Protocol ITFE-2026-C10

A PHASE II PROSPECTIVE, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED AND MULTI-CENTRE CLINICAL TRIAL TO ASSESS THE SAFETY OF 0.005 % ESTRIOL VAGINAL GEL IN HORMONE RECEPTOR-POSITIVE POSTMENOPAUSAL WOMEN WITH EARLY STAGE BREAST CANCER IN TREATMENT WITH AROMATASE INHIBITOR IN THE ADJUVANT SETTING.

"BLISSAFE Study"

Sponsor: ITF RESEARCH PHARMA S.L.U

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Sponsor Study Code: ITFE-2026-C10

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SUMMARY OF THE STUDY PROTOCOL

Study Title: **A PHASE II PROSPECTIVE, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED AND MULTI-CENTRE CLINICAL TRIAL TO ASSESS THE SAFETY OF 0.005% ESTRIOL VAGINAL GEL IN HORMONE RECEPTOR-POSITIVE POSTMENOPAUSAL WOMEN WITH EARLY STAGE BREAST CANCER IN TREATMENT WITH AROMATASE INHIBITOR IN THE ADJUVANT SETTING. "BLISSAFE Study"**

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Countries and number of sites: Spain and Sweden. 5 participating sites approximately

Study Rationale:

Breast cancer is the most frequently diagnosed cancer worldwide and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of total cancer deaths (1). About 80% of primary breast cancers are hormone sensitive as they contain estrogen receptor (ER) and/or progesterone receptor-positive cells (2,3). The presence of hormone receptors makes endocrine manipulation one of the most useful therapeutic options for these women. Antiestrogenic therapy, either by blocking the estrogen receptor (tamoxifen) or by estrogen deprivation (aromatase inhibitor), has become the most effective treatment for endocrine-responsive breast cancer (4,5).

In the setting of postmenopausal hormone receptor (HR) positive breast cancer, treatment with aromatase inhibitors (AIs) is the most effective and well-studied therapy (6). The recommended duration of adjuvant endocrine therapy is 5 years and some patients may benefit from a further 5 years of treatment. It is well established that lack of adherence to the adjuvant treatment is very common due to the side-effects derived of the therapy with AIs, which lead to reduced efficacy and can be the most detrimental factor influencing clinical outcome (7). Due to that, with such long-term treatment duration, it is critical to address morbidity associated with treatment side-effects in an effort to optimize quality of life (QoL).

With regard to AIs, particularly high prevalence of urogenital symptoms such as vaginal dryness and sexual dysfunction due to vaginal atrophy, have been observed (5, 8). Taking into account that many women diagnosed with breast cancer today will be long-term survivors of their disease, the long-term impact of therapy on their well-being and specifically on their sexual adverse effects has become a growing topic for research (9).

In order to overcome symptoms of estrogen deprivation, vaginal moisturizers or, sometimes, low dose topical estrogens are used in daily clinical practice, despite the inexistence of adequate clinical trials that support their use. Non-hormone vaginal moisturizers relieve urogenital symptoms, however their efficacy is significantly lower compared to vaginal estrogens (10). In contrast to oral hormone therapy, some consider that local application of estrogens seems to be safe in postmenopausal breast cancer patients (11). However, the report of a small prospective observation (12) advised caution with the use of vaginal estradiol in breast cancer patients receiving aromatase inhibitors, as an increase of serum estradiol levels was found that potentially

could counteract endocrine breast cancer treatment (13).

A study using lower doses of estradiol presented in the 2013 ASCO Annual Meeting showed no changes in FSH while sporadic elevations of estradiol were seen in 5 out of 26 studied patients. The significance of this is difficult to judge as the full results have not been published yet (14).

According to the publication, "*Practical guidelines for managing menopausal symptoms after breast cancer",* the use of vaginal estradiol in patients treated with aromatase inhibitors was discouraged, but there was a recommendation for the application of vaginal estriol, a much less potent estrogen than estradiol, in case estrogen therapy were deemed necessary (15).

However, safety data of vaginal estriol in breast cancer patients receiving aromatase inhibitors are scarce. Pfeiler et al prospectively investigated the safety of vaginal estriol in 10 postmenopausal breast cancer patients receiving AIs by measuring serum hormone levels before and 2 weeks after daily application of 0.5 mg vaginal estriol. In this study no elevation of serum estriol or estradiol could be detected, but a significant decline in serum follicle stimulating hormone and luteinizing hormone was observed. The conclusion of this study was that vaginal estriol did not lead to a long term elevation of serum estrogen levels (although the analytical methodology employed lacked sufficient sensitivity and the number of patients was very low); nevertheless the significant decline in gonadotropins suggested a systemic effect, which had to be kept in mind when offering vaginal estriol at the dose of 0.5 mg per application to breast cancer patients on aromatase inhibitors (16).

0.005% Estriol vaginal gel is a new formulation for the local treatment of postmenopausal vaginal atrophy, which delivers an ultra-low dose of estriol per application (50 μg), ten times lower than the current dose of this hormone used in clinical practice. It is marketed under the tradename of Blissel® and Gelistrol® in several European countries since 2012. This new formulation has proven to be efficacious in relieving vaginal dryness as well as improving the most typical signs of the atrophy in a cohort of postmenopausal women (17). A pharmacokinetic study compared the systemic absorption of estriol of the new formulation vs the reference product (Ovestinon vaginal cream 0.1%, 0.5 mg of estriol per application). After three weeks daily treatment, it was shown that 0.005% Estriol vaginal gel produced negligible plasma estriol levels and significantly lower than those produced by Ovestinon ($p<0.0001$). In addition, 0.005% estriol formulation did not change serum FSH or LH while a significant decline of serum FSH was observed in women that received Ovestinon ($p=0.0425$) (18,19).

These data have suggested that 0.005% estriol vaginal gel could be safe in postmenopausal patients treated with aromatase inhibitors; however, they were obtained in a cohort of healthy postmenopausal women.

In order to explore the safety of 0.005% estriol vaginal gel in the oncological context, a new safety study is proposed with the hypothesis that 0.005% estriol vaginal gel is a safe therapeutic option to treat the vaginal atrophy caused by AIs, without a significant decline in gonadotropin or increase in systemic estrogen levels in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with non-steroidal aromatase inhibitors (NSAIs) in the adjuvant setting.

Study Design and Treatment:

This is a phase II, prospective, randomized, double-blind, placebo-controlled, international (Spain and Sweden) and multicentre study.

60 patients will be randomized to receive 0.005% estriol vaginal gel (arm A) and placebo moisturizing gel (arm B) in a 4:1 proportion, so 48 patients will enter in arm A and 12 in arm B.

Arm A: Investigational drug product: 0.005% estriol vaginal gel (Blissel®)

Route:	Vaginal. Administration by an applicator inserted deep inside the vagina
Dose:	1 g of gel, containing 50 μg of estriol
Dosage schedule:	Weeks 1-3: single daily application
	Weeks 4-12: twice weekly administration.

Women will be instructed to administer the gel at bedtime.

Arm B: placebo moisturizing gel administered in the same way.

Patients will have hormone determinations (estriol, estradiol, estrone, FSH and LH) analyzed in a central laboratory at baseline and at weeks 1, 3, and 8 from the beginning of the therapy and at week 12 (last day of study therapy). An additional FSH and LH determination will be obtained during the screening period in order to assess the intra-individual variation of these hormones in baseline conditions. Patients will also undergo gynaecological examination (vaginal smear and pH), evaluation of vaginal symptoms and signs, and will answer a female sexual function questionnaire at baseline, at week 3 (from the beginning of therapy) and week 12 (last day of study therapy). Adverse events will be followed along the study and specifically collected at weeks 1, 3 and 8 from the beginning of therapy, at week 12 (last day of study therapy) and at safety visit (30 ±5 days after last day of study therapy).

Primary Objective:

- The primary objective is to evaluate the levels of FSH after treatment with 0.005% estriol vaginal gel in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with NSAIs in the adjuvant setting and symptoms of vaginal atrophy.

Primary End-point:

- Variation in serum levels of FSH from baseline to 12 weeks of treatment

Secondary Objectives:

- To evaluate the levels of estriol, estradiol, estrone, FSH and LH after treatment with 0.005% estriol vaginal gel in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with NSAIs in the adjuvant setting and symptoms of vaginal atrophy.

- To assess the safety and tolerability of 0.005% estriol vaginal gel in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with NSAIs in the adjuvant setting and symptoms of vaginal atrophy.
- To assess the efficacy of 0.005% estriol vaginal gel in the treatment of symptoms and signs of vaginal atrophy in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with NSAIs and symptoms of vaginal atrophy.
- To measure the impact of treatment with 0.005% estriol vaginal gel in sexual function of hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with NSAIs and symptoms of vaginal atrophy.

Secondary End-points:

- Variation in serum levels of FSH at different time points compared to baseline (weeks 1, 3 and 8).
- Variation in serum levels of LH and plasma levels of estriol, estradiol and estrone, at different time points compared to baseline (weeks 1, 3, 8 and 12).
- AEs according to the Medical Dictionary for Regulatory Activities (MedDRA).
- Changes in vaginal dryness and other symptoms and signs of vaginal atrophy; changes in vaginal maturation value and changes in vaginal pH at week 3 and week 12 vs baseline.
- Changes in sexual function measured by the Female Sexual Function Index ($FSFI^{20}$) scale at week 3 and week 12 vs baseline.

Justification of Sample size determination and interim analysis:

This is an exploratory study and there are scarce data to allow a precise sample size calculation. The most relevant data come from Pfeiler (16) who reported that the levels of FSH in patients treated with aromatase inhibitors fell from a mean value of 75.7 to 66.0 mIU/ml after two weeks of daily vaginal treatment with 0.5 g of estriol. Although no figures are reported of the variability of those measurements, an estimation of the standard deviation can be made applying the standard rule of [sd = range/4]. The reported range of FSH values in that report is 45.6 – 134.6, thus a reasonable approximation to the sd is 89/4 = 22.3. Under these assumptions, it can be calculated that a sample size of 44 would provide 80% power to detect with an alpha=0.05 a decrease of FSH levels from 75.7 to 66.0 mIU/ml assuming a standard deviation of 22.3.

The hormone levels in women treated with IA who are not receiving estrogen therapy are likely to suffer physiological temporal oscillations. These variations could make difficult the interpretation of any eventual modification of hormone levels observed in the estriol treated women. In the absence of data of naturally occurring variations in hormone levels of women treated with IA that allow us to interpret the results of hormone determinations under study, it is considered appropriate to include a placebo group to provide reference data. In this case, the placebo is a vaginal moisturizing gel.

It is estimated that a group of 11 women will allow the assessment of physiological fluctuations in hormone levels. Additionally, this sample size for the placebo group will provide 80% power and alpha=0.05 to differentiate between active and placebo (ratio 4:1) on the change in the

vaginal maturation value, which is a secondary variable of the study that provides objective information about the effectiveness of treatment on vaginal atrophy (calculation based on the parameter data from the pivotal Blissel study and considering only the subgroup of women who had severe vaginal dryness: change VM active group 24.9 vs 0.6 points in placebo group, SD = 24.2).

The differentiation between active and placebo in the subjective parameters of efficacy (secondary) would be possible at the expense of a sample size noticeably superior. Since the evaluation of effectiveness is not primary but secondary in this study, it is not justified in this study to increase the sample for this reason.

In accordance with the above, the study would include 55 women (44 will receive vaginal estriol gel and 11 with the placebo moisturizing gel). Taking into account 10% of dropouts, the sample to be considered is of 60 women (48 vaginal estriol gel and 12 placebo moisturizing gel). This is an unbalanced study but with an active: placebo ratio of 4:1, which will support an adequate patient randomization (blocks of 5).

10 additional women will be recruited in a first safety phase of the study (8 with vaginal estriol gel and 2 placebo moisturizing gel). It is expected that none of them will present with significant changes in FSH from postmenopausal to premenopausal levels.

Statistical:

- Demographics and Baseline Characteristics

  Standard descriptive statistics, such as the mean, median, range and proportion, will be used to summarize the patient sample and to estimate parameters of interest. Ninety-five percent confidence intervals will be provided for estimates of interest wherever possible.

- Blood hormone levels

  The variation of the levels of FSH, estriol, estradiol, estrone and LH after treatment with 0.005% estriol vaginal gel will be studied in each woman. The variation of levels between two arms will be analyzed using a non-parametric test (Mann-Whitney-Wilcoxon test) or an ANCOVA (if it must be adjusted by initial value).

- Safety Analyses

  Adverse events data and serious adverse events will be reported in frequency tables (overall and by grades). The safety analysis will be performed in the population that has received at least one dose of the drug. AEs will be graded according to MedDRA. Adverse events will be compared using the chi-square tests (Fisher's Exact test in the case of observing frequencies <5%).

  Symptoms of Vaginal atrophy will be evaluated with a codification from 0 to 3.

- Efficacy Analyses

  The variations in the intensities for each one of the symptoms and signs of the vaginal atrophy, after 3 and 12 weeks, in each treatment arm, will be compared using a non-parametric test (Mann-Whitney-Wilcoxon). Descriptive statistics will be calculated for all variables studied.

- Other analysis: Sexual function measured by the FSFI scale. It will be used an algorithm for determining domain scores and a composite full-scale score.

Study population and main inclusion and exclusion criteria:

Patients with hormone receptor positive and HER2 negative early breast cancer who are on treatment with NSAI and have vaginal atrophy.

Inclusion Criteria:

Patients are eligible to be included in the study only if they **meet all** of the following criteria:

1. Written informed consent prior to beginning specific protocol procedures.
2. Patients must have histological confirmation of breast adenocarcinoma with stage I-IIIA, documented at a local pathology department.
3. The breast tumors must be estrogen-receptor positive and/or progesterone receptor positive (≥1% of stained tumor cells by IHC as determined by the local laboratory) with any HER2 status.
4. Postmenopausal status defined as: 12 months of spontaneous amenorrhea or 6 months of spontaneous amenorrhea with serum FSH levels > 40 mIU/ml or 6 weeks postsurgical bilateral oophorectomy with or without hysterectomy.
5. Patient must be receiving the non-steroidal aromatase inhibitors anastrozole or letrozole as breast cancer treatment in the adjuvant setting for a minimum of 6 months.
6. Women suffering from moderate to severe vaginal dryness according to the FDA guidelines for drug development in postmenopausal women (Center for Drug Evaluation and Research, CDER Jan 2003). A moderate symptom will be considered if the symptom is present, bothersome and annoying, and a severe symptom will be considered if the symptom is present, bothersome and annoying, and interferes with the normal patient activity.
7. Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0 or 1.
8. Adequate bone marrow as defined by the following laboratory values:
    a. Absolute Neutrophil Count (ANC) ≥ 1.5 x 10^9/L.
    b. Platelets (plt) ≥ 100 x 10^9/L.
    c. Hemoglobin (Hgb) ≥ 10 g/dl.
9. Patient has adequate organ function as defined by the following laboratory values:
    d. Serum creatinine ≤ 1.5 x ULN.
    e. Bilirubin ≤ 1.5 × ULN.
    f. Alkaline phosphatase ≤ 2 × ULN.
    g. AST and ALT ≤ 2 × ULN.
10. Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.

Exclusion Criteria:

Patients will be excluded from the study if they **meet any** of the following criteria:

1. Stage IIIB-IV breast cancer or bilateral breast cancer.
2. Treatment with any other current anti-tumoral therapy (chemotherapy, anti-Her2…etc) besides the NSAI. Pamidronate or Alendronate are permitted.
3. Prior history of other malignancy within 5 years of study entry, aside from non-melanoma skin cancer or carcinoma-in-situ of the uterine cervix adequately treated.
4. Postmenopausal uterine bleeding. Vaginal bleeding of unknown etiology.
5. Patients with endometrial thickness equal to or greater than 4 mm measured by transvaginal ultrasound.
6. Patients who have received any type of vulvovaginal treatment in the 15 days prior to the start of the study.
7. Use of any hormone, natural (phytoestrogens) or herbal products for the treatment of menopausal symptoms within the last 3 months.
8. Current or previous history of thromboembolic disease or coagulopathies.
9. Severe cardiovascular or respiratory diseases in the previous 6 months.
10. Renal Impairment.
11. Hepatitis B and/or hepatitis C carriers (unless with normal hepatic function).
12. Known human immunodeficiency virus infection.
13. Known hypersensitivity to NSAI.
14. Other severe acute or chronic medical or psychiatric condition, or laboratory abnormality that would impart, in the judgment of the investigator, excess risk associated with study participation or study drug administration, or which, in the judgment of the investigator, would make the patient inappropriate for entry into this study.
15. Previous investigational treatment for any condition or participation in any clinical trial within 4 weeks of inclusion date.

Table of Contents

Abbreviations and Definitions

AE	Adverse Event
AI	Aromatase Inhibitor
ALT/ALAT (SGPT)	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AP	Alkaline Phosphatase
AR	Adverse Reaction
AST/ASAT (SGOT)	Aspartate Aminotransferase
Compliance	Adherence to all the trial-related requirements, good clinical practice (GCP) requirements, and the applicable regulatory requirements.
CR	Complete Response
CTCAE	Common Terminology Criteria for Adverse Events
CT Scan	Computed Tomography Scan
eCRF	Electronic Case Report Form (sometimes referred to as Clinical Report Form). An electronic form for recording study participants' data during a clinical study, as required by the protocol.
ECOG	Eastern Cooperative Oncology Group
End of Study (Trial)	The end of study (trial) is the date of the last visit or last scheduled procedure shown in the Study Schedule for the last active patient in the study.
Enroll	The act of assigning a patient to a treatment. Patients who are enrolled in the trial are those who have been assigned a registration number and treatment.
Enter	The act of obtaining informed consent for participation in a clinical trial from patients deemed eligible or potentially eligible to participate in the clinical trial. Patients entered into a trial are those who sign the informed consent document directly or through their legally acceptable representatives.
ER	Estrogen Receptor
ERB/IRB	Ethical review board/Institutional review board: A board or committee (institutional, regional, or national) composed of medical professional and nonmedical members whose responsibility is to verify that the safety, welfare, and human rights of the patients participating in a clinical trial are protected.
ESR	Expedited Safety Report
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone

FSFI	Female Sexual Function Index
GCP	Good Clinical Practice
GEICAM	Spanish Breast Cancer Research Group
HER2	Human Epidermal Growth Factor Receptor 2
Hgb	Hemoglobin
HR	Hormone Receptor
ICD	Informed Consent Document
IDMC	Independent Data Monitoring Committee
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
Investigator	A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.
ITF	ITF Research Pharma S.L.U.
ITT	Intent To Treat
LH	Luteinizing Hormone
Legal Representative	An individual, judicial, or other body authorized under applicable law to consent, on behalf of a prospective patient, to the patient's participation in the clinical trial.
MedDRA	Medical Dictionary for Regulatory Activities
MV	Maturation Value
NSAI	Non-Steroidal Aromatase Inhibitor
Patient	A subject with a defined disease.
PgR	Progesterone Receptor
QoL	Quality of Life
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAP	Statistical Analysis Plan
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper Limit of Normal

Study Title: A PHASE II PROSPECTIVE, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED AND MULTI-CENTRE CLINICAL TRIAL TO ASSESS THE SAFETY OF 0.005% ESTRIOL VAGINAL GEL IN HORMONE RECEPTOR-POSITIVE POSTMENOPAUSAL WOMEN WITH EARLY STAGE BREAST CANCER IN TREATMENT WITH AROMATASE INHIBITOR IN THE ADJUVANT SETTING.

"BLISSAFE Study"

1. Introduction

1.1 Overview of Breast Cancer

Breast cancer is the most frequently diagnosed cancer worldwide and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of total cancer deaths (1). About 80% of primary breast cancers are hormone sensitive as they contain estrogen receptor (ER) and/or progesterone receptor (PgR) positive cells (2,3). The presence of hormone receptors makes endocrine manipulation one of the most useful therapeutic options for these women. The role of endocrine therapy in breast cancer is well stablished since more than 100 years. Antiestrogenic therapy, either by blocking the estrogen receptor (tamoxifen, fulvestrant) or by estrogen deprivation (aromatase inhibitor [AI]), has become the most effective treatment for endocrine-responsive breast cancer (4,5).

1.2 Adjuvant endocrine treatment of postmenopausal HR+ early breast cancer patients

Since more than 40 years, tamoxifen administered for 5 years has been considered the gold standard for postmenopausal early breast cancer patients, however in the last 10 years the AIs have been considered the most effective and well-studied therapy (6). The recommended duration of adjuvant endocrine therapy is 5 years and some patients may benefit from a further 5 years of treatment. It is well established that lack of adherence to adjuvant treatment is very common due to the side-effects derived of the therapy with AIs, which lead to reduced efficacy and can be the most detrimental factor influencing clinical outcome (7). Due to that, with such long-term treatment duration, it is critical to address morbidity associated with treatment side-effects in an effort to optimize quality of life (QoL).

1.3 Side effects associated with breast cancer adjuvant endocrine therapy

Across all adjuvant endocrine trials, regardless of the treatment received, vasomotor symptoms such as hot flushes are the most common adverse events. Other frequently reported side effects, such as vaginal discharge, vaginal dryness, dyspareunia and arthralgia, vary in prevalence between tamoxifen and AIs (5). Data from a study specifically addressing menopausal

symptoms after breast cancer showed that hot flushes (41%), night sweats (36%), loss of interest in sex (30%), difficulty sleeping (25%), fatigue (22%) and extreme vaginal dryness (19%) were the most common adverse effects, being the urogenital symptoms such as the vaginal dryness and sexual dysfunction due to vaginal atrophy of particular high prevalence with the treatment with AIs (8).

1.4 Management of sexual adverse effects derived from the treatment of breast cancer patients

Taking into consideration that many women diagnosed with breast cancer today will be long-term survivors of their disease, the long-term impact of therapy on their well-being and specifically on their sexual adverse effects has become a growing topic for research (9).

In order to overcome symptoms of estrogen deprivation, vaginal moisturizers or, sometimes, low dose topical estrogens are used in daily clinical practice, despite the inexistence of adequate clinical trials that support their use. Non-hormone vaginal moisturizers relieve urogenital symptoms, however their efficacy is significantly lower compared to vaginal estrogens (10). In contrast to oral hormone therapy, some consider that local application of estrogens seems to be safe in postmenopausal breast cancer patients (11). However, the report of a small prospective observation (12) advised caution with the use of vaginal estradiol in breast cancer patients receiving aromatase inhibitors, as an increase of serum estradiol levels was found that potentially could counteract endocrine breast cancer treatment (13).

A study using lower doses of estradiol presented in the 2013 ASCO Annual Meeting showed no changes in FSH while sporadic elevations of estradiol were seen in 5 out of 26 studied patients. The significance of this is difficult to judge as the full results have not been published yet (14).

According to the publication "*Practical guidelines for managing menopausal symptoms after breast cancer",* the use of vaginal estradiol in patients treated with aromatase inhibitors was discouraged, but there was recommended the application of vaginal estriol, a much less potent estrogen than estradiol, in case estrogen therapy were deemed necessary (15).

However, safety data of vaginal estriol in breast cancer patients receiving aromatase inhibitors are scarce. Pfeiler et al prospectively investigated the safety of vaginal estriol in 10 postmenopausal breast cancer patients receiving AIs by measuring serum hormone levels before and 2 weeks after daily application of currently marketed dose of 0.5 mg vaginal estriol (Ovestin). In this study no elevation of serum estriol or estradiol could be detected, but a significant decline in serum follicle stimulating hormone and luteinizing hormone was observed (fell from a mean value of 75.7 to 66.0 mIU/ml after two weeks of daily vaginal treatment with 0.5 g of estriol). The conclusion of this study was that vaginal estriol did not lead to a long term elevation of serum estrogen levels (although the analytical methodology employed lacked sufficient sensitivity and the number of patients was very low); nevertheless the significant decline in gonadotropins indicated a systemic effect, which had to be kept in mind when offering vaginal estriol at the dose of 0.5 mg per application to breast cancer patients on aromatase inhibitors (16).

1.5 Role of 0.005% estriol vaginal gel (Blissel®) in the Treatment of vaginal atrophy

0.005% Estriol vaginal gel is a new formulation for the local treatment of postmenopausal vaginal atrophy, which delivers an ultra-low dose of estriol per application (50 μg), ten times lower than the current dose of this hormone currently used in clinical practice. It is marketed under the trade names of Blissel® or Gelistrol® in several European countries since 2012.

This new formulation has proven to be efficacious in relieving vaginal dryness as well as improving the most typical signs of the atrophy in a cohort of postmenopausal women (17). A pharmacokinetic study compared the systemic absorption of estriol of the new formulation vs the reference product (Ovestinon vaginal cream 0.1%, 0.5 mg of estriol per application). After three weeks daily treatment, it was shown that Blissel produced negligible plasma estriol levels and significantly lower than those produced by Ovestinon ($p<0.0001$). In addition, 0.005% estriol formulation did not change serum FSH or LH while a significant decline of serum FSH was observed in women that received Ovestinon ($p=0.0425$) (18,19).

These data suggested that Blissel could potentially be safe in postmenopausal patients treated with aromatase inhibitors; however these results were obtained in a cohort of healthy postmenopausal women.

1.6 Study Rationale

In order to explore the safety of 0.005% estriol vaginal gel in the oncological context, a new safety study is proposed with the hypothesis that 0.005% estriol vaginal gel is a safe therapeutic option to treat the vaginal atrophy caused by AIs, without a significant decline in gonadotropin or increase in systemic estrogen levels in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with non-steroidal aromatase inhibitors (NSAIs) in the adjuvant setting.

2. Objectives

2.1 Primary Objective

The primary objective is to evaluate the levels of FSH after treatment with 0.005% estriol vaginal gel in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with NSAIs in the adjuvant setting and symptoms of vaginal atrophy.

2.2 Primary End-point

Variation in serum levels of FSH from baseline to 12 weeks of treatment.

2.3 Secondary Objectives

- To evaluate the levels of estriol, estradiol, estrone, FSH and LH after treatment with 0.005% estriol vaginal gel in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with NSAIs in the adjuvant setting and symptoms of vaginal atrophy.
- To assess the safety and tolerability of 0.005% estriol vaginal gel in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with NSAIs in the adjuvant setting and symptoms of vaginal atrophy.
- To assess the efficacy of 0.005% estriol vaginal gel in the treatment of symptoms and signs of vaginal atrophy in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with NSAIs and symptoms of vaginal atrophy.
- To measure the impact of treatment with 0.005% estriol vaginal gel in sexual function of hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with NSAIs and symptoms of vaginal atrophy.

2.4 Secondary End-points

- Variation in serum levels of FSH at different time points compared to baseline (weeks 1, 3 and 8).
- Variation in serum levels of LH and plasma levels of estriol, estradiol and estrone, at different time points compared to baseline (weeks 1, 3, 8 and 12).
- AEs according to the Medical Dictionary for Regulatory Activities (MedDRA).
- Changes in vaginal dryness and other symptoms and signs of vaginal atrophy; change in vaginal maturation value and change in vaginal pH at week 3 and week 12 vs baseline.
- Changes in sexual function measured by the Female Sexual Function Index ($FSFI^{20}$) scale at week 3 and week 12 vs baseline.

3. Investigational Plan

3.1 Study Design

This is a phase II, prospective, randomized, double-blind, placebo-controlled, international (Spain and Sweden) and multicentre study.

Sixty patients will be randomized (stratified by site) to receive 0.005% estriol vaginal gel (arm A) and placebo moisturizing gel (arm B) in a 4:1 proportion, so 48 patients will enter in arm A and 12 in arm B. Patients will undergo gynaecological tests, plasma and serum extraction for hormone determinations, adverse events collection and will answer a questionnaire according to what is outlined in Figure 1.

A) Patients randomized to Arm A (experimental arm) will receive:

Investigational drug product: 0.005% estriol vaginal gel (Blissel®)

Route:	Vaginal. Administration by an applicator inserted deep inside the vagina
Dose:	1 g of gel, containing 50 µg of estriol
Dosage schedule:	Weeks 1-3: single daily application Weeks 4-12: twice weekly administration.

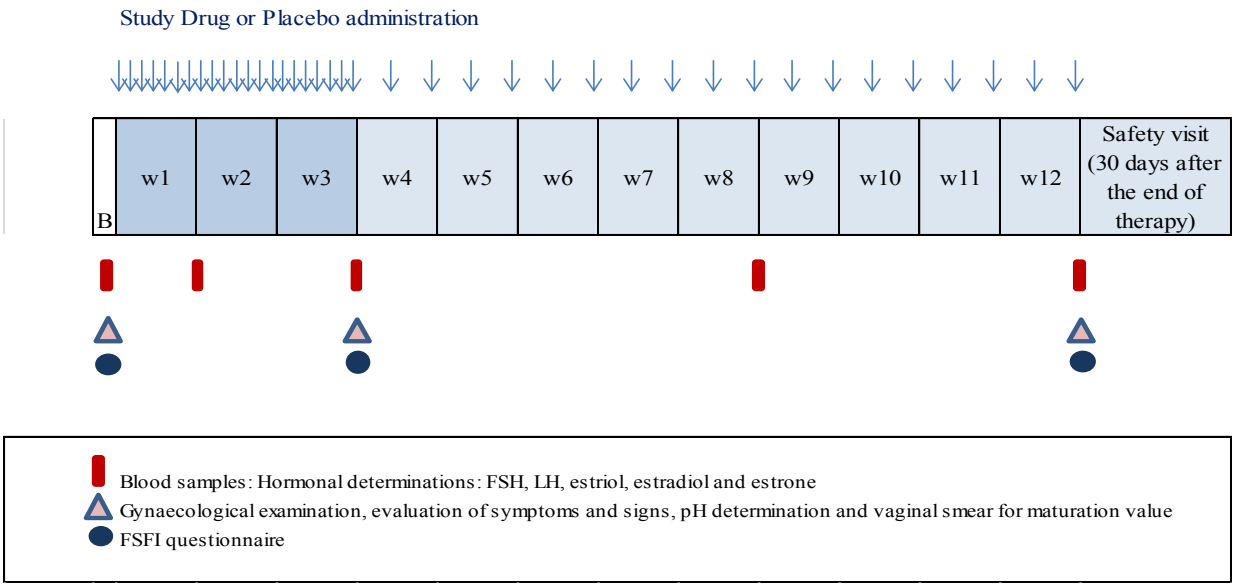
Women will be instructed to administer the gel at bedtime.

B) Patients randomized to Arm B (control arm) will receive:

Placebo moisturizing gel (vehicle of Blissel®).

Same route and dose schedule than Blissel®.

Figure1. Study Design



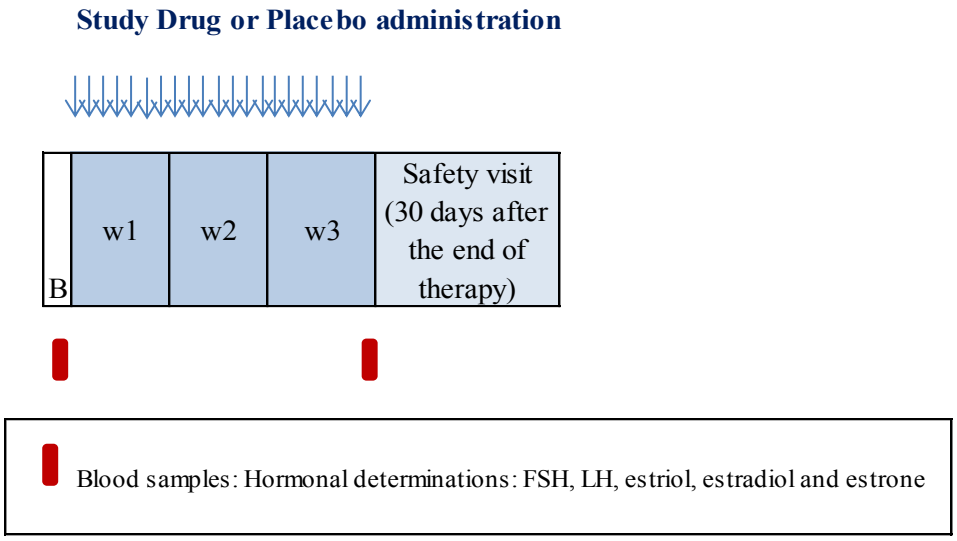
3.1.1 Early Safety Review

There will be an initial safety phase of the study, in which 10 patients will be included (also randomized 4:1) to obtain some preliminary safety data and FSH values prior to the recruitment of the rest of the group under study.

These women will receive the study treatment over 3 weeks (8 will receive estriol vaginal gel and 2 placebo moisturizing gel). FSH levels in serum will be analysed at screening, baseline and at week 3 (day 22 +/- 3 days). An independent data monitoring committee (IDMC) will review the results (together with safety data) which will advise the chief investigators about a go /no-go decision with the other 60 patients to complete the study. The study will continue if no significant changes in FSH levels from postmenopausal to premenopausal levels are observed.

These 10 patients will be recruited in two sites in Spain, 5 patients in each one. These patients will have estrogens, and FSH and LH determined at screening, baseline and at week 3.

Figura 2: Study Design of the Initial Safety Phase



3.2 Duration of the study

It is estimated that the 60 patients will be recruited in approximately 3 months. These patients will receive study therapy for 12 weeks.

The first 10 patients for the early safety review will be recruited in approximately 1 month and treated for 3 weeks. The results of the FSH levels will be available in 2-3 additional weeks.

For safety reasons all patients will have a visit 30 (+/-5) days after finishing treatment with the study drugs/medications.

According to what is outlined above, the length of study will be approximately 11 months.

4. Study Population

Patients with hormone receptor positive and HER2 negative early breast cancer who are on treatment with NSAI and have vaginal atrophy.

4.1 Inclusion Criteria

Patients are eligible to be included in the study only if they **meet all** of the following criteria:

1. Written informed consent prior to beginning specific protocol procedures.
2. Patients must have histological confirmation of breast adenocarcinoma with stage I-IIIA, documented at a local pathology department.
3. The breast tumors must be estrogen-receptor positive and/or progesterone receptor positive (≥1% of stained tumor cells by IHC as determined by the local laboratory) with any HER2 status.
4. Postmenopausal status defined as: 12 months of spontaneous amenorrhea or 6 months of spontaneous amenorrhea with serum FSH levels > 40 mIU/ml or 6 weeks postsurgical bilateral oophorectomy with or without hysterectomy.
5. Patient must be receiving the non-steroidal aromatase inhibitors anastrozole or letrozole as breast cancer treatment in the adjuvant setting for a minimum of 6 months.
6. Women suffering from moderate to severe vaginal dryness according to the FDA guidelines for drug development in postmenopausal women (Center for Drug Evaluation and Research, CDER Jan 2003). A moderate symptom will be considered if the symptom is present, bothersome and annoying, and a severe symptom will be considered if the symptom is present, bothersome and annoying, and interferes with the normal patient activity.
7. Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0 or 1.
8. Adequate bone marrow as defined by the following laboratory values:
    a. Absolute Neutrophil Count (ANC) ≥ 1.5 x 10^9/L.
    b. Platelets (plt) ≥ 100 x 10^9/L.
    c. Hemoglobin (Hgb) ≥ 10 g/dl.
9. Patient has adequate organ function as defined by the following laboratory values:
    d. Serum creatinine ≤ 1.5 x ULN.
    e. Bilirubin ≤ 1.5 × ULN.
    f. Alkaline phosphatase ≤ 2 × ULN.
    g. AST and ALT ≤ 2 × ULN.
10. Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.

4.2 Exclusion Criteria

Patients will be excluded from the study if they **meet any** of the following criteria:

1. Stage IIIB-IV breast cancer or bilateral breast cancer.
2. Treatment with any other current anti-tumoral therapy (chemotherapy, anti-Her2…etc) besides the NSAI. Pamidronate or Alendronate are permitted.
3. Prior history of other malignancy within 5 years of study entry, aside from non-melanoma skin cancer or carcinoma-in-situ of the uterine cervix adequately treated.
4. Postmenopausal uterine bleeding. Vaginal bleeding of unknown etiology.
5. Patients with endometrial thickness equal to or greater than 4 mm measured by transvaginal ultrasound.
6. Patients who have received any type of vulvovaginal treatment in the 15 days prior to the start of the study.
7. Use of any hormone, natural (phytoestrogens) or herbal products for the treatment of menopausal symptoms within the last 3 months.
8. Current or previous history of thromboembolic disease or coagulopathies.
9. Severe cardiovascular or respiratory diseases in the previous 6 months.
10. Renal Impairment.
11. Hepatitis B and/or hepatitis C carriers (unless with normal hepatic function).
12. Known human immunodeficiency virus infection.
13. Known hypersensitivity to NSAI.
14. Other severe acute or chronic medical or psychiatric condition, or laboratory abnormality that would impart, in the judgment of the investigator, excess risk associated with study participation or study drug administration, or which, in the judgment of the investigator, would make the patient inappropriate for entry into this study.
15. Previous investigational treatment for any condition or participation in any clinical trial within 4 weeks of inclusion date.

4.3 Discontinuations

4.3.1 Discontinuation of Study Medication

The criteria for enrollment must be followed explicitly. If a patient who does not meet enrollment criteria is inadvertently enrolled, that patient should be discontinued from the study medication, an exception may be granted if the patient, in the opinion of the investigator, is having benefit from the study medication. In these rare cases, the investigator must obtain documented approval from the sponsor to allow the patient to continue to receive the study drug.

Patients can be discontinued from the study therapy in the following circumstances:

- Patient's own request.
- Unacceptable toxicity.
- Tumor recurrence.
- Any clinical adverse event (AE), laboratory abnormality or inter-current illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the patient.
- Termination of the study by the sponsor.
- Physician's decision, including need of other anti-cancer therapy, not specified in the protocol.
- If the patient is non-compliant with study procedures.
- Loss of ability to freely provide consent through imprisonment or involuntarily incarceration for treatment of either a psychiatric or physical (e.g. infectious disease) illness.
- All patients will be discontinued from the treatment phase and they will perform the safety visit in case of a delay of more than 1 week during the daily administration phase or more than 2 weeks during the twice weekly administration phase, or permanent discontinuation of the study drug unless there is an obvious clinical benefit per the investigator's medical judgment and after discussion with the sponsor.

If possible, and after the permanent discontinuation of treatment, the patients will be assessed using the procedure normally planned for the last dosing day with the study treatment.

All permanent treatment discontinuation should be recorded by the Investigator in the eCRF when considered as confirmed.

4.3.2 Discontinuation of Study Sites

Study Site participation may be discontinued if the sponsor, the investigator or the IRB of the study site judges it necessary for any reason.

4.3.3 Discontinuation of Study

The study may be discontinued by the sponsor if this is medically reasonable and consistent with applicable regulations of Good Clinical Practice (GCP). Stopping the study for medical reasons may be required if patients experienced adverse reactions under the treatment with the study drug or if new information about the safety or effectiveness of the study drug justifies it.

5. Treatment

5.1 Treatments Administered

5.1.1 Blissel® (0.005% estriol vaginal gel)

Blissel® is a vaginal formulation, estriol-based hydrating gel for the treatment of vaginal atrophy.

The 0.005% estriol gel is provided in 30 g aluminium tubes. The tube is intended for multi-use applications, the product dosage being 1 g gel /day (50 µg estriol/g gel), which is applied using disposable single-use applicators.

The list of all components used in the manufacture of the investigational drug, qualitative composition (% and per unit dose) and quality of the inactive ingredients are indicated in table 1.

Table 1: Qualitative and quantitative composition of the drug 0.005% estriol gel (50 µg/g gel)

Component	Function	% (p/p)	Quantity/g product	Reference to standards
Estriol	Drug substance	0.005	50 µg	Ph. Eur.
Polycarbophil AA-1	Gelling polymer	1.5	15 mg	Ph. Eur.
Carbopol 971P	Gelling polymer	0.5	5 mg	Ph. Eur.
Glycerine	Hydrating agent	10	100 mg	Ph. Eur.
Methylparaben sodium	Preservative	0.16	1.6 mg	Ph. Eur.
Propylparaben sodium	Preservative	0.02	0.2 mg	Ph. Eur.
10 %NaOH	pH adjustment	q.s. pH = 4.5-5.5	---	Ph. Eur.
37 % HCl	pH adjustment	q.s. pH = 2.5-3.5	---	Ph. Eur.
Purified water	Carrier	q.s. 100 %	q.s. 1 g	Ph. Eur.

Blissel® will be administered by vaginal route at a dose of 1g/application. Patients will have 1 application/day during weeks 1 to 3 and 2 applications/week during weeks 4 to 12 (i.e. Mondays and Thursdays).

Route:	Vaginal. Administration by an applicator inserted deep inside the vagina
Dose:	1 g of gel, containing 50 µg of estriol
Dosage schedule:	Weeks 1-3: single daily application Weeks 4-12: twice weekly administration.

The treatment will be administered for 12 weeks.

The Principal Investigator, Sub-Investigator, study nurse or the Gynecologist must explain to the patient how to administer the vaginal gel. It is advisable that the first application be performed in presence of the gynecologist or the oncologist. Patients will be instructed to administer the rest of treatment applications at bedtime.

5.1.2 Vaginal gel placebo

The placebo moisturizing gel has the same composition than Blissel® without the active drug substance, estriol.

The placebo moisturizing gel will be administered in the same way.

5.2 Materials and Supplies

For this study, the term "study drug" (Investigational Medicinal Product [IMP]) refers to 0.005% estriol vaginal gel (Blissel®).

Placebo vaginal gel is the control medicinal product.

The Sponsor will provide to the study sites with Blissel® and Placebo for the purpose of this study.

Patients will be given three different packages:

- Baseline: tube of 30 g and 25 administration applicators. For weeks 1-3 of treatment.
- Week 3: tube of 30 g and 12 administration applicators. For weeks 4-8 of treatment.
- Week 8: tube of 30 g and 12 administration applicators. For weeks 9-12 of treatment.

5.2.1 Storage, preparation and administration

Storage conditions stated in the Study Reference Safety Document may be superseded by the label storage.

Investigators and site staff are reminded to continuously monitor room storage temperatures and ensure that thermometers are working correctly as required for proper storage of investigational product. Any temperature excursions must be reported immediately to the Sponsor and documented. Once a deviation is identified, the investigational product MUST be quarantined and not used until the Sponsor provides documentation of permission to use the investigational product.

Blissel® and placebo should be stored at controlled room temperature (≤ 25ºC) in their original container.

Medication should be kept in a secured locked area at the study site in accordance with applicable regulatory requirements. Returned medication should be stored separately from medication that needs to be dispensed.

The investigator`s team will hand over the medication to the patient. Patients will only be given the medication corresponding to each following period in the visits of day 1 (baseline visit) and in the visits of week 3 and week 8.

The Principal Investigator, Sub-Investigator, study nurse or the Gynecologist must explain to the patient how to administer the vaginal gel. It is advisable that the first application be performed in presence of the gynecologist or oncologist.

Women will be instructed to administer the gel themselves, preferably at bedtime, for the rest of applications.

5.2.2 Accountability

The patient will be indicated to return both the tube and the unused applicators as surplus medication. The investigator will collect them and will record the number of unused applicators in each period in the eCRF in order to evaluate dosage compliance.

The surplus medication, the unused applicators in each period, will be stored until the end of the study. At that time they will be returned to the sponsor, which shall destroy them. All medication supplied for the study will only be used for this protocol and in no case for another purpose.

It is the responsibility of the investigator to ensure that a current record of Blissel® and placebo disposition is maintained at each study site where study drug is inventoried and disposed. Records or logs must comply with applicable regulations and guidelines.

GEICAM (on behalf of Sponsor) will provide forms to facilitate inventory control if the staff at the investigational site does not have an established system that meets these requirements.

5.3 Method of Assigning a Patient to a Treatment

All patients will be screened prior to entry on this study. An explanation of the study and discussion of the expected side effects and presentation of the informed consent document will take place. Eligible and consenting patients will be enrolled into the study and a screening code will be assigned to the patients.

All patients who meet all inclusion and exclusion criteria will be randomized to receive 0.005% estriol vaginal gel (Blissel®) or placebo vaginal gel.

A randomization list will be generated taking into consideration blocks of 5 (4 actives 1 placebo). The study medication will be coded according to this randomization list. Study medications will be shipped in blocks of 5 to the sites.

In each site, all patients that fulfil all inclusion and exclusion criteria and are definitely included in the study will be assigned the randomization code. This code will be the one labelled in the medications. Thus, the first patient included in a site will receive the first study medication in the block, the second patient included will receive the second medication in the block, and this procedure will be followed consecutively.

The eligibility checklist must be completed and signed by the investigator prior to inclusion and it must be included into medical history of each patient.

All eligible patients enrolled in the study will be entered in a patient registration log maintained by the Site and in the Trial Master File.

The study treatment must be administered after definite inclusion of the patient in the study.

5.4 Special Treatment Considerations. Dose Adjustments of Study Drug

All dose modifications should be based on the worst preceding toxicity.

Every effort should be made to administer study treatment on the planned schedule. However, in the event of significant treatment-related toxicity, administration of study drug could be interrupted or delayed as described in the following sections.

5.4.1 Dose Interruption

In the event of significant treatment-related toxicity, Blissel® or placebo may be interrupted as described below. Patients are to be instructed to notify investigators at the first occurrence of any adverse sign or symptom.

Patients experiencing the following adverse events should have their treatment interrupted:

- Jaundice or deterioration in liver function
- Significant increase in blood pressure
- New onset of migraine-type headache

Treatment may be held as needed until toxicity resolution. Appropriate follow up assessments should be done until adequate recovery occurs as assessed by the Investigator. Treatment will be resumed once this toxicity completely disappeared; if treatment cannot be resumed within 3 weeks it will be interrupted permanently.

All dose interruptions must be clearly documented in the patient's source notes and in the eCRF.

5.4.2 Special conditions

The following conditions will require a closer revision of the investigator:

- Heart disease or circulation problems
- Hypercholesterolemia

- Hyperglycemia
- Breast changes
- Uterine fibroids or endometriosis
- Epilepsy
- Asthma
- Systemic Lupus Erythematosus
- Otosclerosis

5.5 Medication Errors and Overdose

Medication errors may result in this study from the administration at the wrong time. Such medication errors occurring to a study participant are to be captured on the eCRF. In addition the Sponsor should be notified immediately.

Medication errors are reportable irrespective of the presence of an associated AE/SAE, including:

- ✓ Medication errors involving patient exposure to the product;
- ✓ Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the participating patient.

5.6 General Concomitant Medication and Supportive Care Guidelines

Patients must be instructed not to take any additional medications (over-the-counter or other products) during the study without prior consultation with the investigator. Any medications including herbal supplements, vitamins, or treatment taken by the patient from 28 days prior to the start of study treatment and up to 30 days (±5 days) following the last dose of investigational product and the reason for their administration must be recorded on the eCRF.

Routine postoperative care, such as dressing changes, suture removal, drain removal, or venous access (central or peripheral), does not need to be recorded. Anesthetics used for any surgical procedures performed during the patient's participation in the study can be recorded as "unspecified anesthesia" on the concomitant treatment records; it is not necessary to list the specific anesthetics.

5.6.1 Prohibited Medications

The following treatments are prohibited throughout the duration of the active treatment phase:

- ✓ **Anticancer agents:** No additional investigational or commercial anticancer agents such as chemotherapy, immunotherapy, targeted therapy, biological response modifiers or endocrine therapy (different than the NSAI: anastrozole or letrozole) will be permitted during the active treatment phase. In general, any drugs containing "for the treatment of breast cancer" on the product insert are not permitted on study.

- ✓ **Hormone replacement therapy with estrogens or progestins, tibolone**, topical estrogens (different from the study drug/placebo), phytoestrogen, **megestrol acetate** and **selective estrogen-receptor modulators** (eg, raloxifene, tibolone) are prohibited during the active treatment phase.

5.6.2 Permitted Medications

The following treatments are permitted throughout the duration of the active treatment phase:

- ✓ **Standard therapies** for pre-existing medical conditions, medical and/or surgical complications, and palliation. Any medication intended solely for supportive care (eg, analgesics, antidiarrheals, antidepressants) may also be used at the investigator's discretion. All medications should be recorded.
- ✓ **Bisphosphonates and receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitors** for the treatment of osteopenia/osteoporosis.

5.7 Treatment Compliance

The compliance will be evaluated by the Investigator Team at week 3, 8 and 12 by counting and recording the number of unused applicators returned in each period.

The percentage of pharmacological compliance will be calculated from the count of the applicators.

To evaluate the compliance, the flexibility permitted in the appointments, having to increase or reduce applicators from the theoretical calculation of applicators returned, will be taken into consideration. Checking the patient completion calendar will facilitate this evaluation.

5.8 Blinding and Unblinding

The study drug, 0.005% Estriol vaginal gel, and its vehicle in gel (placebo vaginal gel) will be of identical appearance and will have the same aroma and the same texture in order to maintain the double blind.

Throughout the study, the medication with which the patient 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 blinding due to safety reasons. The unblinding must be done only by the principal investigator of the site or by an authorized person, only when the knowledge of the treatment assignment is deemed essential for the patient's care.

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 the protocol violations have been determined. All personnel involved directly in the study shall be unaware of the treatment assigned to the patients until the database has been closed.

5.8.1 Process for urgent unblinding

In general, unblinding of participants during the conduct of the clinical trial is not allowed unless there are compelling medical or safety reasons to do so.

Any serious adverse event considered by the investigator to be related to the blinded treatment medication (0.005% estriol vaginal gel/placebo vaginal gel) should be reported as such to the sponsor via the appropriate SAE reporting form.

There will be two complete sets of randomization codes. One will be kept by the GEICAM Pharmacovigilance department and the other will be distributed among the investigators. The emergency codes should be kept in a safe but accessible place in the event they should be opened. Each investigator will receive the necessary envelopes closed to randomize a determined number of patients. Each envelope will contain the details of the treatment that the patient is receiving. In the case of emergency the envelope must be opened to determine the treatment being administered.

The randomization envelopes can only be opened in an emergency. When the investigator opens the envelope he/she must record the date, the time and the reason he/she has had to do so and will keep this information with the eCRF documentation. He/she should also immediately inform the study monitor that a treatment code has been opened As per regulatory reporting requirement, it will unblind the identity of the study medication for all unexpected SAES that are considered by the investigator to be related to the study drug. Details of subjects who are unblended during the study will be included in the Clinical Study Report.

6. Efficacy and Safety Evaluations, Sample Collection and Testing (Standard Laboratory Testing) and Appropriateness of Assessments

Study procedures and their timing (including tolerance limits for timing) are summarized in the Study Schedule, Protocol Attachment 1.

Patients will receive the treatment for 12 weeks (3 months).

Patients will be evaluated at baseline (within 2 weeks prior to treatment initiation on day 1), at week 1 (day 8 +/-2 days) at week 3 (day 22 +/- 3 days), week 8 (day 57 +/- 3 days) and week 12 (day 85 +/- 3 days). There will be an additional safety visit 4 weeks (± 5 days) after the last study medication administration.

Before a patient starts her participation in the study, she must have given her written informed consent. Through this consent, it is guaranteed that the person voluntarily expresses their wish to participate in the clinical trial, after having understood all the information that has been provided to her about the study.

6.1 FSH serum levels

The primary endpoint is to evaluate the variation of FSH levels in serum from baseline to 12 weeks of treatment with 0.005% estriol vaginal gel. As a secondary endpoint, we will evaluate the variation at different time points along 8 weeks of treatment with 0.005% estriol vaginal gel.

Blood samples to analyze FSH (in serum) will be collected at six time points:

- Screening: one blood extraction
- Baseline visit: one blood extraction.
- Weeks 1-3: one blood extraction in visits at week 1 (day 8 +/- 2 days) and week 3 (day 22 +/- 3 days).
- Weeks 4–12: one blood extraction in visits at week 8 (day 57 +/- 3 days) and week 12 (day 85 +/- 3 days).

Serum FSH levels will be determined in a Central Laboratory (Laboratorios Echevarne). Chemiluminescent assay will be used for their analysis.

Detailed instructions for the collection, labelling, handling and shipment of samples are outlined in the Sample Management Manual.

6.2 Estriol, estradiol, estrone plasma levels and LH serum levels

Secondary endpoints are to evaluate the variation of estriol, estradiol and estrone levels in plasma and LH levels in serum along 12 weeks of treatment with 0.005% estriol vaginal gel.

Blood samples to analyze LH (in serum) will be collected at the same six time points:

- Screening: one blood extraction

- Baseline visit: one blood extraction
- Weeks 1-3: blood extraction in visits at week 1 (day 8 +/- 2 days) and week 3 (day 22 +/- 3 days)
- Weeks 4–12: blood extraction in visits at week 8 (day 57 +/- 3 days) and week 12 (day 85 +/- 3 days)

Blood samples to analyze estriol, estradiol, estrone (in plasma) will be collected at five time points:

- Baseline visit: one blood extraction
- Weeks 1-3: blood extraction in visits at week 1 (day 8 +/- 2 days) and week 3 (day 22 +/- 3 days)
- Weeks 4–12: blood extraction in visits at week 8 (day 57 +/- 3 days) and week 12 (day 85 +/- 3 days)

Plasma estradiol, estriol, estrone levels and serum LH levels will be determined in Central Laboratories (Laboratorios Echevarne; Pharm-Analyt). Due to the extremely low levels of plasma estrogens expected, a newly developed and validated ultrasensitive LC-MS/MS assay will be used for the analysis of estradiol, estriol and estrone at Pharm-Analyt. Chemiluminescent assay will be used for the analysis of Serum LH at Laboratorios Echevarne.

Detailed instructions for the collection, labelling, handling and shipment of samples are outlined in the different Sample Management Manuals.

6.3 Safety assessments

Investigators are responsible for monitoring the safety of patients who have entered this study and for alerting GEICAM to any event that seems unusual.

The investigator is responsible for appropriate medical care of patients during the study.

The investigator remains responsible for following, through an appropriate health-care option, adverse events that are serious or that caused the patient to discontinue before completing the study. The patient should be followed until the event is resolved or explained. Frequency of follow-up evaluation is left to the discretion of the investigator.

During the course of the study, all patients entering the trial must be evaluated according to the schedule outlined in the flow charts and described below. The results of the evaluation will be recorded in the eCRF pages until the patients are not followed anymore.

6.3.1 Timing of Assessments

Physical and gynecological examination including vital signs assessments (blood pressure and pulse), will be performed at screening, week 1 (day 8 +/- 2 days), week 3 (day 22 +/- 3 days), week 8 (day 57 +/- 3 days), week 12 (day 85 +/- 3 days) and 4 weeks after the last study drug

administration. Height and weight will be registered at screening. Gynaecological examination will comprise breast examination and pelvic examination (examination of uterus and appendages), and will be performed at baseline and at weeks 3 and 12. Endometrial ultrasound will be performed at screening and at week 12.

The following safety laboratory assessments will be performed by the local laboratories, at screening and at week 12 and 4 weeks (+/- 5 days) after the last study drug administration:

- Hematology: hemoglobin, WBC, absolute neutrophils, platelet count.
- Blood Chemistry: fasting glucose, alkaline phosphatase, ALT, AST, total bilirubin, serum creatinine and BUN (or urea).
- Urine test: density, pH, glucose, proteins, cetones, bilirubin, blood, nitrites, urobilinogen and leukocites.

All AEs (and their relatedness to the study drug) occurring during the study will be documented in the eCRF at baseline, week 1 (day 8 +/- 2 days), week 3 (day 22 +/- 3 days), week 8 (day 57 +/- 3 days), week 12 (day 85 +/- 3 days) and 4 weeks after the last study drug administration. AEs will be graded according to MedDRA for each type of adverse event will be recorded. Additionally, the relationship of the adverse event(s) to the study treatment will be taken into consideration.

6.3.2 Safety Definitions

The safety definitions are described in the table 2.

Table 2: Safety definitions

Concept	Definition
Adverse Event (AE)	Any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product. Laboratory abnormalities should be reported as AE only in case they lead to an action on study treatment or if they are serious.
Adverse Reaction (AR)	All untoward and unintended responses to a medicinal product related to any dose administered. All expected ARs are listed in the Summary of Product Characteristics [SmPC]. If the nature or the severity of an

	adverse reaction is not consistent with the applicable product information, the AR is defined as unexpected. The basis for the decision is the current version of the corresponding reference document that has been submitted and approved by the competent authority and the ethics committees. **Accountability criteria** The sponsor will classify the adverse event, based in their causation relation with the investigational product, following the Karch y Lasagna (1977) algorithm, as: o Final: there is reasonable temporal sequence between the drug administration and the existence of the adverse event. This event matches with the adverse reaction described for the investigational product, improves with the omission and reappears after its re-administration and can't be explained by other causes. o Probable: there is reasonable temporal sequence between the investigational product administration and the appearance of the adverse event. This event matches with the adverse reaction described for the drug, improves with the omission and can't be explained by other causes. o Possible: there is reasonable temporal sequence between the investigational product administration and the appearance of the adverse event. This event matches with the adverse reaction described for the drug but can be explained by other causes. o Conditional or improbable: there is reasonable temporal sequence between the investigational product administration and the appearance of the adverse event. This event does not matches with the adverse reaction described for the drug and can be explained by other causes. o Not related: there is no reasonable temporal sequence between the investigational product administration and the appearance of the adverse event. This event does not matches with the adverse reaction described for the drug and can be explained by other causes. For expedited reporting purposes it is considered as related the categories: final, probable and possible from Karch y Lasagna (1977) algorithm and as not related the category conditional or

	improbable of that algorithm. The determination of the possible relation with the study treatment is responsibility of the principal investigator of the site or the person designated by him.
Serious Adverse Event (SAE) and Serious Adverse Reaction (SAR)	Any adverse event or adverse reaction that, at any dose: o is fatal (results in death), o initial or prolonged inpatient hospitalization, o a life-threatening experience (that is, immediate risk of dying, defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe), o persistent or significant disability/incapacity, o congenital anomaly/birth defect or o an important medical event, defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (eg. medical, surgical) to prevent one of the other serious outcomes listed above. Do not confuse the concept "serious", described before, with "severe" which refers to the intensity of the AE or AR (minor/mild/severe). Any temporary increase in the severity of a symptom or previous sickness that happens after the baseline of the study is considered also as an adverse event.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	Any serious adverse reaction whose nature, intensity or consequences do not correspond with the reference information for the investigational product (Summary of Product Characteristics [SmPC]). The unexpected nature of an adverse reaction is based in the fact of not being observed previously and not in what could be advanced based on the pharmacological properties of the drug.

6.3.3 Management, Timing and Assessment of Adverse Events

AE Classification	Adverse events should be classified following MedDRA. The investigators team must have access to MedDRA. The causal relation between the investigational product and the AE will be assessed by the investigator using the Karch y Lasagna (1977) algorithm. Note that, for the purpose of this protocol, the following circumstances – with or without associated adverse event/reaction - should be included the data base and will be submitted to the sponsor - that is, managed in the same way and time as adverse events/reactions: • Overdose, abuse, misuse • Off-label use • Medication error
Procedure to notify an AE to Sponsor	The site will enter the following events in the eCRF: o All adverse events that occur after enrollment. o Preexisting conditions that get worse during the study. o The evaluation of the possible relationship of each adverse event to the study drug or protocol procedure. o The circumstances and data that causes the suspension of the treatment of a patient due to an adverse event. o The events leading to the clinical outcome of death from disease progression will be included in the efficacy analysis unless and are not recorded as adverse events, unless the investigator believes they could have been caused by the study drug. Data Management of GEICAM will notify all these events to the sponsor as agreed between the parties.
Timing and assessment of AE (see Protocol Attachment 3)	The site staff will report on the eCRF the information of the AE in the following periods: • **Baseline (after ICD and before study treatment):** study site personnel will note the occurrence and nature of each patient´s medical condition(s) and preexisting conditions in the appropriate section of the eCRF. If a patient never receives study treatment but experiences an adverse event after the ICD is signed, ONLY events

	the investigator believes may have been caused by a protocol procedure will be reported to GEICAM via eCRF. • **During treatment with the study treatment:** during the study, site personnel will record any change in the condition(s) and the occurrence and nature of any adverse events. A CTCAE grade rating will be assigned before each cycle for any adverse event experienced during the previous cycle. • **30-day post-discontinuation follow-up period:** each patient will have a 30-day post-discontinuation follow-up evaluation approximately 30 days following the discontinuation of study treatment. Patients should be closely followed for study treatment adverse events in order to detect delayed toxicity. If drug-related toxicity is present beyond 30 days post-discontinuation, patients must be followed until the toxicity resolves or improved to baseline, the relationship is reassessed as unrelated, the investigator confirms that no further improvement can be expected, another therapy is initiated, or death. • **Long-Term Follow-up Period (after the 30-day post-discontinuation):** only new and ongoing SAEs thought to be related to study treatment or protocol procedures should be documented on the eCRF and immediately reported to GEICAM via the designated transmission method, even if the study has been closed.

6.3.4 Management, Timing and Assessment of SAEs

Timing of SAEs (see Protocol Attachment 3)	All the SAEs (either spontaneously or during the trial visits) will be collected since the patient signs the Informed Consent Document (ICD). All the SAEs must be documented in the medical record of the patient and in the eCRF. A follow up of all the SAEs should be done until they are solved or until the toxicity is considered irreversible.
SAEs which do not need to be notified to the Pharmacovigilance Department of the Sponsor	The following events are not considered SAEs: o 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). o Elective surgery planned before signing consent. o Hospitalization which is due solely to a planned study visit and without prolongation. o Routine health assessment requiring admission for baseline/trending of health status (eg. routine colonoscopy). o Medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases. o Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg. lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative). o Progression of the malignancy during study (including signs and symptoms of progression), unless the outcome is fatal and death occurred before end of treatment. Thereafter death due to disease progression has not to be reported as SAE. o Hospitalization due to signs and symptoms of disease progression. o An overnight stay in the hospital that is only due to transportation, organization or accommodation problems and without medical background. They will be reported in the eCRF and in the patient record. The rest of SAEs must be notified as described as follows.
Procedure to notify a SAE to the Pharmacovigilance Department of GEICAM and Sponsor	The SAEs must be notified to the Pharmacovigilance Department of GEICAM and to the Sponsor at the same time. A member of the investigator team must complete and sign the SAE notification form which will be sent by fax/mail, immediately and always during the 24 hours following knowledge of the SAE:

	Pharmacovigilance Department of GEICAM Fax: +34 916 510 406 farmacovigilancia@geicam.org **ITF RESEARCH PHARMA S.L.U** Fax: +34 91 6572372 Email: drugsafety@itfsp.com Once received, GEICAM and the Sponsor will contact each other to make sure that both parties have received the SAE. The SAE will be reviewed by the Pharmacovigilance Department of the Sponsor and, if necessary, will ask more information to the investigator. When additional information is obtained about the SAEs, or this is solved or is improbable it will change, a follow-up report must be also completed and sent by fax/mail, immediately and always during the following the 24 hours to the Pharmacovigilance Department of Sponsor. If the Sponsor suspects that the SAE could be a SUSAR, the investigator should give the follow up information requested. All SAEs from the time the patient have the first dose of the study drug through 30 days following the last administration of study drug must be reported according to the procedure described below. All SAE regardless of timing must be reported, if considered related to study drug. Likewise, progression of a patient's underlying condition leading to one of the above should also not be reported as a SAE, but documented as primary study endpoint. All SAEs will be followed-up by the investigator until satisfactory resolution. Annually all SARs will be reported as the DSUR to the competent authorities and the leading ethics committee, including all SUSARs. Withdrawal from further treatment shall be at the discretion of the investigator.
Death on Study	Any death occurring during the active treatment part of the study and within 30 days following the last treatment must be reported to GEICAM and to the sponsor within 24 hours, regardless of the relation to study drug(s), and has to be

	reported on the death report form section of the eCRF. The cause of death should be documented (cancer-related, treatment-related, cancer- and treatment-unrelated). Autopsy reports should be collected whenever possible and sent to GEICAM. Deaths that occur due to tumor progression do not have to be reported as a SAE unless they occurred before end of treatment. Deaths after the end of study which are considered to be related to study treatment have to be reported as SAEs. To the extent feasible sufficient information including relevant laboratory values, ECG, scan, biopsy or autopsy results must be provided by the investigator in the SAE narrative (even if investigator determines the SAE is not related) so as to permit an independent causality assessment by a Competent Authority.

6.3.5 Management, Timing and Assessment of SUSARs

Expedited Notification of SUSAR to the Competent Authorities/IRB	The Pharmacovigilance Department of the GEICAM (on behalf of the sponsor) is responsible to notify to each of the competent authorities and IRBs of the participating countries, all the SUSARs collected in the study, following the procedures shown in the current legislation.
Timing of notification	The deadline for reporting a SUSAR shall be 15 calendar days from when the Sponsor becomes aware of it. When suspected SUSAR caused the death of the patient or endangered her life, the Sponsor will send the information within 7 calendar days from the date on which it becomes aware. This information must be completed, if possible, in the next 8 days.
Expedited reporting of other relevant safety information	Sponsor will also notify, expeditiously, all the information that could modify the balance benefit/risk of the investigational product, or determine changes in its administration pattern or in the study performance, such as: o A qualitative change or an increase in the percentage of occurrence of the SAR expected, which are considered clinically significant. o The SUSAR occurring after completion of the study and are reported by the investigator to the sponsor.

	○ New events related to the conduct of a trial or the development of an IMP likely to affect the safety of patients, such as: ✓ SAE that could be related with the study procedure and could modify the conduct of the trial. ✓ A significant risk to patients such as lack of efficacy in a drug used to treat a life-threatening illness. ✓ A major safety finding from a newly completed animal study (such as carcinogenicity). ✓ A temporary halt of a trial for safety reasons if the trial is conducted with the same investigational medicinal products in another country and if this information is known by Sponsor. ✓ Any recommendation of the IDMC that is relevant to the safety of patients (if applicable). This relevant information shall be notified as soon as possible and no later than 15 days after Sponsor becomes aware of it. Additional information will also be notified as quickly as possible.
Annual Safety Reports	The annual safety reports that include the SAEs and SUSARs collected during the study will be sent by the Sponsor to the Competent Authorities at the time established by the current legislation.
Notification to investigators	Sponsor will communicate to the investigators any safety information that may affect the safety of trial patients, as soon as possible. Information on SUSAR occurred during the study will be sent at the same time that it is notified to competent authorities but without submitting any information on the treatment arm. They will be informed also, throughout the entire study, of any safety aspect that impacts the performance on the clinical trial or in the product development, including the interruption or modification in the development program of the protocol safety-related.

6.4 Efficacy assesments

The following assessments will be performed at baseline, week 3 (day 22 +/- 3 days) and week 12 (day 85 +/- 3 days) or early withdrawal visit.

- Symptoms of vaginal atrophy (vaginal dryness, dyspareunia and pruritus).
- Signs of vaginal atrophy (mucosa with thinning or flattening of folds, dryness or fragility of the vaginal mucosa).
- Measurement of vaginal pH on the vaginal secretion using a reactive strip (pH 2.0 - 9.0, provided by the sponsor). The investigator team will insert the strips in the vagina and will moisten them as much as possible with the vaginal wall secretion. Then, when the strip is coloured, the investigator will check the combination of colours on the strip and check the pH value to which it corresponds, recording this value in the eCRF. The reading should be made whilst the strip is still damp. The strips will be discarded once the pH has been recorded in the eCRF.
- Vaginal cytology sample to evaluate the vaginal Maturation Value. Two smears (with two different spatulas provided by the sponsor one to take the sample and the other to take the countersample) will be made per patient (sample and countersample), which will be obtained from the bottom of the right and left vaginal sac respectively. Each smear will be suitably labelled. The vaginal smears will be fixed whilst they are still moist with a water-soluble fixation spray for cytodiagnosis (provided by the sponsor). The cytologic samples will be sent to central laboratory (full instructions will be given in a laboratory manual). For the cytologic evaluation, the number of parabasal, intermediate and superficial cells will be calculated in duplicate on 100 consecutive cells of vaginal cytology. The average of the two percentages obtained for each cell type will be calculated, which will serve to determine the maturation value (MV) based on the following formula:

  0.2 x (% parabasal) + 0.6 x (% intermediate) + 1.0 x (% superficial).

6.5 Impact on sexual function

Patient reported outcomes of health related with the sexual function will be assessed using the **FSFI (Female Sexual Function Index)** (see attachment 4). It was developed for the specific purpose of assessing domains of sexual functioning (e.g. sexual arousal, orgasm, satisfaction, pain) in clinical trials. It is not a measure of sexual experience, knowledge, attitudes, or interpersonal functioning in women. It is not designed for use as a diagnostic instrument and should not be used as a substitute for a complete sex history in clinical evaluation. However, when initial assessment reveals a sexual concern, using the FSFI (or an equivalent set of questions) can help both the physician and the patient measure the nature and severity of the problem as well as identify an appropriate course of treatment.

The FSFI is a brief multidimensional scale with 19-item questionnaire composed of three multi-item functional subscales (sexual activity, sexual intercourse and sexual stimulation).

Patients will complete each questionnaire at baseline, week 3 (day 22 +/- 3 days) and week 12 (day 85 +/- 3 days) or early withdrawal visit.

Completed questionnaires are always considered source document and must be filed accordingly.

Patients must preferably complete these instruments in the hospital and prior to having any tests and to any discussion of their progress with healthcare personnel at the site. Interviewer administration in clinic may be used under special circumstances (eg, patient forgot their glasses or feels too ill).

7. Data Quality Assurance

To ensure accurate, complete and reliable data, the Sponsor or delegated people on behalf of the sponsor will do the following:

- provide instructional material to the study sites, as appropriate
- sponsor a start-up training session to instruct the investigators and study coordinators. This session will give instructions on the protocol, the completion of the eCRFs, and study procedures
- make periodic visits to the study site to review study progress, investigator and patient compliance with the clinical trial protocol requirements and any emergent problems.
- be available for consultation and stay in contact with the study site personnel by mail, telephone, and/or fax
- review and evaluate eCRF data and use standard computer edits to detect errors in data collection
- conduct a quality review of the database
- verify the quality of the data

To ensure the safety of participants in the study and to ensure accurate, complete, and reliable data, the investigator will keep records of laboratory tests, clinical notes, and patient medical records in the patient files as original source documents for the study. If requested, the investigator will provide GEICAM (on behalf of the sponsor), applicable regulatory agencies, and applicable ethical review boards with direct access to original source documents.

7.1 Data Management and Registries file

Data for this study will be recorded via an electronic data capture system using eCRFs. Data will be transcribed by the site from the paper or electronic source documents onto the eCRF. In no case the eCRF is to be considered as source data for this trial. The eCRFs must be completed in an electronic Data Base called ORACLE. That electronic Data Base carries out with the regulatory authorities requirements. It is the responsibility of the Investigator to maintain adequate and accurate eCRFs (according to the technology used) designed by GEICAM (on behalf of the sponsor) to record (according to GEICAM instructions) all observations and other data pertinent to the clinical investigation. All eCRFs should be completed in their entirety in a neat, to ensure accurate interpretation of data.

Should a correction be made, the corrected information will be entered in the eCRF overwriting the initial information and including a reason for the change. An audit trail allows identifying the modification.

Data are available within the server of GEICAM as soon as they are entered in the eCRF.

The computerized handling of the data by GEICAM when available in the eCRF may generate additional requests to which the Investigator is obliged to respond by confirming or modifying the data questioned. The requests with their responses will be managed through the eCRF.

8. Sample Size and Statistical Methods

8.1 Determination of Sample Size

This is an exploratory study and there are scarce data to allow a precise sample size calculation. The most relevant data come from Pfeiler[16](16) who reported that the levels of FSH in patients treated with aromatase inhibitors fell from a mean value of 75.7 to 66.0 mIU/ml after two weeks of daily vaginal treatment with 0,5 g of estriol. Although no figures are reported of the variability of those measurements, an estimation of the standard deviation can be made applying the standard rule of [sd = range/4]. The reported range of FSH values in that report is 45.6 – 134.6, thus a reasonable approximation to the sd is 89/4 = 22.3. Under these assumptions, it can be calculated that a sample size of 44 would provide 80% power to detect with an alpha=0.05 a decrease of FSH levels from 75.7 to 66.0 mIU/ml assuming a standard deviation of 22.3.

The hormone levels in women treated with IA who are not receiving estrogen therapy are likely to suffer physiological temporal oscillations. These variations could make difficult the interpretation of any eventual modification of hormone levels observed in the estriol treated women. In the absence of data of naturally occurring variations in hormone levels of women treated with IA that allow us to interpret the results of hormone determinations under study, it is considered appropriate to include a placebo group to provide reference data. In this case, the placebo is a vaginal moisturizing gel.

It is estimated that a group of 11 women will allow the assessment of physiological fluctuations in hormone levels. Additionally, this sample size for the placebo group will provide 80% power and alpha=0.05 to differentiate between active and placebo (ratio 4:1) on the change in the vaginal maturation value, which is a secondary variable of the study that provides objective information about the effectiveness of treatment on vaginal atrophy (calculation based on the parameter data from the pivotal Blissel study and considering only the subgroup of women who had severe vaginal dryness: change VM active group 24.9 vs 0.6 points in placebo group, SD = 24.2).

The differentiation between active and placebo in the subjective parameters of efficacy (secondary) would be possible at the expense of a sample size noticeably superior. Since the evaluation of effectiveness is not primary but secondary in this study, it is not justified in this study to increase the sample for this reason.

In accordance with the above, the study would include 55 women. 44 will receive vaginal estriol gel and 11 with the placebo moisturizing gel). Taking into account 10% of dropouts, the sample to be considered is of 60 women (48 vaginal estriol gel and 12 placebo moisturizing gel). This is an unbalanced study but with an active: placebo ratio of 4:1, which will support an adequate patient randomization (blocks of 5).

10 additional women will be recruited in a first safety phase of the study (8 with vaginal estriol gel and 2 placebo moisturizing gel).

8.2 Statistical and Analytical Plans

8.2.1 General Considerations

Statistical analysis of this study will be the responsibility of GEICAM (on behalf of the sponsor). The interpretation of study results will be the responsibility of the chief investigator of the study together with the sponsor.

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a Statistical Analysis Plan (SAP), which will be dated and maintained by GEICAM (on behalf of the sponsor). This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition and/or its analysis will also be reflected in a protocol amendment.

All the analysis will be performed using the statistical software SAS Enterprise Guide 5.1

8.2.1.1. Patient Populations

Intent to treat population (ITT): The ITT population will include all patients who are randomized, with study treatment (Estriol gel vaginal 0.005% or placebo) assignment designated according to initial randomization.

The ITT population will be the primary population for evaluating patient characteristics, hormone levels, efficacy and the FSFI questionnaire.

Per-protocol population: a subset of the ITT population that received at least one dose of study drug and completed the study without any major protocol violations.

Safety population: will include all patients randomized in the study who received at least one dose of treatment, and they will be analyzed according to the actual treatment received. This population is for the safety analysis.

8.2.2. Patient Disposition

A detailed description of patient disposition will be provided. It could include the following:

- summary of patients entered and by site
- total number of patients entered
- total number of patients enrolled
- summary of reasons for patients entered, but not enrolled
- total number of patients treated
- summary of reasons for patients enrolled, but not treated.

A detailed summary of reasons for patient discontinuation from study treatment will be provided.

A summary of all identified important protocol violations will be provided.

8.2.3. Patient Characteristics

Patient characteristics could include a summary of the following:

- patients demographics
- time of menopause
- baseline disease characteristics
- preexisting conditions/secondary conditions
- prior therapy

Other patient characteristics will be summarized as deemed appropriate.

The categorical variables will be summarized by contingency tables. The continuous variables will be described by the number of patients with valid values (n), mean, standard deviation, median and range in both arms.

The significance level of all statistical tests is established at 0.05.

The effect of the treatment on continuous variables will be assessed by paired-t analysis (or Mann-Whitney-Wilcoxon in case of lack of normality). Categorical variables will be analyzed by chi-cuadrado or Fisher's exact test.

8.2.4. Concomitant Therapy

A summary of concomitant therapies will be generated in the safety population.

8.2.5. Treatment Compliance

The percentage of complying patients will be calculated at weeks 3, 8 and 12, defined as those with a compliance percentage of 80-110%, in the group of patients treated with 0.005% Estriol vaginal gel and in the placebo group. The overall compliance of the study drug will be calculated from these partial calculations. It will be summarized the patients and reasons of those patients which did not complete the 80% of medication of the study.

8.2.6. Hormone levels

All these analysis will be based on the ITT population.

The primary endpoint and one of the secondary endpoints are to evaluate the variation from baseline of FSH, LH, estriol, estradiol and estrone blood levels in patients treated with 0.005% estriol vaginal gel/placebo.

The variation of the levels of FSH, estriol, estradiol, estrone and LH after treatment with 0.005% estriol vaginal gel will be studied in each woman. The variation of levels between two arms will be analyzed using a non-parametric test (Mann-Whitney-Wilcoxon test) or an ANCOVA (if it must be adjusted by initial value).

Two determinations of FSH and LH before treatment with 0.005% estriol vaginal gel will be studied in each woman in order to study the intra-individual variation. Screening and baseline determinations will be used for this purpose.

Changes in hormone levels will be studied in each woman along the treatment (see sections 6.1 and 6.2).

8.2.7. Efficacy

The efficacy analysis will be evaluated in the ITT population.

The primary endpoint, the variation of FSH between week12 and baseline and comparing by arm will be analyzed using a non-parametric test (Mann-Whitney-Wilcoxon test).

An ANCOVA analysis will be used to corroborate the results obtained for the primary endpoint. The ANCOVA analysis will compare the variation of FSH 12 weeks adjusted by FSH basal between the two arms. This analysis will be performed if the relation between FSH 12 and FSH basal is lineal.

The variations in the intensities for each one of the symptoms and signs of the vaginal atrophy, after 3 and 12 weeks, in each treatment arm, will be compared using the non-parametric test Mann-Whitney-Wilcoxon.

8.2.7.1 Signs and symptoms of vaginal atrophy

The symptoms evaluated will be the following:

- Vaginal dryness
- Pruritus or itching
- Dyspareunia

Each symptom will be scored in a numeric scale from 0 to 3, as shown below:

- 0 Absence. The symptom is not present
- 1 The symptom is of mild intensity, without interfering in the patient's activity
- 2 The symptom is of moderate intensity, causing obvious discomfort to the patient
- 3 The symptom is stated as very irritating and severe in intensity

A Global Symptoms Score will be evaluated. It will be calculated by summing the intensities of all the three symptoms of vaginal atrophy in a certain time point, thus ranging between 0 and 9. The variation in the Global Symptom Score vs baseline will be assessed at weeks 3 and 12.

The signs evaluated will be the following:

- Vaginal mucosa with flattening of folds or thinning
- Dryness of the mucosa
- Fragility of the mucosa

Each one of these signs will be scored by the investigator on a numerical scale in accordance with their presence and degree of severity as follows:

- 0 Absence. The sign is not present.
- 1 The sign is present and is considered a mild alteration
- 2 The sign is present and is considered a moderate alteration
- 3 The sign is present and is considered a severe alteration

8.2.7.2 Vaginal Maturation value and pH

The averages of the differences between the baseline and the final MV and pH of the 0.005% Estriol vaginal gel and placebo groups will be shared by a non-parametric test., to determine the possible superiority of the 0.005% Estriol vaginal gel treatment compared with placebo administration.

These same tests will be used to analyse the change in the MV and pH after the initial observation period of 3 weeks.

All of the above secondary analyses will be conducted at a two-sided 0.05 level of significance.

8.2.8. Safety Analyses

The toxicity and tolerability of the study drug will be evaluated in the safety population. Safety analyses will include summaries of the incidence of adverse events by MedDRA that occur during the study treatment period or within 30 days (+/- 5 days) of the last dose of study treatment, regardless of causality and according to the relationship to study drug as assessed by the investigator. Additionally, the following safety-related outcomes will be summarized:

- study treatment discontinuations due to adverse events.
- deaths
- SAEs
- hospitalizations and transfusions
- use of key concomitant medications or growth factors.

Analyses for data with discrete dates, for example, deaths, transfusions, and concomitant medications, will be done through 30 days after each patient's last dose of study treatment. Adverse events will also be analyzed in this timeframe; that is, if an event starts within 30 days of discontinuation from study treatment, but after 30 days after the last dose of study treatment, it will not be included.

Adverse events data and serious adverse events will be presented in frequency tables by grade. Hematological and clinical biochemistry toxicities will be assessed from laboratory test parameters. The safety analysis will be performed in the safety population.

Adverse events data and serious adverse events will be reported in frequency tables (overall and by grades). Adverse events will be compared using the chi-square tests (Fisher's Exact test in the case of observing frequencies <5%).

8.2.9. Other Analyses: Patient Reported Outcomes: FSFI

Sexual function measured by the FSFI scale. It will be used an algorithm for determining domain scores and a composite full-scale score. The individual domain scores and full scale score of the FSFI are derived by a computational formula outlined in the SAP. Individual domain scores are obtained by adding the scores of the individual items that comprise the domain and multiplying the sum by the domain factor. The full scale score is obtained by adding the six domain scores. It should be noted that within the individual domains, a domain score of zero indicates that no sexual activity was reported during the previous evaluation.

8.2.10. Interim Analysis

There will be an interim analysis after the first 10 included patients have completed 3 weeks of therapy. These patients will be evaluated after 3 weeks according to the study scheme.

Only safety data and the analytical values of FSH of these 10 firsts patients will be reviewed. It is expected that no significant changes from the postmenopausal to the premenopausal range in any patient.

The 10 patients included in the interim analysis will not be included in the final analysis, as they will not complete 4 months of therapy. For that reason, it will not be necessary to perform a correction of the final alpha.

8.3 Data Monitoring Committee

The study will use an IDMC. The IDMC membership and governance is outlined in a separate charter.

The IDMC will be responsible to review the FSH and safety data from the first 10 patients included in the study and to advise the sponsor and chief investigator about a go /no-go decision with the other 60 patients to complete the calculated sample size.

8.4 Criteria for End of Study

This study will be considered complete following the data cut-off date and datalock for the final analysis. The data cut-off date for the final analysis will occur after all enrolled patients have completed the safety visit 30 (+/-5) days after the last study drug administration.

9. Informed Consent, Confidentiality, Responsibility Insurance and Regulatory Considerations

9.1. Informed Consent

The investigator is responsible for ensuring that the patient understands the risks and benefits of participating in the study, including answering any questions the patient may have throughout the study and sharing any new information that may be relevant to the patient's willingness to continue her participation in the trial in a timely manner.

The informed consent document will be used to explain the risks and benefits of study participation to the patient in simple terms before the patient is entered into the study, and to document that the patient is satisfied with her understanding of the risks and benefits of participating in the study and desires to participate in the study.

The investigator is responsible for ensuring that informed consent is given by each patient or legal representative. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study treatment.

As used in this protocol, the term "informed consent" includes all consent and assent given by patients or their legal representatives.

If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All patients (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

9.2. Respect of Confidentiality

The investigator will be responsible for preserving the suitable information about each patient (for example, name, address, telephone number, social security number and study identification) so that the competent authorities can have access to said information if necessary. These records must be confidentially preserved for the time indicated by the legislation.

The investigators and the sponsor will maintain the confidentiality of all patients participating in the study, according to Good Clinical Practice, GCP and local legislation.

9.3. Responsibility Insurance

The Sponsor has signed an insurance policy to cover the responsibilities of the investigator and those of other parties participating in the study.

9.4. Regulatory Considerations

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with good clinical practices and the applicable laws and regulations. The investigator, head of the medical institution, or designee will promptly submit the protocol to applicable ethical review board(s).

9.4.1 Investigator Information

Physicians with a specialty in medical oncology and gynecology will participate as investigators in this clinical trial.

If investigators are added after the study has been approved by the sponsor, an ERB/IRB, or a regulatory agency, these additions will not be considered changes to the protocol.

9.4.2 Protocol Signatures

After reading the protocol, each principal investigator will sign the protocol signature page and send a copy of the signed page to Sponsor.

10.Practical Considerations

10.1. Monitoring, Audit and Inspections

The study will be monitored by means of regular visits of the patients. During the visits to the center, the monitor must review the original records of the patients, the records of medication stocks and document preservation. The monitor must also evaluate the study procedures and discuss the possible problems with the investigator. During the course of the study, audit visits can be carried out in the participating centers. The investigator will allow direct access to the source documents/data for the tasks of monitoring, audit, reviewed by the ERB and the inspection by the Competent Authorities.

10.2. Preservation of Study Documentation

The copies of all the relevant information will be preserved by the investigator for a period of at least 5 years after the end of the study, according to current legislation.

10.3. Protocol Modification

Once it has been authorized by the ERB and the competent authority any protocol modification must be documented by writing, in the form of an amendment.

The amendments must be duly identified, by its chronological order number, dated and signed by the sponsor and the chief investigator.

All the protocol amendments must be notified to the ERBs involved in the trial and to the competent authority. If the modifications are relevant, the authorization of the involved ERBs and/or the competent authority will be necessary before their application.

After reading the protocol amendment, each principal investigator will sign the protocol amendment signature page and send a copy of the signed page to Sponsor.

10.4. Use of the Information and Publication

All the information concerning the study treatment provided by the sponsor in relation to this study, and not previously published, is considered to be confidential information with property right of the sponsor. This information comprises the basic information about the product, the clinical protocol, the work forms where appropriate, the e-CRFs, the assessment methods, the technical methodology and the basic scientific data. This confidential information will be the property of the sponsor, it must not be disclosed to third parties without the prior written consent of the sponsor and it must not be used other than for the purposes of the study.

The information developed during the practice of this clinical study is also considered to be confidential. This information can be disclosed to the extent considered necessary by the sponsor.

To allow the use of the information derived from this study and to ensure the compliance with the current rules, the investigator is obliged to provide GEICAM (on behalf of the sponsor) with all the results of examinations and all the data developed in this study. Except in that required by law, the information obtained during the study can only be provided to the doctors and to the

competent authorities by the Sponsor or delegated people on behalf of the sponsor. The sponsor commits to publish the results of the study and to present them in scientific meetings either if they are positive or negative.

10.5. Ethics Committees

The protocol and the informed consent document will be reviewed by the involved ERBs. The single decision of the ERBs referring to the development of the study will be provided in writing to GEICAM (on behalf of the sponsor).

GEICAM (on behalf of the sponsor) will submit the required reports of the progress of the study to the ERB and will communicate the possible SAE, the life-threatening adverse events and deaths. At the end of the study, GEICAM (on behalf of the sponsor) will inform the ERB of trial closure.

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Protocol Attachment 1. Study Schedule

Study Schedule of Events and Timelines. ITFE-2026-C10 (BLISSAFE Study)	**During Study Treatment. All visits (except week 1) +/- 3 days of scheduled treatment day.**	**Post-treatment (4 weeks) from the last study drug dose**

Visits	**Screening**	**Baseline**	**Week 1**	**Week 3**	**Week 8**	**Week 12 or early withdrawal visit**	**Post-treatment (4 weeks) from last study drug dose**
Day	Within 14 days prior to the registration	**1º**	**8** (+/- 2 days)	**22** (+/-3d)	**57** (+/-3d)	**85** (+/- 3d)	**+30** (+/-5d)
Procedure/Laboratory/ Diagnostic Test							
ICD for Entry (before any study specific tests)[a]	**X**						
Medical and surgical history and demographics[b]	**X**						
Physical examination[c]	**X**		**X**	**X**	**X**	**X**	**X**
ECOG PS	**X**		**X**	**X**	**X**	**X**	**X**
Hematology[d]	**X**					**X**	**X**
Blood Chemistry[e]	**X**					**X**	**X**
Urine Test[f]	**X**					**X**	**X**
Endometrium by transvaginal ultrasound[g]	**X**					**X**	
Moderate or severe vaginal dryness	**X**						
Review Inclusion/Exclusion Criteria	**X**	**X**					
Concomitant medications	**X**	**X**	**X**				

Visits	**Screening**	**Baseline**	**Week 1**	**Week 3**	**Week 8**	**Week 12 or early withdrawal visit**	**Post-treatment (4 weeks) from last study drug dose**
Day	Within 14 days prior to the registration	**1º**	**8** (+/- 2 days)	**22** (+/-3d)	**57** (+/-3d)	**85** (+/- 5d)	**+30** (+/-5d)
AEs and SAEs[h]	**X**	**X**	**X**				
Blood (serum) samples for FSH and LH determination[i]	**X**	**Xp**	**X**	**X**	**X**	**X**	
REGISTRATION / RANDOMIZATION		**X**					
Blood (plasma-EDTA) samples for estriol, estradiol and estrone determination[i]		**Xp**	**X**	**X**	**X**	**X**	
Gynaecological examination[j]		**Xp**		**X**		**X**	
Symptoms and signs of vaginal atrophy[k]		**Xp**		**X**		**X**	
Measurement vaginal pH[l]		**Xp**		**X**		**X**	
Vaginal cytology sample to evaluate the vaginal Maturation Value[m]		**Xp**		**X**		**X**	
FSFI questionnaire[n]		**Xp**		**X**		**X**	
Study drug/ placebo dosing[ñ]		**Xñ**	**Xñ**				

Study Schedule of Events and Timelines. Protocol ITFE-2026-C10 (BLISSAFE Study)

a	Signed, written informed consent (approved by ERB) obtained prior to any study specific procedure.
b	Includes previous treatments, disease characteristics, age, time of menopause.
c	Physical examination includes measurements of height and weight (Baseline only), area surface body, blood pressure and pulse rate.
d	Hemoglobin, WBC, absolute neutrophils, platelet count.
e	Tasting glucose, alkaline phosphatase, ALT, AST, total bilirubin, serum creatinine and BUN (or urea).
f	pH, glucose, proteins, cetones, bilirubin, blood, nitrites, urobilinogen and leukocites
g	It is advisable that the endometrial ultrasound be performed as the last of the screening procedures
h	After informed consent form signature, but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected. Adverse events to be monitored continuously during the treatment period. All AEs occurring during the study and until the treatment discontinuation visit 30 days after the last study drug to be recorded according to MedDRA, thereafter all study drug-related SAEs should continue to be collected.
i	Hormonal determinations will be performed by a Central Laboratory.
j	It includes breast examination; pelvic examination (examination of uterus and appendages); speculum inspection of vagina and exocervix, vulva and rectovaginal component.
k	Symptoms of vaginal atrophy (vaginal dryness, dyspareunia and pruritus). Signs of vaginal atrophy (mucosa with thinning or flattening of folds, dryness or fragility of the vaginal mucosa).
l	Measurement of vaginal pH on the vaginal secretion using a reactive strip (pH 2.0 - 9.0, Merck, provided by the sponsor). The investigator team will insert the strips in the vagina and will moisten them as much as possible with the vaginal wall secretion. Then, when the strip is coloured, the investigator will check the combination of colours on the strip and check the pH value to which it corresponds, recording this value in the eCRF. The reading should be made whilst the strip is still damp. The strips will be discarded once the pH has been recorded in the CRF.
m	Two smears (with two different spatulas provided by the sponsor one to take the sample and the other to take the countersample) will be made per patient for each sample (sample and countersample), which will be obtained from the bottom of the right and left vaginal sac respectively. Each smear will be suitably labelled with the corresponding labels provided to the investigator. The vaginal smears will be fixed whilst they are still moist with a water-soluble fixation spray for cytodiagnosis. The cytologic samples will be sent to central laboratory (full instructions will be given in the Laboratory Manual).
n	FSFI: Female Sexual Function Index questionnaire; patients must preferably complete these instruments in the hospital and prior to having any tests and to any discussion of their progress with healthcare personnel at the site. Interviewer administration in clinic may be used under special circumstances (eg, patient forgot their glasses or feels too ill).

ñ	Dosage schedule: Weeks 1-3: single daily application Weeks 4-12: twice weekly administration. The treatment will be administered for 12 weeks. The Principal Investigator, Sub-Investigator, study nurse or the Gynecologist must explain to the patient how to administer the vaginal gel. It is advised that the first application consider be performed in presence of the gynecologist or oncologist. Women will be instructed to administer the gel themselves for the rest of applications. The 10 patients included in the safety phase to obtain some preliminary safety data and FSH values prior to the recruitment of the rest of the group under study. These women will receive the study treatment over 3 weeks. These 10 patients will only perform the determinations of all hormone (FSH, LH, estriol, estradiol and estrone)
o	The baseline visit may be performed in two consecutive days (no more), although it will be best performed it in one day. Treatment administration will always be the last of the baseline visit procedures.
p	These tests should be performed after the definite inclusion of the patient in the study, as part of the baseline visit.

Protocol Attachment 2. Eastern Cooperative Oncology Group Performance Status

ECOG Performance Status

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. 1982. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5(6):649-65.

Protocol Attachment 3. Adverse Events / Serious Adverse Events Assessment Guide

Time	**After ICD Before Drug**	**During Therapy**	**30-Day Post-discontinuation Follow-up Period**	**Long-Term Follow-up Period**
Events to Collect	AE/SAEs Related to Procedures	New/Ongoing AE/SAEs Regardless of Relatedness to Study Treatment or Procedures		New/Ongoing SAEs Related to Study Treatment or Procedures

Abbreviations: AE = adverse event, ICD = informed consent document, SAE = serious adverse event.

Protocol Attachment 4. FSFI Questionnaire

In separate document.