The University of Arizona Cancer Center
Hematology Oncology Section
Breast Cancer Program

A Single-arm Phase II Trial to Evaluate Serum Estradiol Levels in Patients with Breast Cancer Treated with Vaginal Estrogen, Estring

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CLINICAL PROTOCOL SYNOPSIS

Title of Study: A Single-arm Phase II Trial to Evaluate Serum Estradiol Levels in Patients with Breast Cancer Treated with Vaginal Estrogen, Estring

Principal Investigator:
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Biostatistics:
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Study Center: University of Arizona Cancer Center

Primary Objective: To evaluate if there is a significant change in serum estradiol levels in patients with breast cancer when treated with vaginal estrogen preparation, Estring

Secondary Objective:
1. To assess if there is improvement in vaginal symptoms on treatment with Estring

Primary Endpoint: To measure changes in serum estradiol level compared to their baseline in patients with breast cancer when treated with vaginal estrogen preparation, Estring

Secondary Endpoint:
1. To measure changes in vaginal dryness symptom questionnaire compared to their baseline while on treatment with Estring

Study Design: This study is a prospective single arm Phase II trial to evaluate changes in serum estradiol levels in patients with breast cancer when treated with vaginal estrogen preparation, Estring. We will also collect data from a chart review of patients who were treatment in a similar manner as a part of their routine standard of care.

Number of Patients: We plan to consent 14-20 patients, with the goal of accruing a total of 14 patients to receive treatment on this prospective single arm, Phase II trial.

Main Criteria for Inclusion/Exclusion:

To be eligible for inclusion, each patient **must have**:
- Diagnosis of estrogen receptor positive breast cancer as defined by FDA guidelines
- Attained menopause as defined by World Health Organization
- On adjuvant hormonal therapy with aromatase inhibitors, Anastrozole or Letrozole or Exemestane
- Be informed of the nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to any study specific procedures
- Be willing and able to comply with the treatment plan, scheduled clinic visits, laboratory tests and other study procedures
- Greater than 18 years of age

To be included each patient **must not have**:

- Metastatic disease

Intervention: Patients with breast cancer treated at the University of Arizona Cancer Center will be enrolled in this trial. Patients who are currently being treated with adjuvant aromatase inhibitors (anastrozole, letrozole or exemestane) will be screened by their physicians for genitourinary symptoms. Once identified, they will be consented to this study and be treated with Estring for their symptoms.

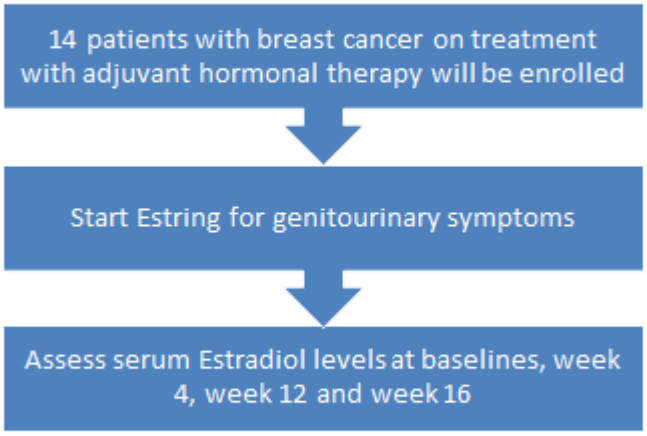
Duration of Intervention: Patients will get a baseline serum estradiol level measured prior to receiving Estring. We will repeat serum estradiol levels at 4 weeks, 12 weeks and 16 weeks. Estring will be stopped if there is evidence of continued rise in serum estradiol. Also, it will be discontinued if there is development of persistent $\geq$ grade 3 vaginal discomfort or bleeding or if the patient chooses.

Statistical Methods: This study is a prospective single arm Phase II trial to assess changes in serum estradiol levels when treated with vaginal estrogen preparation, Estring. We plan to enroll 14 patients into this study.

Analytic plan for primary objective:
There is no statistical significant difference in the serum estradiol levels at week 16 compared to baseline.

Analytic plan for secondary objective:
Vaginal dryness questionnaire will be completed and scores will be compared between baseline and 16 weeks.

Schema


14 patients with breast cancer on treatment with adjuvant hormonal therapy will be enrolled
Start Estring for genitourinary symptoms
Assess serum Estradiol levels at baselines, week 4, week 12 and week 16

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1. Introduction

1.1. Breast Cancer

Breast cancer is the most common life threatening malignancy in women, with over 200,000 new cases diagnosed in the United States annually.[1] With early detection and novel treatment strategies survival has increased, increasing the number of survivors experiencing the short- and long-term consequences of estrogen deficiency.

1.2. Background

Majority of these cancers express estrogen receptors (75%) and/or progesterone receptors making endocrine manipulation a very effective therapeutic option. Several endocrine options with proven efficacy are currently available for treatment. Factors guiding the oncologist's decision on the type and duration of endocrine therapy include the patient's menopausal status, response to prior trials of endocrine treatment, and consideration of side-effect profiles. Tamoxifen, a selective ER modifier, may be used in pre-, peri-, and postmenopausal women.[2,3] Alternative options for premenopausal and perimenopausal women who have contraindications to tamoxifen use include ovarian suppression and ablation.[4] In postmenopausal women, where the majority of estrogen is derived from the peripheral conversion of androgens to estrogen, the incorporation of aromatase inhibitors (AIs) into treatment regimens has shown benefits in the early[5-8] and advanced settings.[9,10] Aromatase inhibitors or selective estrogen receptor modulators are the standard adjuvant treatment for hormone receptor positive breast cancer in post-menopausal women. The aromatase inhibitors work by decreasing systemic estrogen production leading to lower circulating estrogen levels.

Effects of normal menopause on the urogenital system

Estrogen is fundamental for the normal development of multiple organ systems including the reproductive tract, mammary glands, bone, and blood vessels. To date, two ERs have been isolated, ER-α and ER-β, with a varied proportion of each type of receptor in various tissues.[11] In general, the activation of these receptors at the nuclear level by estrogen and/or other cofactors is needed to induce cell growth and differentiation. ERs are heavily concentrated in the vulva, vagina, pelvic floor musculature, endopelvic fascia, bladder, and urethra.[12,13] As a result, the urogenital system is exquisitely sensitive to estrogen deprivation. A decline in estradiol concentrations results in a reduction in squamous epithelial cells in the vulvovaginal area and uroepithelial lining with a predominance of basal cells.[14] Moreover, collagen, glycogen, mucopolysaccharides, and hyaluronic acid significantly decline in hypoestrogenemic urogenital epithelium. The vaginal walls therefore become thin, friable, pale, and hyposecretory, and lose their elasticity with progressive stenosis, while the urethra develops increased atrophy and laxity.[14] The uterus, ovaries, vagina,and vulva also shrink in size.[15] Symptoms of estrogen deprivation include: vaginal dryness,

itchiness, discharge, incontinence, burning, and pain during sexual intercourse. Concomitant with these changes is a reduction in the vaginal *Lactobacillus* population, which in turn leads to greater vaginal alkalinity (pH >5) and further contributes to the greater risk for urinary tract infections.[16]

Prevalence of genitourinary symptoms in breast cancer patients

The prevalence and severity of genitourinary symptoms among women with a prior history of breast cancer have been reported using a variety of quality of life scales, making an overview analysis challenging. Nonetheless, the literature has demonstrated a clear increase in the incidence and progression of gynecologic symptoms following chemotherapy and/or endocrine therapy. Ganz et al. reported that up to 50%–75% of breast cancer survivors experience one or more urogenital symptoms.[17] Treatment with both chemotherapy and tamoxifen seems to compound the severity of symptoms versus treatment with either agent alone.[18] Vulvovaginal atrophy in turn contributes to increasing sexual dysfunction, reported as dyspareunia, diminished libido, and decreased sexual satisfaction.[19,20] In a separate study, the severity of sexual symptoms and vaginal dryness negatively correlated with self-perceived quality of life, and perception of partner quality of life [32].[21]In recent years, multiple endocrine therapy trials have prospectively evaluated the prevalence of gynecologic symptoms in studies comparing tamoxifen with AIs. Reported results of urogenital side effects from AIs suggest less tolerable vulvovaginal symptoms in comparison with those observed with tamoxifen. Morales et al. found an increase in severe or intolerable dyspareunia among women started on nonsteroidal AIs, from 11% at baseline to 25% after 3 months of treatment, which was significantly associated with vaginal dryness.[22] Similarly, a cohort of women in the Arimidex, Tamoxifen, Alone or in Combination trial after 5 years of treatment with anastrozole reported rates of dyspareunia, diminished libido and decreased sexual satisfaction, and vaginal dryness of 17.3%, 34.0%, and 18.5%, respectively.[23] In comparison, among patients taking tamoxifen, reported side effects for dyspareunia, diminished libido and decreased sexual satisfaction, and vaginal dryness were significantly lower at 8.1%, 26.1%, and 9.1%, respectively ($p < .05$).[23] The incidence of urinary tract infections among menopausal women varies depending on the presence of risk factors such as diabetes[24] or the presence of urinary prolapse[25], with reported incidences in the range of 5%–8%.[25,26] Similar cystitis rates have been reported among women on estrogen deprivation therapy. Rates of urinary tract infections among women taking the AI anastrozole have ranged between 3.5% [38] and 8%, while the incidence of urinary tract infections among women taking tamoxifen has been reported to be as high as 10%.[27]

Treatment options for genitourinary symptoms

Non-hormonal vaginal moisturizers and lubricants are the recommended first line treatment for postmenopausal vaginal symptoms however results on their efficacy have been inconsistent. They adhere to the vaginal epithelium creating a moisture barrier but do not reverse the atrophic changes, which are the root cause of the symptoms moreover; they have minimal effect on urinary symptoms.[28] Their efficacy in relief of genitourinary symptoms in breast cancer patients receiving aromatase inhibitors has not been studied.

Vaginal estrogen has been used effectively to treat postmenopausal genitourinary symptoms. Estrogen can be delivered locally as vaginal cream (Estrace and Premarin), ring (Estring and Femring) or tablet (Vagifem). The optimal treatment regimen, minimum effective dose and duration have not been established. A Cochrane review of 16 clinical trials conducted on 2,129 postmenopausal women concluded all formulations of local estrogen therapy had efficacy in relieving subjective genitourinary symptoms.[29] There was also an improvement in vaginal elasticity, fluid volume and moisture unlike vaginal moisturizers which provide only symptomatic relief but do not alter objective atrophic findings. North American Menopause Society (NAMS) published a position statement in 2007 on the role of local vaginal estrogen for treatment of vaginal atrophy in postmenopausal women.[30] NAMS systematically reviewed all relevant medical literature and concluded that postmenopausal women with moderate to severe vaginal atrophy symptoms who have failed the first line therapy with non-hormonal vaginal moisturizers may be candidates for local estrogen treatment. However the main concern in using vaginal estrogen in breast cancer patients is related to their absorption and systemic levels of estrogen (estradiol, E2) and the theoretical concern that these levels could stimulate breast cancer growth.

Clinical Studies using topical estrogen preparations in breast cancer patients

A drop in serum estradiol levels following consistent use with either Estring or Vagifem has also been well documented among menopausal women without a prior history of breast cancer [51, 64].[31,32] This fall in serum estradiol levels is likely a reflection of vaginal epithelial maturation, which further inhibits systemic absorption of estrogen. Of concern is whether even minor changes in serum estradiol levels from vaginal absorption of topical estrogen replacement agents increase breast cancer recurrence. Among postmenopausal women, the Endogenous Hormones and Breast Cancer Collaborative Group found an association between high levels of endogenous estrogen and higher breast cancer risk. However, this overview found a large range of median estrogen values of 21.7–101 pmol/l among the control group resulting from variations in laboratory analyses.[33] Given the variation in results, a threshold estradiol level that was clearly associated with elevated breast cancer risk could not be established. Further highlighting the need for a sensitive estradiol assay for women taking AIs was a small study of 10 patients before and after AI use who had up to a 70% difference in estradiol levels when measured by direct assay versus being measured after pre-extraction with an organic solvent.[34] Retrieval and storage of serum may also impact estradiol levels; delaying blood sample processing by 1 day has been shown to spuriously increase estrogen levels by 7.1% (95% CI, 3.2%–11.3%).[35]

Few studies have investigated the impact of local vaginal estrogen preparations for the treatment of gynecologic symptoms with regard to breast cancer recurrence and mortality. A small cohort study of 69 women, some of whom were taking tamoxifen, suggested that vaginal estrogen cream or tablets did not increase the risk for recurrent breast cancer and resulted in a hazard ratio for disease recurrence of 0.57 (95% CI, 0.20 –1.58; p = .28).[36] A separate study of seven women taking AIs with subsequent intolerable urogenital side effects documented serum estradiol levels after treatment with Vagifem 25 µg. Estradiol levels rose from a mean baseline level of < 5 pmol/l to a mean of 72 pmol/l at 2 weeks, and then declined at 4 weeks to a median of 16 pmol/l. Two women had high estradiol levels after 7 weeks of treatment.[37] In contrast, Santen et al have reported that at 12 weeks, serum estradiol levels were within

postmenopausal range of 3-10 pg/ml when treated with vaginal estradiol at a dose of 10µg.[38] Recently, data presented on 26 breast cancer patients at San Antonio Breast Cancer conference, showed no significant rise in serum estradiol level in breast cancer patients on aromatase inhibitors being treated by Vagifem 10µg tablets at week 12 compared to baseline.[39] There have been no prospective studies reporting serum estradiol levels in breast cancer patients being treated with aromatase inhibitors and concurrent use of Estring. We propose an investigator-initiated clinical trial at the university of Arizona cancer center to evaluate changes in serum estradiol level in patients with breast cancer treated with vaginal estrogen, Estring for their genitourinary symptoms.

1.3. **Study Population**

This study will enroll patients with stage I-III estrogen receptor positive breast cancer who are on adjuvant hormonal treatment with aromatase inhibitors. They will be screened for genitourinary symptoms and will be offered to participate in this study. Enrolled patients will be treated with vaginal estrogen preparation, Estring, for their symptoms.

2. **Trial Objectives**

Primary Objective: To evaluate if there is a significant change in serum estradiol levels in patients with breast cancer when treated with vaginal estrogen preparation, Estring

Secondary Objective:

1. To assess if there is improvement in vaginal symptoms on treatment with Estring

3. **Trial Design**

3.1. **Study Endpoints**

Primary Endpoint: To measure changes in serum estradiol level compared to their baseline in patients with breast cancer when treated with vaginal estrogen preparation, Estring

Secondary Endpoint:

1. To measure changes in vaginal dryness symptom questionnaire compared to their baseline while on treatment with Estring

3.2. **Study Design** This study is a prospective single arm Phase II trial to changes in serum estradiol levels in patients with breast cancer treated with aromatase inhibitors when treated with vaginal estrogen preparation, Estring. We will also collect data from a chart review of patients who were treatment in a similar manner as a part of their routine standard of care.

3.3. **Study Duration and Follow up**

All patients will be treated with vaginal estrogen preparation, Estring for at least 4 mths. Patients will get baseline serum estradiol value measured. It will be repeated at week 4, week 12 and week 16 (calculated from day 1 which is the first day of Estring insertion).

Patients will also fill out a vaginal dryness symptom questionnaire at baseline and week 16.

4. Selection and Withdrawal of Patients

4.1. **Inclusion Criteria**: To be eligible for inclusion, each patient must have:

- 4.1.1 Stage I-III estrogen receptor positive breast cancer (positive for estrogen receptor (ER)) with positivity defined as immunohistochemical staining in ≥ 10% of cells) on adjuvant hormonal therapy with aromatase inhibitors (anastrozole, letrozole or exemestane)
- 4.1.2 Adults over 18 years of age with a life expectancy of at least 3 months
- 4.1.3 Attained menopause as defined by World Health Organization Criteria (defined as permanent cessation of menstruation resulting from the loss of ovarian follicular activity. This is recognized to occur after 12 consecutive months of amenorrhea, for which there is no other obvious pathological or physiological cause.)
- 4.1.4 Persistent genitourinary symptoms causing discomfort for more than 2 weeks prior to the visit with the physician.
- 4.1.5 Tried at least 1 prior pharmacological/ non-pharmacological treatment for their genitourinary symptoms
- 4.1.6 Be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to any study specific procedures
- 4.1.7 Be willing and able to comply with the treatment plan, scheduled clinic visits, laboratory tests and other study procedures

4.2 Exclusion Criteria

- 4.2.1 Patients with metastatic breast cancer
- 4.2.2 Have a concurrent active non-breast malignancy except for non-melanoma skin cancer
- 4.2.3 Patients with vaginal stenosis
- 4.2.4 Patients unable to apply Estring

4.3 Study Exit

Patients will continue with study visits until study exit. Patients will exit the study at 16 weeks or withdrawal. Each patient has the right to withdraw from the study at any time without prejudice. Should a patient withdraw from the study prior to completion, the reason(s) must be stated in the chart and on the case report form. All procedures (laboratory evaluations, study questionnaires and adverse events reporting) should be performed at the study exit visit. If a patient stops Estring prior to the study completion then they will go off study at that time of discontinuation.
If a patient is noted to have a serum estradiol level of >10pg/ml at week 16 then Estring will be discontinued.

Patients may be withdrawn from the study early due to:

a. Development of toxicity which in the Investigator's judgment precludes further study participation

b. Significant protocol violations or noncompliance on the part of the patient or Investigator
c. Discontinuation, in the judgment of the Investigator, is in the patient's best interest
d. The patient is beginning another treatment for breast cancer
e. Refusal of the patient to continue treatment or follow-up
f. Loss to follow-up

4.4 Patient Registration

Patients must be registered prior to initiation of treatment. Patients will be registered through a Breast Team Clinical Research Coordinator (CRC) from 8:00 a.m. to 5:00 p.m., Mountain Standard Time, Monday through Friday, (excluding holidays)

4.5 Study Patient Identification

Patients who have been consented and are undergoing study screening will be identified on study-related documentation and forms by their initials (first/middle and last name initials). All patients registered on this trial will be identified by their study initials and a unique study identification number. A unique study number will only be assigned to patients who meet the eligibility requirements and have completed the screening visit. The unique number will begin with the following prefix: VE. The prefix will be followed by the patient identification number beginning with # 001. These numbers will be issued to patients sequentially and no patient identification numbers will be re-assigned in the event that the subject withdraws from the protocol.

5. Study Procedures

5.1. Pretreatment

Patients will be consented and evaluated for participation based on the following procedures which will be performed within 28 days of study entry (time of consent) before being registered and starting treatment

- Documentation that patient has genitourinary symptoms

5.2. Patient Registration

Patients must be registered prior to initiation of treatment. Patients will be registered through a Breast Team Clinical Research Coordinator (CRC) from 8:00 a.m. to 5:00 p.m., Mountain Standard Time, Monday through Friday, (excluding holidays)

Registration Guidelines
Before a subject participates in the trial, the investigator or delegate is responsible for obtaining written informed consent after adequate explanation of the aims, methods, anticipated benefits, subject responsibilities and potential hazards of the study and before any protocol-specific screening procedures or any study required medications are administered. All patients must meet the eligibility requirements and complete the screening visit prior to registration. Once registration is complete treatment may begin.

5.3. Treatment Phase

The following tests and observations will be performed during the treatment phase of the study. For the intervals below day 1 is the first day of Estring

Test	Interval
Symptom questionnaire	Baseline and week 16 (+/- 7days)
Serum Estradiol level	Baseline, week 4, 12& 16 (+/- 7 days)

SAEs that would be considered/classified as possible, probably or definitely related to study procedures or study required medication only will be followed.

5.4. **Follow Up**

Patients who have experienced a serious adverse event that has been attributed as possibly, probably or definitely related to study procedures or study required medication will have a follow up visit or a phone call 30 days (+/-_ 7 days) calculated from the week 16 visit.

6. Criteria for Evaluation

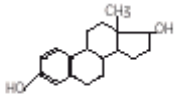
The primary endpoint is the change in serum estradiol level compared to baseline. Other efficacy measures are changes in vaginal dryness questionnaire. The SAEs will be assessed at time of week 4, 12 and 16 (+/- 7 days) by visit with the health care provider or phone call by the research RN.

Evaluation Criteria Definitions

6.1. **Treatment related toxicity** is an adverse effect that is clearly related to Estring.

7. Drug Information

ESTRING® (estradiol vaginal ring) is a slightly opaque ring with a whitish core containing a drug reservoir of 2 mg estradiol. Estradiol, silicone polymers and barium sulfate are combined to form the ring. When placed in the vagina, ESTRING releases estradiol, approximately 7.5 mcg per 24 hours, in a consistent stable manner over 90 days.
Estradiol is chemically described as estra-1,3,5(10)-triene-3,17β-diol. The molecular formula of estradiol is $C_{18}H_{24}O_2$ and the structural formula is:



The molecular weight of estradiol is 272.39.

Pharmacokinetics

A. Absorption

Estrogens used in therapeutics are well absorbed through the skin, mucous membranes, and the gastrointestinal (GI) tract. The vaginal delivery of estrogens circumvents first-pass metabolism.

In a Phase I study of 14 postmenopausal women, the insertion of ESTRING (estradiol vaginal ring) rapidly increased serum estradiol (E2) levels. The time to attain peak serum estradiol levels (Tmax) was 0.5 to 1 hour. Peak serum estradiol concentrations post-initial burst declined rapidly over the next 24 hours and were virtually indistinguishable from the baseline mean (range: 5 to 22 pg/mL). Serum levels of estradiol and estrone (E1) over the following 12 weeks during which the ring was maintained in the vaginal vault remained relatively unchanged (see Table 1).

The initial estradiol peak post-application of the second ring in the same women resulted in ~38 percent lower Cmax, apparently due to reduced systemic absorption via the treated vaginal epithelium. The relative systemic exposure from the initial peak of ESTRING accounted for approximately 4 percent of the total estradiol exposure over the 12-week period.

The release of estradiol from ESTRING was demonstrated in a Phase II study of 222 postmenopausal women who inserted up to four rings consecutively at three-month intervals. Systemic delivery of estradiol from ESTRING resulted in mean steady state serum estradiol estimates of 7.8, 7.0, 7.0, 8.1 pg/mL at weeks 12, 24, 36, and 48, respectively. Similar reproducibility is also seen in levels of estrone. The systemic exposure to estradiol and estrone was within the range observed in untreated women after the first eight hours.

In postmenopausal women, mean dose of estradiol systemically absorbed unchanged from ESTRING is ~8 percent [95 percent CI: 2.8–12.8 percent] of the daily amount released locally.

B. Distribution

The distribution of exogenous estrogens is similar to that of endogenous estrogens. Estrogens are widely distributed in the body and are generally found in higher concentrations in the sex hormone target organs. Estrogens circulate in the blood largely bound to sex hormone binding globulin (SHBG) and albumin.

C. Metabolism

Exogenous estrogens are metabolized in the same manner as endogenous estrogens. Circulating estrogens exist in a dynamic equilibrium of metabolic interconversions. These transformations take place mainly in the liver. Estradiol is converted reversibly to estrone, and both can be converted to estriol, which is the major urinary metabolite. Estrogens also undergo enterohepatic recirculation via sulfate and glucuronide conjugation in the liver, biliary secretion

of conjugates into the intestine, and hydrolysis in the intestine followed by reabsorption. In postmenopausal women, a significant proportion of the circulating estrogens exist as sulfate conjugates, especially estrone sulfate, which serves as a circulating reservoir for the formation of more active estrogens.

D. Excretion

Estradiol, estrone, and estriol are excreted in the urine along with glucuronide and sulfate conjugates.

Mean percent dose excreted in the 24-hour urine as estradiol, 4 and 12 weeks post-application of ESTRING in a Phase I study was 5 percent and 8 percent, respectively, of the daily released amount.

8. Study Statistics

8.1. **Analytic plan for primary objective**: Patients will get a baseline serum estradiol level checked and at week 4, 12 and 16. Serum estradiol level of 10pg/ml is used as cut off point. All patients will have serum estradiol level ≤10pg/ml at baseline. At week 16, the proportion of patients with serum estradiol level >10pg/ml is used to assess the presence of an unacceptable increase change in serum estradiol level. With sample size of 14 patients, if none have serum estradiol level >10pg/ml at week 16, it will be concluded that the probability of serum estradiol level >10pg/ml at week 16 is less than 10% (based on the upper limit of a one-sided 95% confidence interval). The proportion of patients with serum estradiol level >10pg/ml at week 16 and its one-sided 95% confidence interval will be estimated. The mean change in serum estradiol level between baseline and week 16 and its 95% confidence interval will be estimated. In addition, to assess the presumed maximum change in serum estradiol level, the mean change in serum estradiol level between baseline and week 4 with its 95% confidence interval will be estimated.

8.2. **Analytic plan for secondary objective**: The patients will complete the vaginal dryness symptoms questionnaire at baseline and week 16. The mean (standard deviation) and median of scores at baseline and week 16 will be reported. The signed rank test will be used to evaluate if there is a significant change in the score between baseline and week 16.

9. Data and Safety Monitoring Plan

Identification of the DSMB obligated for oversight responsibilities:
The Arizona Cancer Center Data and Safety Monitoring Board (DSMB) will provide ongoing oversight for this trial.

Identification of the entity obligated for routine monitoring duties:
Routine monitoring will be provided by the Quality Assurance/Quality Control (QA/QC) Program to ensure that the investigation is conducted according to protocol design and regulatory requirements.

Monitoring progress and data review process:
Routine monitoring of subject data will be conducted at least every six months.

The first routine monitoring visit will include at a minimum:

- Informed consent – 100% of cases enrolled;
- Subject eligibility - 50% of cases, up to two subjects;
- Data review - 50% of cases, up to two subjects.

All subsequent monitoring visits will consist of randomly selected subject cases based on current enrollment and include continuing review of previously selected cases, as applicable.

A monitoring visit report and follow-up letter will be completed within two weeks of the routine monitoring visit; a copy will be maintained in the study file. A query/finding form will also be completed by the monitor to request additional source documentation, clarification, information or corrections to the CRF and/or regulatory records. The Clinical Research Coordinator or other applicable staff responsible for the study will be given a copy of this form for resolution of queries/findings. The query/finding form will be maintained with a copy of the visit report for follow-up at the next monitoring visit.

The Principal Investigator will ensure the accuracy, completeness, legibility and timeliness of the data reported in the Case Report Form (CRF). Source documentation supporting the CRF data will indicate the subject's participation in the trial and will document the dates and details of study procedures, adverse events, and patient status.

Case report forms, which include the inclusion/exclusion criteria form, adverse event forms and serious adverse event forms should be completed with a black ball-point pen or typed. Corrections to the forms should not obscure the original entry and should be made by striking the incorrect information with a single line. Each strike should be accompanied by the initials of the corrector and the correction date. All subject forms and study files will be stored in a secure area limited to authorized staff.

Note: Routine monitoring of regulatory documents and test article will be conducted at least annually.

Process to implement study closure when significant risks or benefits are identified:

There is no planned interim analysis. Results will be analyzed after 14 patients are enrolled and data is collected for all of them.

Description of adverse events and reporting procedures:

Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical 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 a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Any and all adverse events will be recorded on the UMC adverse events record form and reviewed by the Principal Investigator.

All adverse events will be classified using either the MedDRA term or NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 and will address:

- Grade
- Relationship to study drug(not related, unlikely, possible, probable, definitely)
- Causality other than study drug (disease related, concomitant medication related, intercurrent illness, other)
- Date of onset, date of resolution
- Frequency of event (single, intermittent, continuous)
- Event outcome (resolved, ongoing, death)
- Action taken (none, held, dose reduced, discontinued, medication given)

Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of an existing hospital stay
- Results in disability persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

All SAEs which meet the criteria for a reportable event will be reported to the University of Arizona Human Subjects Protection Program within 10 working days of the event date or receipt of notification of the event.

Non-local Unanticipated Problem Involving Risks to Subjects or Others, which is any information that meets **all three** of the following criteria:

i) Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent; and (b) the characteristics of the subject population being studied. A harm is “unexpected” when its specificity and severity are not accurately reflected in the consent document.

ii) Related or possible related due to participation in this research (possible related means that the outcome may have been caused by the procedures involved in the research); and

iii) Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. A harm is “at least probably related to the Human Research procedures” if in the opinion of the investigator, the research procedures more likely than not caused the harm.

Plan for assuring data accuracy and protocol compliance:

Routine study activity and safety information will be reported to the DSMB every six months, or more frequently if requested. These reports will include:

- Study activity, cumulative and for the period under review;
- Safety (narrative description on non-serious and serious adverse events);
- Predetermined protocol early stopping rules for efficacy/futility;
- Monitoring and protocol compliance;
- Comments;
- Attachments (AE data reviewed by the PI to compile the report, SAE letters and reports, results of any review(s), applicable correspondence with the IRB or other regulatory agencies.

Data, safety and study progress will be reported to:

- Human Subjects Protection Program (IRB) at least annually;
- Sponsor (if applicable) at least every six months.

Identification of the sponsor or funding agency, as applicable:
The PI will immediately notify; in writing, the funding agency, if applicable, any action resulting in a temporary or permanent suspension of the study.

10. **Discipline Review**

There is no discipline review

11. **Data Submission**

Electronic case report forms will be completed in the OnCore system.

12. **Special instructions**

Serum Estradiol will be measured by tandem mass spectrometry and results will be reported in pg/ml. Estradiol assay used is a quantitative high performance liquid chromatography-Tandem mass spectrometry which can report serum estradiol levels between 1pg/ml-2000pg/ml. This is a send out lab and University Medical Center is contracted with ARUP laboratories for this. This test is also done by LabCorp and SonoraQuest diagnostics. We will send out the samples to appropriate laboratories based on patient's insurance approval.

13. **Ethics**

The trial will be conducted in accordance with the Declaration of Helsinki for biomedical research involving human subjects and local regulatory requirements.

Ethical Principles
This study will be conducted in accordance with Title 21 of the Code of Federal Regulations (CFR). Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed by an Institutional Review Board; the study is to be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the patients will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; the results to be reported will be accurate; patients will give

their informed consent and will be competent to do so and not under duress; and the study will comply with the ethical principles in Title 21 of the CFR.

Informed Consent
This study will be conducted in full compliance with the informed consent regulations in 21 CFR 50. The Sponsor-Investigator is responsible for obtaining written consent from potential patients prior to performing any trial tests or assessments required by the protocol.
A copy of the fully executed informed consent form (PHI authorization form and ancillary consent forms if applicable), is given to the subject. One copy is placed in the subject's medical record, another copy is placed in the research chart, and the originals are filed by the protocol number in room 2111 at UACC North Campus.

Institutional Review Board
This study will be conducted in full compliance with the Institutional Review Board (IRB) regulations in 21 CFR 56, in accordance with the Declaration of Helsinki. This protocol will not be initiated unless it and the informed consent form have been reviewed and approved by, and remains open to continuing review by, an IRB meeting the requirements of 21 CFR 56. The IRB shall review and have the authority to approve, require modification in (to secure approval), or disapprove the protocol. The IRB shall notify the Investigator and the institution in writing of its decision. The IRB shall require that the information given to patients as part of the informed consent is in accordance with 21 CFR 50.25. The IRB shall conduct continuing reviews of the protocol at intervals appropriate to the degree of risk, but not less than once per year. At the completion or early termination of the trial, a final report should be sent to the IRB by the Investigator. The Investigator is obligated to maintain an IRB correspondence file.

Confidentiality of Patient Data
The investigator must ensure that patient confidentiality will be maintained. Patients will be identified by initials and a protocol-assigned patient number as described in section 4.4. Permission for direct access to patient data will be sought in writing for the patient by the investigator as part of the informed consent procedure. The patient will be informed that all clinical information is confidential, but that the IRB, and regulatory authorities may inspect these records.

14. Bibliography

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APPENDIX 1

Investigator Agreement

Protocol No.

Protocol Title: A Phase II Trial to Evaluate Serum Estradiol Levels in Patients with Breast Cancer Treated with Estring

By signing below I agree:

1) That my staff and I have read, understand and will adhere to the protocol as written, and that any changes to the protocol will be agreed to and approved by the Sponsor and the Institutional Review Board (IRB), except a change to eliminate an immediate hazard to study subjects, which may be implemented at once, with notice to the Principal Investigator and the IRB;

2) To abide by all obligations stated on the FDA Form 1572 and other documents required by regulation;

3) To conduct this study in accordance with the current International Conference on Harmonization (ICH) guidance, the Good Clinical Practices (GCP) guidance, the Declaration of Helsinki, US FDA regulations and local IRB and legal requirements;

4) To obtain IRB approval of the protocol, any amendments to the protocol, and periodic re-approval as required, and to keep the IRB informed of adverse events and periodically report the status of the study to them;

5) To ensure that each individual enrolled into the trial, or legally authorized representative, has read, understands, and has signed the Informed Consent form;

6) To ensure that I and all persons assisting me with the study are adequately informed and trained about the investigational drug and their study-related duties and functions as described in the protocol;

7) To make prompt reports of SAEs and deaths to the FDA according to the regulations;

8) To prepare and maintain adequate and accurate case histories to document all observations and other data pertinent to the study for each individual enrolled in the clinical trial.

Investigator Signature Date

Investigator Name (Print)

APPENDIX 2

VAGINAL DRYNESS SYMPTOM QUESTIONNAIRE

EVERYDAY PROBLEMS DURING THE PAST 4 WEEKS

We are interested in knowing how much you have been bothered by any of the following problems during the **PAST 4 WEEKS.** *(Check one box on each line. If you do not have the problem, check "not at all".)*

During the **past 4 weeks,** how much were you bothered by:

	Not at all	Slightly	Moderately	Quite a bit	Extremely
1. Hot flashes	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
2. Nausea	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
3. Vomiting	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
4. Difficulty with bladder control when laughing or crying.	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
5. Difficulty with bladder control at other times	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
6. Vaginal dryness	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
7. Pain with intercourse	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
8. General aches and pains	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4

9. Joint pains	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
10. Muscle stiffness	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
11. Weight gain	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
12. Unhappiness with the appearance of your body	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
13. Forgetfulness	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
14. Night sweats	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
15. Difficulty concentrating	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
16. Being easily distracted	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
19. Vaginal discharge	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
20. Vaginal bleeding or spotting	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
21. Genital itching/irritation	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
22. Lack of energy	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
23. Tiredness	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
24. Lack of interest in sex	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4
25. Low sexual enjoyment	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4