endpoints significant	Result	Next step
	Vaginal dryness Vaginal pH Parabasal cells Superficial cells	No	Not enough evidence to conclude a difference exists at 0.0008% estriol gel	Stop testing

7.8.2 Analysis of Secondary Efficacy Endpoints

The change from Baseline to Week 12 in the severity of individual vaginal symptoms (dyspareunia, pruritus, burning and dysuria) and signs (pallor, friability, thinning or flattening of folds, petechiae, and dry mucosa) will be assessed using the same Wilcoxon Rank Sum test as for the co-primary endpoint of vaginal dryness at Week 12. Missing values will be estimated using LOCF. Only post-Baseline values will be carried forward.

The change from Baseline to Week 12 in the Global Symptom Score will be assessed using the same parametric ANCOVA as for the co-primary endpoints of vaginal pH and proportions of parabasal and superficial cells at Week 12. Missing values will be estimated using LOCF. Only post-Baseline values will be carried forward.

The change from Baseline to Week 3 in the severity of individual vaginal symptoms (vaginal dryness, dyspareunia, pruritus, burning and mucosa) and signs (pallor, friability, thinning or flattening of folds, petechiae, and dry mucosa) will be assessed using the same Wilcoxon Rank Sum test as for the co-primary endpoint of vaginal dryness at Week 12. Missing data will not be imputed.

The change from Baseline to Week 3 in the vaginal pH, the proportion of parabasal cells, the proportion of superficial cells, and the Global Symptom Score will be assessed using the same parametric ANCOVA as for the co-primary endpoints of vaginal pH and proportions of parabasal and superficial cells at Week 12. Missing data will not be imputed.

7.8.3 Analyses of Exploratory Efficacy Endpoint

Endpoints of acceptability of therapy and final subjective global perception will be summarized by treatment group.

7.8.4 Safety Analyses

The assessment of safety will be based on the incidence of AEs occurring during the 12-weeks on study treatment, and changes from Baseline to Week 12 in laboratory values and ECG QTc determinations. Changes from Baseline to Week 12 in transvaginal ultrasound findings will be summarized for women with an intact uterus. Changes in endometrial histological evaluation at Week 12 will be summarized for women with an intact uterus.

7.8.4.1 Pooling of Study Sites

Sites will be pooled according to country for inclusion as an effect in the statistical models. It is recognized that the pooling of sites in this manner preserves the inclusion of all randomized subjects in the analysis. However, the result of pooling is a loss of the "individuality" of each site being pooled as well as the potential for the pooled site to be associated with an inflated sample size compared to sites that do not require pooling. The effect of this cannot be determined a priori but it is an acceptable approach for the retention of subjects in the analysis.

7.8.5 Interim Analyses

Interim analyses will not be performed.

7.9 Data Quality Assurance

Suitably qualified and trained clinical research personnel of the Sponsor or designee will visit the trial site at regular intervals during the trial for monitoring purposes and to assist the research staff with any queries. The eCRFs and source documentation will be available for review during monitoring visits to the trial site. The function of this monitoring is to ensure compliance with the protocol, adherence to regulatory and GCP obligations, proper maintenance of records including study drug accountability records, correct administration of study drug including storage conditions and accurate reporting of AEs.

After data from the eCRFs have been entered onto the database, they will be reviewed and the data verified against the subject's source data. Any discrepancies will be tracked by paper or electronically with an electronic audit trail.

The Sponsor or designee has an obligation to audit a proportion of study sites. A department other than the clinical department will undertake this obligation. Therefore the Sponsor, an independent auditor, or a regulatory authority may audit the trial site and trial documentation. These audits may take place while the trial is being conducted or up to several years later.

7.9.1 Data Management

As part of the responsibilities assumed by participating in the study, the investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories. These source documents may include laboratory reports, histology reports, and ultrasound findings.

Investigative site personnel will enter subject data into Medidata Rave (eCRF). The analysis data sets will be a combination of these data and data from other sources (eg, laboratory data).

Clinical data management will be performed in accordance with applicable [redacted] standards and data cleaning procedures to ensure the integrity of the data, eg, removing errors and inconsistencies in the data. Adverse events and concomitant medication terms will be coded using the MedDRA.

8 Ethics

8.1 Independent Ethics Committee or Institutional Review Board

Federal regulations and the International Conference on Harmonisation (ICH) guidelines require that approval be obtained from an IRB/IEC before participation of human subjects in research studies. Before study onset, the protocol, informed consent, advertisements to be used for the recruitment of study subjects, and any other written information regarding this study to be provided to the subject or the subject's legal guardian must be approved by the IRB/IEC. Documentation of all IRB/IEC approvals and of the IRB/IEC compliance with ICH Harmonised Tripartite Guideline E6 (R1/R2): Good Clinical Practice (GCP) will be maintained by the site and will be available for review by the Sponsor or its designee.

All IRB/IEC approvals should be signed by the IRB/IEC chairman or designee and must identify the IRB/IEC name and address, the clinical protocol by title or protocol number or both, and the date approval or a favorable opinion was granted.

The investigator is responsible for providing written summaries of the progress and status of the study at intervals not exceeding 1 year or otherwise specified by the IRB/IEC. The investigator must promptly supply the Sponsor or its designee, the IRB/IEC, and, where applicable, the institution, with written reports on any changes significantly affecting the conduct of the study or increasing the risk to subjects.

8.2 Ethical Conduct of the Study

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki (Section 12.1), ICH Guideline for GCP E6 (R1/R2), and all applicable regulations.

8.3 Subject Information and Consent

A written informed consent in compliance with ICH Guideline for GCP E6 (R1/R2) and US Title 21 Code of Federal Regulations (CFR) Part 50 shall be obtained from each subject before entering the study or performing any unusual or non-routine procedure that involves risk to the subject. An informed consent template may be provided by the Sponsor to investigative sites. If any institution-specific modifications to study-related procedures are proposed or made by the site, the consent should be reviewed by the Sponsor or its

designee or both before IRB/IEC submission. Once reviewed, the consent will be submitted by the investigator to his or her IRB/IEC for review and approval before the start of the study. If the ICF is revised during the course of the study, all active participating subjects must sign the revised form. Reconsent will be needed before rescreening a patient.

Before recruitment and enrollment, each prospective subject or his or her legal guardian will be given a full explanation of the study and be allowed to read the approved ICF. Once the investigator is assured that the subject/legal guardian understands the implications of participating in the study, the subject/legal guardian will be asked to give consent to participate in the study by signing the ICF.

The investigator shall retain the signed original ICF and give a copy of the signed original form to the subject or legal guardian.

9 Investigator's Obligations

The following administrative items are meant to guide the investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Changes will be reported to the IRB/IEC but will not result in protocol amendments.

9.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain subject confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the subject (or the subject's legal guardian), except as necessary for monitoring and auditing by the Sponsor, its designee, the IRB/IEC, or the US FDA.

The investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

9.2 Financial Disclosure and Obligations

Investigators are required to provide financial disclosure information to allow the Sponsor to submit the complete and accurate certification or disclosure statements required under FDA 21 CFR 54 (even though the study is being conducted in the European Union). In addition, the investigator must provide to the Sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

The Sponsor is not financially responsible for further testing or treatment of any medical condition that may be detected during the screening process. In addition, in the absence of specific arrangements, the Sponsor is not financially responsible for further treatment of the subject's disease.

9.3 Investigator Documentation

Prior to beginning the study, the investigator will be asked to comply with ICH GCP E6 (R1/R2) 8.2 by providing the following essential documents, including but not limited to:

- IRB/IEC approval
- Original investigator-signed investigator agreement page of the protocol
- Curriculum vitae for the investigator and each sub-investigator participating in the study
- Financial disclosure information to allow the Sponsor or designee to submit complete and accurate certification or disclosure statements required under ICH GCP E6 (R1/R2). In addition, the investigators must provide to the Sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study.
- IRB/IEC-approved informed consent, samples of site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the subject or legal guardian, and
- Laboratory certifications and normal ranges for any local laboratories used by the site, in accordance with GCP

9.4 Study Conduct

The investigator agrees that the study will be conducted according to the principles of ICH Guidelines for GCP E6 (R1/R2). The investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations. Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

9.5 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with ICH Guidelines for GCP E6 (R1/R2) and all applicable guidelines and regulations.

9.6 Adverse Events and Study Report Requirements

By participating in this study the investigator agrees to submit reports of SAEs according to the time line and method outlined in the protocol. In addition, the investigator agrees to submit annual reports to the study site IRB/IEC as appropriate.

9.7 Investigator's Final Report

Upon completion of the study, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB/IEC with a summary of the study's outcome and the Sponsor and regulatory authority(ies) with any reports required.

9.8 Records Retention

Following completion of the study, the investigator will retain copies of the approved study protocol, ICF, relevant source documents, and all other supporting documentation related to the study according to the ICH guidelines, according to local regulations, or as specified in the Clinical Study Agreement (CSA), whichever is longer.

An investigator shall retain records required to be maintained under this part for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. The documentation should be retained for longer if required by regulatory requirements or by agreement with the sponsor.

If the investigator can no longer maintain the archive of study records (e.g., due to retirement or relocation), the sponsor must be informed in writing about any change in responsibility for record retention, including the name of the new responsible party, contact information, and location of the study records. Records may not be destroyed without prior written consent from the sponsor.

9.9 Publications

The investigator will not be allowed to publish findings from study participation without prior written consent of the Sponsor.

10 Study Management

10.1 Monitoring

The clinical monitor, as a representative of the Sponsor, has the obligation to follow the study closely. In doing so, the monitor will visit the investigator and study site at periodic intervals, in addition to maintaining necessary telephone and letter contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the investigator and personnel.

All aspects of the study will be carefully monitored, by the Sponsor or its designee, for compliance with applicable government regulation with respect to current GCP and current standard operating procedures.

10.1.1 Inspection of Records

Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB/IEC review, and regulatory inspections by providing direct access to all study records. In the event of an audit, the investigator agrees to allow the Sponsor, representatives of the Sponsor, or a regulatory agency (e.g., FDA or other regulatory agency) access to all study records.

The investigator should promptly notify the Sponsor of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the Sponsor.

10.2 Management of Protocol Amendments and Deviations

10.2.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the subject, must be reviewed and approved by the Sponsor. Amendments to the protocol must be submitted in writing to the investigator's IRB/IEC for approval before subjects can be enrolled into an amended protocol. Amendments to the protocol may require changes to the informed consent document and must then be reviewed and approved by the investigator's IRB/IEC before subjects can be enrolled into the amended protocol (see Section 8.3).

10.2.2 Protocol Deviations

The investigator or designee must document and explain in the subject's source documentation any deviation from the approved protocol. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study subjects without prior IRB/IEC approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendments should be submitted to the IRB/IEC for review and approval, to the Sponsor for agreement, and to the regulatory authorities, as applicable.

A significant deviation from the protocol is an unintended or unanticipated departure from the procedures or processes approved by the Sponsor and the IRB/IEC and agreed to by the investigator. A significant deviation occurs when there is non-adherence to the protocol by the subject or investigator that results in a significant, additional risk to the subject. Significant deviations can include non-adherence to inclusion or exclusion criteria, or non-adherence to ICH GCP guidelines or FDA regulations, and will lead to the subject being withdrawn from the study (Section 4.2).

Protocol deviations will be documented by the study site staff, the investigator, and the clinical monitor throughout the course of the study. Principal investigators will be notified in writing by the monitor of deviations. The IRB/IEC should be notified of all protocol deviations in a timely manner, as applicable.

10.3 Study Termination

The Sponsor reserves the right to modify or terminate the study at any time. Possible reasons for termination are unsatisfactory enrollment of subjects, new scientific knowledge becomes known that makes the objectives of the study no longer feasible, or safety reasons such as if the incidence of AEs in this study or other study using the same investigational drug indicates there is a potential health risk for subjects.

The Sponsor will provide a written statement to the IRB/IEC and the relevant regulatory authority with the reasons for modification or termination of the study. This termination will be conducted within 15 days of the decision to terminate the study. If the study is terminated, a close out visit will be performed at each active site and applicable procedures will be carried out to close the trial site(s). Although the Sponsor has every intention of

completing the study, ITF Research Pharma SLU reserves the right to discontinue the study at any time for clinical or administrative reasons.

The end of the study is defined as the date on which the last subject completes the last visit (includes Follow-up Visit).

10.4 Final Report

Whether the study is completed or prematurely terminated, the Sponsor will ensure that the clinical study reports are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The Sponsor will also ensure that the clinical study reports in marketing applications meet the standards of the ICH Harmonised Tripartite Guideline E3: Structure and content of clinical study reports.

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results.

Upon completion of the clinical study report, the Sponsor will provide the Investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate. The study results will be posted in publicly available clinical trial registers.

11 Reference List

The 2007 Hormone Therapy Position Statement of the North American Menopause Society (2007). *Menopause*, 14:357-369 .

The 2012 Hormone Therapy Position Statement of the North American Menopause Society. (2012). *Menopause*, 19:257-271.

Bergink EW, Kloosterboer HJ, van der Vies J (1984). Oestrogen binding proeins in the female genital tract. *J Steroid Biochem*, 20:1057-1060.

Cano A, Estevez J, Usandiziga R, et al (2012). The therapeutic effect of a new ultra low concentration estriol gel formulation (0.005% estriol vaginal gel) on symptoms and signs of postmenopausal vaginal atrophy: results from a pivotal phase III study. *Menopause*, 19:1130-1139.

Cardozo L, Bachmann G, McClish D, et al (1998). Meta-analysis of estrogen therapy in the management of urogenital atrophy in postmenopausal women: second report of the Hormones and Urogenital Therapy Committee. *Obstet Gynecol*, 92:722-727.

Fleiss JL (1986). *The Design and Analysis of Clinical Experiments.* New York: John Wiley & Sons.

Gruber CJ, Tschuqquel W, Schneeberger C, et al (2002). Production and actions of estrogens. *N Engl J Med*, 346:340-352.

Hayashi T, Ito I, et al (2000). Estriol (E3) replacement improves endothelial function and bone mineral density in very elderly women. *J Gerontol A Biol Sci Med Sci*, 55:B183-B190.

ICH Expert Working Group. ICH Harmonised Tripartite Guideline, Choice of Control Group and Related Issues in Clinical Trials E10. 20 July 2010

Fleiss JL (1986). *The Design and Analysis of Clinical Experiments.* New York: John Wiley & Sons.

Karch FE, Lasagna L (1977). Towards the operational identification of adverse drug reactions. *Clin Pharm Ther*, 21:247-254.

Kurman RJ, Hedrick Ellenson L, Ronnett BM (eds) (2011). *Blaustein's Pathology of the Female Genital Tract, 6th Edition.* New York: Springer.

Nakayama H, Yano T, Sagara Y, et al (1999). Estriol add-back therapy in the long-acting gonadotropin-releasing hormone agonist treatmetn of uterine leiomyomata. *Gynecol Endocrinol*, 13:382-389.

Padwick ML, Siddle NC, Lane G, et al (1986). Oestriol with oestradiol versus oestradiol alone: a comparison of endometrial, symptomatic and psychological effects. . *Br J Obstet Gynaecol*, 93:606-612.

Raz R, Stamm WE (1993). A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. *N Engl J Med*, 329:753-756.

Reynolds TM, Nix AB, Bunstan FD, et al (1993). Age-specific detection and false-positive rates: an aid to counselling in Down syndrome risk screening. *Obstet Gynecol*, 81:447-450.

Takahashi K, Manabe A, Okada M, et al (2000). Efficacy and safety of oral estriol for managing postmenopausal symptoms. *Maturitas*, 34:169-177.

US Dept. Health and Human Services (2003). Guidance for Industry: Estrogen and estrogen/progestin drug products to treat vasomotor symptoms and vulvar and vaginal atrophy symptoms - Recommendations for Clinical Evaluation. Draft Guidance. Washington: U.S. Department of Health and Human Services. http://www.fda.gov/downloads/ScienceResearch/SpecialTopics/WomensHealthResearch/UCM133343.pdf

van Haaften M, Donker GH, Sie-Go DM, et al (1997). Biochemical and histological effects of vaginal estriol and estradiol applications on the endometrium, myometrium and vagina of postmenopausal women. *Gynecol Endocrinol*, 11:175-185.

12Appendices

12.1 WMA Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

INTRODUCTION

The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics

declares that, "A physician shall act in the patient's best interest when providing medical care."

Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

In medical practice and in medical research, most interventions involve risks and burdens.

Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant

sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the Sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or

community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has

understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give

informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or

Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

12.2 Investigator's Statement and Agreement

I (as Investigator) have read and understood all sections of the study protocol Version 5.0, dated 07 June 2017, and the accompanying Investigator's Brochure, [redacted].

I hereby agree to supervise all aspects of the protocol and to conduct the clinical investigation in accordance with the Final Protocol, the International Conference on Harmonisation Harmonised Tripartite Guideline E6 (R1/R2): Good Clinical Practice and all applicable government regulations.

I will not make changes to the protocol before consulting with ITF Research Pharma SLU or implement protocol changes without independent ethics committee approval except to eliminate an immediate risk to subjects. I agree to administer study drug only to subjects under my personal supervision or the supervision of a sub-investigator. I will not supply the investigational drug to any person not authorized to receive it. Subject identity will not be disclosed to third parties or appear in any study reports or publications.

It is further agreed that the details of this trial and all information provided to me by ITF Research Pharma SLU will be held in the strictest confidence and will not be revealed for any reason without written authorization from ITF Research Pharma SLU except to those who are to participate in the conduct of the trial.

I agree that all data, the case report forms and all materials provided for the conduct of the trial are the property of ITF Research Pharma SLU unless specified otherwise in writing. It is understood that ITF Research Pharma SLU will utilize the information derived from this trial in various ways, such as for submission to government regulatory agencies, required internal reports and presentations without violating patient/ subject confidentiality in any way.

I further agree that ITF Research Pharma SLU or its representatives will be permitted access in accordance with current Good Clinical Practice Guidelines, to all data generated by this trial and the source documents from which case report form data were generated.

Principal Investigator Printed Name: _____

Signature: _____ Date: _____ Site Nº or Name: _____

12.3 Screening Criteria – Allowed Pre-study Therapies

Medication/Therapy	Route of Administration	Discontinuation Prior to Screening
Non-hormonal vulvovaginal treatment (including cosmetics expected to have an impact on vaginal pH such as special feminine wash gels)	Topical	7 days
Phytoestrogens	Any route	1 month
Hormone therapy	Vaginal	1 month
Hormone therapy: estrogen alone progestin alone estrogen/progestin combination	Oral, vaginal, intrauterine, or transdermal	2 months
Progestational implants	Subcutaneous	3 months
Estrogen	Injectable	3 months
Estrogen/progestational products	Injectable	3 months
Estrogen pellet therapy	Subcutaneous	6 months
Progestin therapy	Injectable	6 months
Estrogen lotions/gels	Percutaneous	1 month
Testosterone or testosterone derivatives	Any route	2 months
Dehydroepiandrosterone	Any route	2 months
Tibolone	Any route	2 months
Selective estrogen receptor modulators	Any route	2 months

12.4 Screening Criteria - Excluded Medications

Medication/Therapy
Anti-epileptic drugs, including: Barbiturates Hydantoins Carbamazepine
Rifampicin
Rifabutin
Tetracyclines
Ketoconazole
Evirapine
Efavirenz
Ritonavir
Nelfinavir
Phenyobutazone
Preparations based on medicinal plants that contain St. John's Wort (*Hypericum perforatum*)