SUMMARY OF THE RESULTS OF THE CLINICAL TRIAL ITFE-2026-C10 – "BLISSAFE STUDY":

A-CLINICAL TRIAL INFORMATION:

1. **Clinical trial identification:**

   Protocol number: ITFE-2026-C10

   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"

2. **Identifiers:**

   EudraCT Number: 2014-004517-84

3. **Sponsor details:**
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   Cooperative Group:
   Spanish Breast Cancer research Group – GEICAM (Fundación Grupo Español de Investigación en Cáncer de Mama)
   GEICAM Study Code: GEICAM/2014-05

4. **Paediatric regulatory details:**
   Not applicable, the study population is postmenopausal women.

5. **Result analysis stage:**
   An initial safety phase of the study, which started on March 30th, 2015 (date of First Visit First Patient), was conducted on 10 patients. The results obtained were evaluated by an Independent Data Monitoring Committee that met on September 3rd, 2015 obtaining the conclusion that there were no safety concerns to continue with the next phase of the study. The results of this safety phase were published as a poster in the 10th European Breast Cancer Conference on March 9th 2016.

   The treatment phase comprised 61 patients, the first patient was included on October 16th, 2015 and the study ended globally on February 10th, 2017. The study results, provided confidence of efficacy and safety use of the product and were published as an oral communication on the North American Menopause Society Symposium held in

October 2017, and as a poster on the San Antonio Breast Cancer Symposium held in December 2017.

6. General Information about the trial:

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. About 80% of primary breast cancers are hormone sensitive as they contain estrogen receptor (ER) and/or progesterone receptor-positive cells. 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.

In the setting of postmenopausal hormone receptor (HR) positive breast cancer, treatment with aromatase inhibitors (AIs) is the most effective and well-studied therapy. 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. 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. 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.
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. In contrast to oral hormone therapy, some consider that local application of estrogens seems to be safe in postmenopausal breast cancer patients. However, the report of a small prospective observation 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.

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.

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.

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.

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. 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 ($p=0.0425$).

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.

Trial Design:

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

61 patients were randomized to receive 0.005% estriol vaginal gel (arm A) and placebo moisturizing gel (arm B) in a 4:1 proportion, so 50 patients entered in arm A and 11 in arm B.

Arm A: Investigational drug product: 0.005% estriol vaginal gel

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 were instructed to administer the gel at bedtime.

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

Patients had 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 were obtained during the screening period in order to assess the intra-individual variation of these hormones in baseline conditions. Patients also underwent gynecological examination (vaginal smear and pH), evaluation of vaginal symptoms and signs, and answered 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 were 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).

Objectives:

Primary objective:

- 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.

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.

Date of start of the clinical trial:

The initial safety phase of the trial started on March 30th, 2015 and the treatment phase of the trial started in October 16th, 2015

Measures of protection of subjects taken: Not applicable, it was not necessary to applied extra measures for protection of the subjects out of the good clinical practice environment

Background therapy:

Patients were 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 were recorded for analysis purposes.

Routine postoperative care, such as dressing changes, suture removal, drain removal, or venous access (central or peripheral), did not need to be recorded. Anaesthetics used for any surgical procedures performed during the patient's participation in the study was recorded as "unspecified anaesthesia".

The following treatments were 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) were permitted during the active treatment phase. In general, any drugs containing "for the treatment of breast cancer" on the product insert were 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) were prohibited during the active treatment phase.

The following treatments were 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 (analgesics, antidiarrheals, antidepressants, etc) could also be used at the investigator's discretion. All medications should had to be recorded.

- Bisphosphonates and receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitors for the treatment of osteopenia/osteoporosis.

Statistical methods:

- Demographics and Baseline Characteristics

  Standard descriptive statistics, such as the mean, median, range and proportion, were used to summarize the patient sample and to estimate parameters of interest. Ninety-five percent confidence intervals were 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 was studied in each woman. The variation of levels between two arms were analysed using a non-parametric test (Mann-Whitney-Wilcoxon test) or an ANCOVA (if it must be adjusted by initial value).

  For FSH and LH the intra individual variation of these hormones (differences between the pre study determinations screening and baseline) was compared to the variation between baseline and the values obtained at every study visit.

- Safety Analyses

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

- Efficacy Analyses

  Symptoms and signs of vaginal atrophy were evaluated with a codification from 0 to 3.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, were compared using a non-parametric test (Mann-Whitney-Wilcoxon). Descriptive statistics were calculated for all variables studied.

  The averages of the differences between the baseline and the final Maturation Value (MV) and pH of the 0.005% estriol vaginal gel and placebo groups were 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 were used to analyse the change in the MV and pH after the initial observation period of 3 weeks.

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

7. **Population of subjects:**

Table 1. COUNTRY RANDOMIZATION				
		Active (N = 50)	Placebo (N = 11)	Total (N = 61)
Country				
Spain	n (%)	33 (66.0%)	7 (63.6%)	40 (65.6%)
Sweden	n (%)	17 (34.0%)	4 (36.4%)	21 (34.4%)

Table 1 .SITE RANDOMIZATION				
		Active (N = 50)	Placebo (N = 11)	Total (N = 61)
Site				
Karolinska Institute	n (%)	17 (34.0%)	4 (36.4%)	21 (34.4%)
Complejo Hospitalario de Jaén	n (%)	16 (32.0%)	4 (36.4%)	20 (32.8%)
Complejo Hospitalario Universitario A Coruña	n (%)	7 (14.0%)	1 (9.1%)	8 (13.1%)
Instituto Catalán de Oncología de Barcelona	n (%)	4 (8.0%)	1 (9.1%)	5 (8.2%)
Hospital Universitario Sanchinarro CIOCC	n (%)	4 (8.0%)	0 (0.0%)	4 (6.6%)
Hospital Clínico Universitario de Valencia	n (%)	2 (4.0%)	1 (9.1%)	3 (4.9%)

B-SUBJECT DISPOSITION:

1. **Recruitment:**

Subjects screened, randomized and withdrawn:

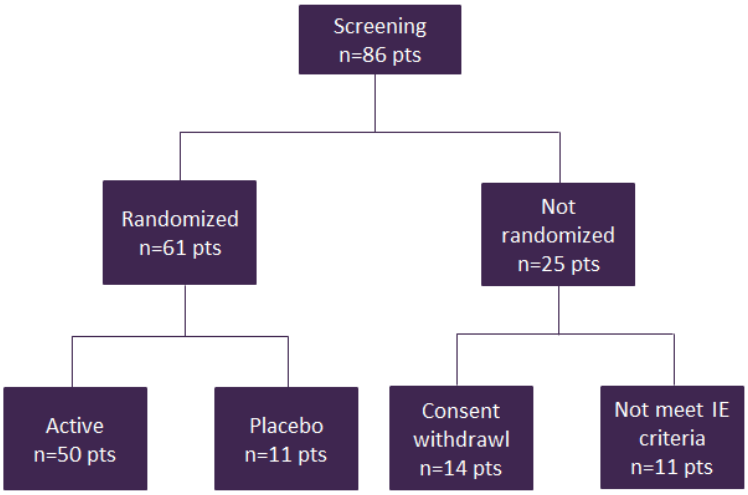

Table 3. STUDY DISCONTINUATIONS				
		Active (N = 50)	Placebo (N = 11)	Total (N = 61)
End of study according to protocol				
No	n (%)	7 (14.0%)	2 (18.2%)	9 (14.8%)
Yes	n (%)	43 (86.0%)	9 (81.8%)	52 (85.2%)
If no, specify Reason for Discontinuation				
Adverse Event: Lymphoma	n (%)	1 (2.0%)	0 (0.0%)	1 (1.6%)
Other deviation from protocol: Stopped Anastrozol Treatment	n (%)	0 (0.0%)	1 (9.1%)	1 (1.6%)
Other: Patient Consent Withdrawal	n (%)	2 (4.0%)	0 (0.0%)	2 (3.3%)
Patient decision:	n (%)	4 (8.0%)	1 (9.1%)	5 (8.2%)
• *PATIENT DECIDES NOT TO CONTINUE TREATMENT*	*n (%)*	*1 (2.0%)*	*0 (0.0%)*	*1 (1.6%)*
• *THE PATIENT CAN NOT MEET THE STUDY VISITS*	*n (%)*	*1 (2.0%)*	*0 (0.0%)*	*1 (1.6%)*
• *SHE MOVED TO THE NORTH OF SWEDEN*	*n (%)*	*1 (2.0%)*	*0 (0.0%)*	*1 (1.6%)*
• *DISAPPOINTED, NO IMPROVEMENT*	*n (%)*	*0 (0.0%)*	*1 (9.1%)*	*1 (1.6%)*
• *NO IMPROVEMENT*	*n (%)*	*1 (2.0%)*	*0 (0.0%)*	*1 (1.6%)*

Inclusion criteria:

Patients were eligible to be included in the study only if they **met 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:

    a. Serum creatinine ≤ 1.5 x ULN.

    b. Bilirubin ≤ 1.5 × ULN.

    c. Alkaline phosphatase ≤ 2 × ULN.

    d. 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 were excluded from the study if they **met 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…) 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.

Randomization and blinding details:

Patients were randomized to receive 0.005% estriol vaginal gel or placebo vaginal gel, in a 4:1 proportion. The study drug, and its vehicle in gel (placebo vaginal gel) were of identical appearance and had the same aroma and the same texture in order to maintain the double blind.

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

In each site, all patients that fulfilled all inclusion and exclusion criteria and were definitely included in the study were assigned the randomization code. This code was the one labelled in the medications. Thus, the first patient included in a site received the first study medication in the block, the second patient included received the second medication in the block, and this procedure was followed consecutively.

Throughout the study, the medication with which the patient was treated was kept strictly confidential and only authorized persons had access to this information, unless it was necessary to break the blinding due to safety reasons. The unblinding must have been done only by the principal investigator of the site or by an authorized person, only when the knowledge of the treatment assignment was deemed essential for the patient's care.

The randomization codes were unblinded and made available for the project statistician to perform the data analysis, when the study was finalized, the database was verified and the protocol violations had been determined. All personnel involved directly in the study had to be unaware of the treatment assigned to the patients until the database had been closed.

In case unblinding was necessary, there were two complete sets of randomization codes. One was kept by the CRO Pharmacovigilance department and the other was distributed among the investigators. The emergency codes had to be kept in a safe but accessible place in the event they had to be opened. Each investigator received the necessary envelopes closed to randomize a determined number of patients. Each envelope contained the details of the treatment that the patient was receiving. In the case of emergency the envelope had to be opened to determine the treatment being administered.

The randomization envelopes could only be opened in an emergency. If the investigator had to open the envelope he/she must record the date, the time and the reason he/she had to do so and kept this information with the trial documentation. He/she should also immediately had to inform the study monitor that a treatment code had been opened.

Investigational medicinal product:

The 0.005% estriol vaginal gel was provided in 30 g aluminum tubes. The tube was intended for multi-use applications, the product dosage was 1 g gel /day (50 µg estriol/g gel), which was applied using disposable single-use applicators. The product was administered by vaginal route at a dose of 1g/application. Patients had 1 application/day

during weeks 1 to 3 and 2 applications/week during weeks 4 to 12 (i.e. Mondays and Thursdays).

The placebo moisturizing gel had the same composition than the estriol vaginal gel without the active drug substance, estriol, and was administered in the same way.

2. **Pre-assignment Period:**

Patient underwent a 14 days screening period prior to the registration on the trial.

3. **Post assignment period:**

Patients underwent 12 weeks of treatment and performed a safety visit (post-treatment visit) 30 days after the end of the therapy.

During treatment phase the following visits were done: baseline, week 1, week 3, week 8, week 12 and post-treatment visit.

C- BASELINE CHARACTERISTICS:

1- Baseline characteristics age and study specific characteristics:

Table 4. BASELINE CHARACTERISTICS: AGE AND MENOPAUSAL STATUS

	Active (N = 50)	Placebo (N = 11)	Total (N = 61)
Age from Birth to Rando			
N	50	11	61
Missing	0	0	0
Mean (SD)	58.9 (7.6)	61.4 (4.7)	59.3 (7.1)
Median	58.5	63.0	59.0
Q1 ; Q3	53.0 ; 65.0	56.0 ; 65.0	53.0 ; 65.0
Min ; Max	45.0 ; 77.0	52.0 ; 66.0	45.0 ; 77.0
Postmenopausal Status			
>=12 months of Spontaneous Amenorrhea n (%)	44 (88.0%)	11 (100.0%)	55 (90.2%)
At least 6 weeks Postsurgical Bilateral Oophorectomy with or Without Hysterectomy n (%)	5 (10.0%)	0 (0.0%)	5 (8.2%)
6 months of Spontaneous Amenorrhea with serum FSH Increased levels > 40 mIU/ml n (%)	1 (2.0%)	0 (0.0%)	1 (1.6%)

2- Baseline Characteristics Gender: all included patients were postmenopausal women.

D- END POINTS:

1- End point definitions and statistical analysis:

Primary end-point:

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

  Statistical analysis: The variation of FSH levels was analysed using a non-parametric test (Mann-Whitney-Wilcoxon test) or an ANCOVA (if it must be adjusted by initial value).

Secondary End-Points:

- Variation in serum levels of FSH at different time points compared to baseline (weeks 1, 3 and 8).

  Statistical analysis: The variation of FSH levels was analysed using a non-parametric test (Mann-Whitney-Wilcoxon test) or an ANCOVA (if it must be adjusted by initial value).

- 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).

  Statistical analysis: The variation of LH, estriol, estradiol and estrone levels was analysed using a non-parametric test (Mann-Whitney-Wilcoxon test) or an ANCOVA (if it must be adjusted by initial value).

- Adverse Events (AEs) according to the Medical Dictionary for Regulatory Activities (MedDRA).

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

- 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.

  Statistical analysis: 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, were compared using a non-parametric test (Mann-Whitney-Wilcoxon). Descriptive statistics were calculated for all variables studied.

- Changes in sexual function measured by the Female Sexual Function Index (FSFI) scale at week 3 and week 12 vs baseline.

  Statistical analysis: Sexual function measured by the FSFI scale. An algorithm was used for determining domain scores and a composite full-scale score.

2- Results:

Safety results:

Primary objective:

No significant differences were found between the variation of FSH serum levels between baseline and week 12 [-5.2 (-10.9; 10.3) mUI/ml [median (Q25; 75)]] from the natural physiological variation of FSH before treatment measured at screening and baseline points -1.0 (-4.8; 2.2) mUI/ml in the 0.005% estriol vaginal gel arm; being the difference between them -2.8 [-13.1; 7.4] mUI/ml (p-value Wilcoxon = 0.10).

Secondary objectives:

Small oscillations in FSH values are shown over treatment with 0.005% estriol vaginal gel in the different time points from 55.3 (45.5; 68.5) mUI/ml at baseline [median (Q25; 75)] to 52.8 (38.6; 63.5) mUI/ml at week 12 reaching the lowest FSH value, 47.6 (37.5; 59.5) mUI/ml, at week 1, but always within postmenopausal ranges (21, 7 – 153 mUI/ml).

Regarding the variation of FSH serum levels between baseline and the different time points (week 1, 3 or 8) from the natural physiological variation of FSH before treatment in the 0.005% estriol vaginal gel arm statistical significant differences were found in the variation of levels between week 1 and baseline and week 3 and baseline compared to physiological variation but not for week 8 and baseline compared to physiological variation.

Small oscillations were observed in LH levels over the treatment with 0.005% estriol vaginal gel from 19.8 (14.8; 30.9) mUI/ml at baseline [median (Q25; 75)] to 19.3 (14.4; 27.6) mUI/ml at week 12 reaching the lowest LH value, 17.7 (13.9; 26.2) mUI/ml at week 8, but always within postmenopausal ranges (11.3 to 39.8 mUI/ml).

Moreover, no significant differences were found in LH values comparing the variation of LH before treatment (physiological variation) and the variation between baseline and visits week 1, 3, 8 and 12 in estriol arm.

Women that received 0.005% estriol vaginal gel slightly increased estriol levels from 0.5 (0.5; 1.0) pg/mL at baseline to 3.9 (0.5; 12.1) pg/mL at week 1 and 1.9 (0.5; 6.8) pg/mL at week 3. Always within postmenopausal levels (< 5 pg/mL). Then, values went back to 0.5 (0.5; 6.0) pg/mL at week 8 and 0.5 (0.5; 7.3) pg/mL at week 12. Estriol levels of 0.5 pg/mL were even lower as levels below limit of quantitation (< 1 pg/mL) were convened as 0.5 pg/mL.

Comparing estriol values between both treatment arms, statistical significant differences are found at every study point (w1, w3 and w8), including at baseline before initiating any study treatment (p- U-Mann-Whitney = 0.04), with the exception of week 12 (p- U-Mann-Whitney = 0.15).

Estradiol and estrone were not affected by treatment with 0.005% estriol vaginal gel and remain below LOQ along treatment

No significant adverse events were shown during the study and no serious adverse events related to treatment were reported. An unrelated Serious adverse event (lymphoma) was reported but before study treatment administration. Related adverse events included breast tenderness, vulvovaginal inflammation, vulvovaginal pruritus, burning sensation, diarrhoea, vomiting, mucosal dryness and pyrexia. All of them reported only once in the 0.005% estriol vaginal gel arm. Three subjects discontinued the study due to safety reasons; one due to the lymphoma reported before initiating the study treatment and 2, one in the estriol arm and one in the placebo arm, due to no improvement.

Initial safety study:

From the initial 10 subjects included in the study and treated for 3 weeks, FSH levels and safety were evaluated in order to have a go decision for the following Study Phase (n 60). Two subjects were excluded as didn´t satisfy an exclusion criteria because they were participating in another clinical trial.

In the 0.005% estriol vaginal gel arm, there were no differences between week 3 and baseline FSH values (p = 0.94) and no changes from postmenopausal to premenopausal values were shown.

Additionally, neither serious nor significant adverse events were reported in any subject.

After the evaluation of the results of this initial safety phase, the Independent Data Monitoring Committee decided to continue the study with the 60 subject – 12 weeks treatment phase.

Efficacy Results:

Secondary objectives:

There was an improvement in vaginal pH. Statistical significant differences between treatment arms were found both at week 3 and at week 12 (p- U-Mann-Whitney <0.0001 and <0.01 respectively). Looking at the change in vaginal pH between baseline and the different time points (week 3 and 12), statistical significant differences between treatment arms were found at week 3 and tendency at week 12 (p- U-Mann-Whitney <0.01 and = 0.057 respectively).

Compared to placebo, there were also statically significant differences in vaginal maturation values in the variation between the value at baseline and at week 3 (p- U-Mann-Whitney <0.0001) and in the variation between the value at baseline and at week 12 (p- U-Mann-Whitney <0.01) when compared to placebo.

Comparing the estriol arm to placebo, the change of symptoms total score was statistically significant in both, the change from baseline to week 3 (median 3.0 vs. 1.0, p- U-Mann-Whitney =0.03) and to week 12 (median 4.5 vs. 2.0, p- U-Mann-Whitney =0.04). Looking at specific symptoms, vaginal dryness resulted to be statistically significant different in the change from baseline to week 12 (median 2.0 vs. 1.0, p- U-Mann-Whitney <0.01) compared to placebo.

Regarding signs, the comparison between estriol and placebo arm in the change of scores from baseline to week 12 resulted to be statistically significant different for total sign score, dryness of the mucosa, fragility of the mucosa and vaginal mucosa with flattening of folds or thinning (p- U-Mann-Whitney < 0.01 for all of them) and in the

change from baseline to week 3 was statistically significant different for total sign score, dryness of the mucosa, and vaginal mucosa with flattening of folds or thinning (p- U-Mann-Whitney < 0.001 for all of them).

E- ADVERSE EVENTS:

1- Adverse events information:

No relevant adverse events occurred in this study.
Urinary tract infection was the most common adverse event presented during the study, that occurred in three patients (6.0%) treated with the 0.005% estriol vaginal gel (none of them related to the study drug) and in one patient (9.1%) treated with the placebo. Diarrhoea was the second more common adverse event presented during the study, that occurred in two patients (4.0%) treated with the 0.005% estriol vaginal gel (one of them related to the study drug) and in none patient treated with the placebo.

2- Adverse event reporting group:

Adverse events were classified following MedDRA. The causal relation between the investigational product and the AE was assessed by the investigator using the Karch y Lasagna (1977) algorithm. No serious reportable adverse reaction occurred during the trial.

3- Serious adverse event:

Only one serious adverse event occurred in the study. The event started before study treatment administration.

Table 05. SERIOUS ADVERSE EVENTS

	Arm A N=50	Arm B N=11	Total N= 61
Neoplasms benign, malignant and unspecified (incl cysts and polyps)			
Lymphoma	1 (2.0%)		1 (1.6%)

4- Non-serious adverse events:

Table 06. ADVERSE EVENTS PER PATIENT

	Active N=50	Placebo N=11	Total N=61
Reproductive system and breast disorders			
Atrophic vulvovaginitis	1 (2.0%)		1 (1.6%)
Breast tenderness	1 (2.0%)		1 (1.6%)

Table 06. ADVERSE EVENTS PER PATIENT			
	Active N=50	Placebo N=11	Total N=61
Vaginal discharge	1 (2.0%)		1 (1.6%)
Vulvovaginal inflammation	1 (2.0%)		1 (1.6%)
Vulvovaginal pruritus	1 (2.0%)		1 (1.6%)
Musculoskeletal and connective tissue disorders			
Back pain		1 (9.1%)	1 (1.6%)
Pain in extremity	1 (2.0%)		1 (1.6%)
Nervous system disorders			
Burning sensation	1 (2.0%)		1 (1.6%)
Respiratory, thoracic and mediastinal disorders			
Cough	1 (2.0%)		1 (1.6%)
Gastrointestinal disorders			
Diarrhoea	2 (4.0%)		2 (3.3%)
Vomiting	1 (2.0%)		1 (1.6%)
Infections and infestations			
Gastroenteritis		1 (9.1%)	1 (1.6%)
Gastroenteritis viral	1 (2.0%)		1 (1.6%)
Influenza	1 (2.0%)		1 (1.6%)
Pharyngitis	1 (2.0%)		1 (1.6%)
Urinary tract infection	3 (6.0%)	1 (9.1%)	4 (6.6%)
Viral upper respiratory tract infection	1 (2.0%)		1 (1.6%)
Vulvovaginal candidiasis		1 (9.1%)	1 (1.6%)
General disorders and administration site conditions			
Mucosal dryness	1 (2.0%)		1 (1.6%)
Polyp	1 (2.0%)		1 (1.6%)
Pyrexia	1 (2.0%)		1 (1.6%)
Ear and labyrinth disorders			
Vertigo positional	1 (2.0%)		1 (1.6%)

Table 07. RELATED ADVERSE EVENTS			
	Active N=50	Placebo N=11	Total N=61
Reproductive system and breast disorders			
Breast tenderness	1 (2.0%)		1 (1.6%)
Vulvovaginal inflammation	1 (2.0%)		1 (1.6%)
Vulvovaginal pruritus	1 (2.0%)		1 (1.6%)
Nervous system disorders			
Burning sensation	1 (2.0%)		1 (1.6%)
Gastrointestinal disorders			
Diarrhoea	1 (2.0%)		1 (1.6%)
Vomiting	1 (2.0%)		1 (1.6%)
General disorders and administration site conditions			
Mucosal dryness	1 (2.0%)		1 (1.6%)
Pyrexia	1 (2.0%)		1 (1.6%)

F- ADDITIONAL INFORMATION:

1- **Global substantial modifications:** non global substantial modifications were performed during the course of the study

2- **Global interruptions and re-starts:** non global interruptions and re-starts.

3- **Limitations, addressing sources of potential bias and imprecisions and caveats:** none performed

4- **Declaration :** please see signature page

SUMMARY OF THE RESULTS OF THE CLINICAL TRIAL ITFE-2026-C10 – "BLISSAFE STUDY" SIGNATURE PAGE

Study title:

A phase II Prospective, randomized, double-blind, placebo-controlled and multicentre 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"

Protocol number: ITFE-2026-C10

EudraCT Number: 2014-004517-84

I confirm that to the best of my knowledge this summary accurately describes the conduct and results of the study

Medical Director, ITF Research Pharma SLU
Concepción Nieto, MD, PhD
ITF Research Pharma SLU

Signature

8 Feb 18

Date