A Phase II Study of Vaginal Testosterone Cream vs. the ESTRING for Vaginal Dryness or Decreased Libido in Early Breast Cancer Patients Treated with Aromatase Inhibitors

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PROTOCOL SYNOPSIS

A Phase II Study of Vaginal Testosterone Cream vs. the ESTRING for Vaginal Dryness or Decreased Libido in Early Breast Cancer Patients Treated with Aromatase Inhibitors

Principal Investigators

Michelle Melisko, MD and Mindy Goldman, MD

Study centre(s) and number of subjects planned

University of California, San Francisco 70 evaluable patients

Study period

10/1/07-3/31/10

Phase of development

Phase II

Objectives

Primary Objective

To evaluate the safety, based on serial measurements of serum estradiol levels, of intravaginal testosterone cream or the ESTRING administered for relief of vaginal dryness and/or decreased libido related to aromatase inhibitor therapy in early breast cancer patients.

Secondary Objectives

1. To document the systemic estradiol and testosterone levels at several time points in early breast cancer patients on aromatase inhibitors and being treated with intravaginal testosterone cream or the ESTRING.
2. To compare standard clinical laboratory measurements of serum estradiol with values of serum estradiol as measured by an ultrasenstive assay in a research laboratory.
3. To evaluate changes in gynecologic and sexual quality of life using validated questionnaires (the CARES Sexuality Subscales) in early stage breast cancer patients using intravaginal testosterone cream or the ESTRING for relief of vaginal dryness and/or decreased libido related to aromatase inhibitor therapy.

General design

Two-arm, randomized, open-label study.

Target subject population

Stage I-III breast cancer patients receiving aromatase inhibitors as adjuvant hormonal therapy and who have complaints of vaginal dryness, dyspareunia, or decreased libido. For this study, decreased libido is defined as a patient having decreased interest in sexual intercourse, decreased sexual arousal, or inability to achieve an orgasm.

Study treatments, dosage and mode of administration

- Testosterone Cream 1% micronized in velvachol - 0.5 gm of cream vaginally 3 times a week for total of 12 weeks of treatment
- ESTRING 2mg ring inserted vaginally once every 12 weeks

Duration of treatment

12 weeks

Safety

Patients will be monitored throughout the study for evidence of vaginal irritation, bleeding, or other side effects potentially related to the study treatments. Measurements of serum estradiol by standard clinical laboratory assays will be obtained at baseline, and at 4 and 12 weeks into treatment to assure that estradiol levels remain in the postmenopausal range. Throughout this protocol, a "persistently elevated estradiol level above the post-menopausal range" will be defined as the following:

1. An elevation of serum estradiol outside of the post-menopausal range of≤10 pg/ml, and at least 10 pg/ml higher than the baseline value on two consecutive collections at least four weeks apart, and not including the baseline value which is drawn prior to the intervention is initiated.
2. If a patient's baseline (day 1) serum estradiol level is >10 pg/ml, the subsequent levels must be >10 pg/ml higher than the baseline value to be considered a significant elevation outside the post-menopausal range.

Study Design

Up to 80 patients will be randomized to receive either testosterone or ESTRING so that 35 evaluable patients will receive each treatment. Randomization will attempt to balance the number of patients in each group based on self reported or medically documented depression, use of antidepressants, relationship status (single vs. partnered). Estradiol will be measured at baseline, at 4 weeks, and at 12 weeks into the study. If a patient is subsequently found to have a serum estradiol level outside of the post-menopausal range on day 1 of the study before the intervention begins (despite having a post-menopausal value during screening labs), this patient will be allowed to stay on study, and the subsequent estradiol level must be >10 pg/ml higher then the baseline value to be considered a significant elevation outside the post-menopausal range. If a patient has an estradiol level outside of the post-menopausal range on the week 4 draw, or if there is a >10 pg/ml increase in estradiol levels in patients with a baseline value above the post-menopausal range as described previously, they will have an additional lab draw at week 8.

Statistical methods

The primary outcome variable for determining safety at the end of the study is serial serum estradiol measurement. The test statistic is the number of patients that have a persistently elevated estradiol level above the post-menopausal range as described above. In the medical literature, there are no data to suggest what is "safe" or "unsafe" in terms of postmenopausal breast cancer patients being exposed to levels of estrogen above the postmenopausal range for a short duration of time (such as 12 weeks). No data exist to suggest that short term exposure to levels of estrogen above the post-menopausal range for up to 12 weeks would impact breast cancer recurrences. Therefore, it is fairly arbitrary to conclusively state that a certain rate of patients experiencing a short term increase in estradiol exposure with either of the treatment interventions (ESTRING or testosterone cream) is safe or unsafe. Taking this into consideration and after consulting with a number of breast cancer experts, we will consider each intervention "safe" if no more than 25% of patients in each arm have persistently elevated serum estradiol levels above the postmenopausal range after exposure to either of the study interventions.

The trial is designed to test the null hypothesis that a treatment is unsafe (25% or more of patients have a persistently elevated estradiol above the postmenopausal range) at a 5% level of significance. The alternate hypothesis is that a treatment is safe (i.e. fewer than 25% of patients have a persistently elevated estradiol above the postmenopausal range). Each treatment arm is tested separately. The trial is designed to have 98% power of concluding that a treatment is safe if the true failure rate is 5% and 70% power when the true failure rate is 10%.

Secondary Outcomes

A secondary outcome variable is the change in scores on the CARES Sexual Dysfunction Subscale from baseline to week 12. The ESTRING and testosterone cream treatment groups will be analyzed independently. A paired t-statistic will be used to compare week 12 to baseline values. With 35 patients in each treatment group (ESTRING and testosterone cream), we will have 90% power to detect an effect size of 0.41 based on a two-sided paired t-test at 5% level of significance.

Another secondary outcome variable will be the difference in the mean change in CARES Sexual Dysfunction Subscale from baseline to week 12 between the ESTRING and testosterone cream treatment groups. Using a two-sample t-test, and with 35 patients in each treatment group, we would have 90% power to detect a difference of 0.57 in the change scores (e.g. a reduction of 0.1 in one group vs. a reduction of 0.67 in the other group). Currently, there is no published research to suggest an expected effect size for these interventions. However, after carrying out this trial we will have estimates for the reduction for each treatment type and could use these estimates to plan a larger study to determine if one intervention is actually superior to the other.

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1. INTRODUCTION

1.1 Background

Aromatase Inhibitors in Breast Cancer

Approximately 211,300 new cases of breast cancer are diagnosed per year in the United States. Many breast cancer patients receive multi-modality therapy including surgery, chemotherapy and hormonal therapy. Hormonal therapy options for post menopausal women with early breast cancer include tamoxifen and aromatase inhibitors. Aromatase inhibitors are a class of compounds that act systematically to reduce the synthesis of estradiol from androgens in peripheral tissues. These compounds prevent estrogen biosynthesis by inhibiting the enzyme aromatase, which catalyses the conversion of adrenal androgens (androstenedione and testosterone) to estrogens (estrone and estradiol). Consequent reductions in estrogen levels have been shown to achieve objective responses in subjects with advanced breast cancer (1-3) and reductions in the risk of recurrence or disease progression in subjects with early breast cancer with hormone-receptor-positive disease (4).

Aromatase inhibitors have been shown to be an effective in reducing the risk of both local and distant recurrences in women with early breast cancer, and are approved as first line adjuvant hormonal therapy in post-menopausal women (5), as adjuvant hormonal therapy after 2-3 years of tamoxifen (6), and as extended adjuvant hormonal therapy in postmenopausal women who have completed five years of tamoxifen (7).

Sexual Dysfunction in Breast Cancer Patients

In the context of improved survival that has been observed in breast cancer patients over the last decade, it is important to address the long-term effects of breast cancer treatments on quality of life. There is a growing body of scientific literature to suggest that sexual functioning is one of the most distressing problems experienced by breast cancer survivors. One study of breast cancer patients who had surgery one year previously and had completed chemotherapy and radiation therapy found that 64% percent of the women experienced an absence of sexual desire, 38% had dyspareunia, and 42% lubrication problems (8). A study of 864 breast cancer survivors showed that scores on a 36 item general health survey were as good or better than those of healthy, age-matched women, and the frequency of depression was similar to general population samples. Marital/partner adjustment was similar to normal healthy samples, and sexual functioning mirrored that of healthy, age-matched postmenopausal women. However, sexual dysfunction occurred more frequently in women who had received chemotherapy, and in younger women who were no longer menstruating (9). In a much smaller study, the level of relationship distress among breast cancer survivors was the most significant variable affecting arousal, orgasm, lubrication, satisfaction, and sexual pain. This study also found that depression was an important determinant of lower sexual desire, and survivors on antidepressants had higher levels of arousal and orgasm dysfunction. Women who were older had significantly more concerns about vaginal lubrication and pain (10).

Estrogen Deprivation and Urogenital Atrophy in Breast Cancer Patients

Although many factors contribute to sexual dysfunction, a number of studies have shown that vaginal dryness is one of the most important predictors of sexual functioning for women with a history of breast cancer (11, 12). In the setting of estrogen deprivation, the mucosal and stromal tissues of the vagina, urethra, and trigone of the bladder undergo atrophy, resulting in decreased tissue elasticity and fluid secretion. This may lead to symptomatic vaginal dryness and irritation as well as dyspareunia. Estrogen deprivation also leads to an elevation in vaginal pH which may increase the risk of urinary tract infections.

While aromatase inhibitors are generally well tolerated, the incidence of vaginal dryness, dyspareunia, and loss of sexual interest in women taking this class of drugs is significant and particularly bothersome in women who have become post-menopausal rapidly due to systemic chemotherapy (13). These symptoms have a significant impact on quality of life and may lead to discontinuation of the drug in some patients. However, little has been published on the management of vaginal symptoms and sexual dysfunction related to hormonal therapy treatments for breast cancer.

Management of Urogenital Atrophy in Postmenopausal Women

Review of the literature on management of vaginal symptoms in post-menopausal women identifies several therapies that appear to be effective in improving or eliminating vaginal dryness and decreasing dyspareunia. Non-hormonal vaginal moisturizers have been shown to relieve urogenital symptoms, but are not as effective as vaginal estrogens in randomized trials (14). A multicenter, open-label, randomized, parallel-group study compared the efficacy and safety of 25-microgram 17beta-estradiol vaginal tablets (Vagifem) with 1.25-mg conjugated equine estrogen vaginal cream (Premarin Vaginal Cream) for the relief of atrophic vaginitis in 159 post-menopausal women (15). Both treatments provided equivalent relief of the symptoms of atrophic vaginitis based on composite scores of vaginal symptoms (dryness, soreness, and irritation). At weeks 2, 12, and 24, increases in serum estradiol concentrations and suppression of follicle-stimulating hormone were observed in significantly more patients who were using the vaginal cream than in those who were using the vaginal tablets ($p < 0.001$). Vaginal tablet therapy resulted in greater patient acceptance and lower withdrawal rates compared with vaginal cream therapy. In a double-blind placebo-controlled, 1612 post-menopausal patients with urogenital complaints were randomized to receive the Vagifem insert or placebo tablet once a day over a period of 2 weeks, and then twice a week for a total of the 12 months (16). The overall success rates of Vagifem vs. placebo on subjective and objective symptoms of vaginal atrophy were 85.5%, and 41.4%, respectively. A significant improvement of urinary atrophy symptoms was also seen in the Vagifem treated group as compared with the beginning of the study (51.9% vs. 15.5%, P=0.001). Therapy with the Vagifem insert did not raise serum estrogen level nor stimulated endometrial growth in this study.

An estradiol releasing vaginal ring (Estring) has been compared to a topical estriol cream (Synapause) in a 12 week treatment study in postmenopausal women. The Estring was found to be well tolerated, produced equivalent results in reducing vaginal symptoms, and was preferred by patients as less messy and easier to use (17). Clinical trials with Estring have shown that there is minimal systemic absorption of estradiol and the range of serum estradiol

levels measured at various time points fell within the post-menopausal range, making this an attractive option for women with a history of hormone receptor positive breast cancer (18).

A recent prospective randomized study compared the Estring to the Vagifem insert for relief of estrogen deficiency symptoms in post menopausal women over a period of 12 months (19). The primary endpoint was endometrial safety, based on the results of ultrasound measurement of endometrial thickness and a progestogen challenge test at baseline and week 48. Efficacy was determined by subjective assessment of urogenital estrogen deficiency symptoms and assessment of signs of vaginal epithelial atrophy by the clinician. There was no statistical difference between the groups in the alleviation of symptoms and signs of urogenital estrogen deficiency, and after 48 weeks of treatment, there was no statistically significant difference in endometrial thickness between the two groups. A statistically smaller proportion of bleeding/spotting occurred in the Estring group (n = 0) compared to the Vagifem users (n = 4). Estradiol and total estrone serum levels increased during treatment in both groups but remained within the normal postmenopausal range.

Safety of Vaginal Estrogen Preparations in Breast Cancer Patients

While the intravaginal estrogen preparations (creams, tablet inserts, and rings) are all reasonably effective in many post-menopausal patients, the level of systemic estrogen absorption is variable. One small study has raised concerns about a rapid rise in serum estradiol levels with the use of the Vagifem insert in post-menopausal breast cancer patients (20). Serum estradiol, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were followed in seven postmenopausal women using the Vagifem insert while being treated with aromatase inhibitors for breast cancer. Serum estradiol levels, as measured by an assay specifically developed for measuring low levels in postmenopausal women, rose from baseline levels </=5 pmol/l to a mean 72 pmol/l at 2 weeks into Vagifem treatment. By 4 weeks estradiol levels had decreased to <35 pmol/l in the majority (median 16 pmol/l) although two women continued to have further rises in estradiol levels. The authors concluded that Vagifem significantly raises systemic estradiol levels, at least early in the course of treatment for vaginal atrophy, and that in their opinion, this reversal of estradiol suppression is contraindicated in women being treated with aromatase inhibitors for breast cancer. It should be noted, however, that the impact of a very transient and unsustained rise in serum estradiol levels on the risk of recurrence in hormone receptor positive breast cancer is unknown. Furthermore, no studies have evaluated serum estradiol levels in post-menopausal breast cancer patients using the ESTRING for relief of urogenital symptoms. It is likely that due to its slow release mechanism, the levels of absorbed estrogens would be much lower than a rapidly dissolving tablet like the Vagifem insert.

Relationship Between Testosterone, Urogenital Symptoms and Sexual Function

In humans, the adrenal glands and the ovaries represent the main sources of circulating androgens in women. The adrenal steroid dehydroepiandrosterone (DHEA) represents the crucial precursor of human sex steroid biosynthesis. DHEA and its sulfate ester (DHEAS) are the most abundant steroids in the human circulation. DHEA, DHEAS and androstenedione do not have androgenic activity unless they are converted to testosterone and DHT, which can both bind and activate the androgen receptor. Testosterone can be converted either to dihydrotestosterone (DHT) or it can be aromatized towards estrogens. Therefore, an increase

in the circulating testosterone pool may be associated with increased estrogen generation within peripheral target tissues of sex steroid action. DHEA of adrenal origin may still be converted to testosterone within the postmenopausal ovary. A large cross-sectional study found no evidence of a significant decrease in circulating androgens during the menopause transition (21). While naturally occurring menopause does not appear to affect circulating androgen levels, oophorectomy and premature ovarian failure (such as that seen after chemotherapy for breast cancer), may lead to androgen deficiency (22, 23).

The issue of whether circulating androgen levels are associated with decrease sexual desire and arousability is hotly debated. A recently published cross-sectional study in a large cohort of Australian women (n = 1423) found no significant correlation of circulating androgen levels with self-reported perception of sexual desire and sexual satisfaction (24). Another large cohort study found that higher circulating testosterone levels were only minimally associated with increased sexual desire (25). The currently available data suggest that women with significant, near-total depletion of androgens (such as following a bilateral oophorectomy or with adrenal insufficiency) and concurrent complaints of impaired well-being and libido are the most likely to benefit from androgen replacement therapy (26). Beneficial effects on libido and mood has been reported in studies on testosterone replacement in surgically menopausal women (27-31). One of the most well conducted studies in this field evaluated 75 women who had undergone oophorectomy. Patients received conjugated equine estrogens (at least 0.625 mg per day orally) and then placebo, 150 micrograms of testosterone, and 300 micrograms of testosterone per day transdermally for 12 weeks each (32). The higher testosterone dose resulted in significant increases in scores for frequency of sexual activity and pleasure-orgasm compared to placebo. However, in almost all of these studies, androgens were administered concurrently with estrogen replacement, and in most patients, testosterone administration resulted in supraphysiological serum androgen concentrations. The impact of testosterone replacement in an estrogen depleted state has not been adequately addressed. Little is known about the effect of testosterone replacement in women with breast cancer, and nothing has been published documenting how effective it might be for relief of urogenital symptoms in women in an estrogen deprived state such as that created by aromatase inhibition.

Quality of Life Measurements in Breast Cancer Patients Receiving Adjuvant Therapy

As outcomes continue to improve in women with breast cancer, there has been an increasing interest in long term survivorship issues and the impact of adjuvant therapies on quality of life in breast cancer patients. A number of quality of life questionnaires have been tested and validated in breast cancer patients, including the FACT-B (Functional Assessment of Cancer-Breast) (33), and several have been developed that focus specifically on the endocrine, gynecologic, and sexual components of quality of life. These questionnaires include the FACT-ES (Functional Assessment of Cancer – Endocrine Subscale), and the CARES (Cancer Rehabilitation Evaluation System) Sexual Subscales. Using these questionnaires, and other survey tools, numerous studies have documented that adjuvant chemotherapy and hormonal therapies for breast cancer result in decreases in quality of life, particularly in relation to vasomotor symptoms and sexual functioning (8, 9, 11, 34, 35).

Side effects and quality of life issues associated with tamoxifen therapy have been well-described (36, 37). More recently, several large randomized trials of adjuvant hormonal

therapy have incorporated quality of life sub-studies, and the findings of these studies have been very informative in counselling patients on the significance of side effects, and in assisting in decision making about treatment and supportive care needs.

A quality of life subprotocol was conducted among 1,021 women participating in the Arimidex, Tamoxifen, Alone or in Combination (ATAC) Adjuvant Breast Cancer Trial during the first 2 years of treatment (13). Patients completed the Functional Assessment of Cancer Therapy-Breast (FACT-B) plus endocrine subscale (ES) at baseline and 3, 6, 12, 18, and 24 months, or until disease recurrence. Although endocrine symptoms increased between baseline and 3 months, overall quality of life improved for all groups during the 2-year period. Compared with patients receiving tamoxifen only, patients receiving anastrozole only reported more vaginal dryness, painful intercourse, and loss of sexual interest. A similar quality of life analysis was conducted among 582 patients in the Intergroup Exemestane Study (38). While quality of life was generally good and stable over 2 years, prevalence of severe endocrine symptoms at trial entry was high for vasomotor complaints and sexual problems, and these symptoms persisted for both the tamoxifen and exemestane treated patients throughout the study.

1.2 Rationale for this Study

As described above, vaginal dryness, dyspareunia, and decreased libido are common complaints among breast cancer patients treated in our clinic. With increasing use of aromatase inhibitors which are associated with a higher rate of vaginal dryness than tamoxifen, these problems are becoming even more prominent. Medical oncologists at UCSF do not recommend the use of oral or vaginal cream forms of estrogen replacement therapy in breast cancer patients. However, given the minimal estrogen absorption that has been documented with treatments like the ESTRING, breast cancer physicians at UCSF support the use of certain preparations of intravaginal estrogen for breast cancer patients suffering from severe vaginal dryness or sexual dysfunction resulting in significant decreases in quality-of-life. Based on available evidence, we generally recommend the ESTRING **or** a specially compounded 1% testosterone cream as an initial intervention for urovaginal symptoms. Since loss of libido is increasingly common in this patient population, testosterone cream may offer some benefit in addition to relief from vaginal dryness, although the actual amount of systemic absorption of vaginal testosterone cream is unknown. If patients do not have sufficient symptomatic relief with the initial intervention, patients are then prescribed the alternate treatment.

In severe cases when both these treatments have failed, patients may be offered the Vagifem insert, and their serum estradiol levels are followed carefully to assure that they remain in the postmenopausal range. In the context of recent studies demonstrating elevations in serum estradiol levels to outside the post-menopausal range within two weeks after the initial treatment with the Vagifem insert, patients are counselled that the significance of this transient elevation of estradiol is unknown. Patients are advised that ongoing systemic estrogen absorption is believed to be low with both the ESTRING and the Vagifem insert, but there are no data on the routine use of these treatments in breast cancer patients and how they may impact breast cancer recurrence.

This study will evaluate the safety and tolerability of the ESTRING and 1% testosterone cream administered vaginally as treatments for vaginal dryness and/or decreased libido in women receiving an aromatase inhibitor for Stage I-III breast cancer. This study will serially measure serum estradiol and testosterone levels in breast cancer patients being treated the ESTRING and 1% testosterone cream. The primary objective is to evaluate the safety of these treatments by determining whether an unacceptable number of patients receiving either of these treatments are found to have serum estradiol level outside of the post-menopausal range using standard clinical laboratory measurements. This study will also explore whether either of these treatments are effective in improving gynecologic or sexual quality of life in this patient population. This study may answer a number of important questions regarding these therapies which have not been well-investigated. If either of the treatments is found to be effective, and their short term safety and tolerability are confirmed in this study, further examination into the management of the difficult and increasing problem of urogenital atrophy and decreased libido in breast cancer survivors will be encouraged. Furthermore, improvements in the management of side effects associated with aromatase inhibitor treatment should lead to improved patient compliance, decreased discontinuation of treatment, and better outcomes.

2. STUDY OBJECTIVES

2.1 Primary Objective

To evaluate the safety, based on serial measurements of serum estradiol levels, of intravaginal testosterone cream or the ESTRING administered for relief of vaginal dryness and/or decreased libido related to aromatase inhibitor therapy in early breast cancer patients.

2.2 Secondary Objectives

1. To document the systemic estradiol and testosterone levels at several time points in early breast cancer patients on aromatase inhibitors and being treated with intravaginal testosterone cream or the ESTRING.
2. To compare standard clinical laboratory measurements of serum estradiol with values of serum estradiol as measured by an ultrasenstive assay in a research laboratory.
3. To evaluate changes in gynecologic and sexual quality of life using validated questionnaires (the CARES Sexuality Subscales) in early stage breast cancer patients using intravaginal testosterone cream or the ESTRING for relief of vaginal dryness and/or decreased libido related to aromatase inhibitor therapy.

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design

This will be a single institution open label randomized Phase II study evaluating intravaginal testosterone cream vs. the Estring for vaginal dryness and/or decreased libido in early breast cancer patients treated with an aromatase inhibitor. Up to 80 early stage breast cancer patients will be enrolled over 36-48 months in order to have a total of 70 evaluable patients. To be considered evaluable, patients must complete their baseline evaluation and week 4 safety blood draw. Randomization of patients will attempt to balance the number of patients in each group based on self reported or medically documented depression, use of antidepressants, and relationship status (single vs. partnered). Patients will be randomized to a 12 week course of treatment with either:

- Testosterone Cream 1% micronized in velvachol - 0.5 gm of cream vaginally 3 times a week for total of 12 weeks of treatment
- Estring 2mg ring inserted vaginally once every 12 weeks

Patients will complete quality of life surveys including the CARES Sexual Interest and Sexual Dysfunction Subscales (Appendix A), which includes a single item sexual satisfaction measure previously tested by Ganz, at day 1 (baseline), week 4, and at the end of week 12 (the survey may be done either in the clinic, or at home and then mailed/faxed). Patients will undergo a gynecologic exam at baseline and at the end of week 12 (or sooner if they withdraw from the study or experience unexpected side effects). During this exam, the vaginal epithelium will be assessed using a 3-point scale evaluating color, petechiae, friability and dryness (Appendix B). Within each treatment group, composite scores of the vaginal epithelium at baseline will be compared to composite scores at study termination.

Serum estradiol assays sent to the UCSF clinical laboratory are sent out to Quest Diagnostics, which uses extraction chromatography RIA for their ultra-sensitive estradiol. Day 1 and week 4 samples will also be stored temporarily and then sent in batches to a specialized research lab which has developed an assay to quantify low levels of estradiol found in post-menopausal women. This assay is a radioimmunoassay after ether extraction, and has a sensitivity limit of 3pmol/l. Serum testosterone levels will also be sent to Quest, which uses an ultrasensitive testosterone measurement which is run by liquid chromatography tandem mass spectrometry.

The primary outcome variable in this study is the number of breast cancer patients being treated with either intravaginal testosterone cream or the ESTRING for a 12 week period who are found to have a persistently elevated serum estradiol level outside the post-menopausal range as defined by:

1. An elevation of serum estradiol outside of the post-menopausal range and at least 10 pg/ml higher than the baseline value on two consecutive collections at least four weeks apart. The post-menopausal range at UCSF is ≤10 pg/ml (as measured by Quest Diagnostics, the lab utilized by the UCSF clinical laboratory).

2. If a patient's baseline (day 1) serum estradiol level is >10 pg/ml, the subsequent levels must be >10 pg/ml higher than the baseline value to be considered a significant elevation outside the post-menopausal range.

A secondary outcome variable is the change in scores on the CARES Sexual Dysfunction Subscale from baseline to week 12. The ESTRING and testosterone cream treatment groups will be analyzed independently.

3.2 Study plan

Visit	Screening/Baseline	Week 4[a,b]	Week 8-10[c]	Week 12[a]	Week 16-18[c]	Early Withdrawal
Day	**-28 to 1**	**23-33**		**79-89**		
Sign Informed Consent	X					
Medical History	X					
Inclusion /exclusion criteria	X					
Gynecologic Exam[d]	X			X[e]		X
Physical Exam[f]	X					
ECOG status	X					
Concurrent Medication	X			X		X
Chemistry/Hematology[g]	X					
Serum Hormone Measurements[h]	X	X	X	X	X	X
Completion of QOL questionnaires	X	X		X		X
Dispense study medication	X[i]					
Adverse Events		X		X		X

a. May occur ± 5 days from day 28 (week 4) or day 84 (week 12).
b. Clinic visit is optional.
c. Visit is only for patients with abnormal estradiol levels at week 4 or week 12. Test does not include testosterone levels. [If the week 4 or week 12 estradiol level is outside the postmenopausal range (or if there is a >10 pg/ml increase in estradiol levels in patients with an estradiol level above the post-menopausal range at baseline), a repeat sample will be sent within 4-6 weeks. Therefore, estradiol will be repeated by week 10 for a patient with an elevation at week 4, and by week 18 for a patient with an elevation at week 12.]
d. The physician's assessment of the vaginal epithelium will include a 3-point scale to evaluate color, petechiae, friability and dryness.
e. Performed sooner if patient drops out or experiences unexpected side effects
f. Limited exam consisting of cardiac and lung auscultation and abdominal exam only
g. Includes CBC with differential, AST, alkaline phosphatase, total bilirubin. Abnormal results will be followed up at the investigator's discretion as per standard of care.
h. Includes estradiol levels for all patients and serum testosterone levels only for patients randomized to the testosterone cream arm.
i. Must be done on Day 1.

3.3 Selection of study population

3.3.1 Study selection record

Patients receiving an aromatase inhibitor for early breast cancer who have complaints of vaginal dryness, dyspareunia, or decreased libido will be invited to participate. For this study, decreased libido is defined as a patient having decreased interest in sexual intercourse, decreased sexual arousal, or inability to achieve an orgasm. Ongoing logs will be kept to document the number of patients who declined to participate or who were found to be ineligible for the study during the screening process.

3.3.2 Inclusion criteria

1. Patients with histologically or cytologically confirmed Stage I-III breast cancer who are taking an aromatase inhibitor as adjuvant hormonal therapy. Patient must have started the aromatase inhibitor at least 30 days prior to enrolling.
2. Postmenopausal status as defined by:

   - History of bilateral oophorectomy

   OR

   - No menses for 12 months

   A. If patient is amenorrheic due to ongoing use of a GnRH agonist, the patient must have a documented serum estradiol level in the post-menopausal range while on an aromatase inhibitor at some time in the past AND the serum estradiol level must be <30 pg/ml during the screening period.
   B. If a pre-menopausal patient was rendered amenorrheic by adjuvant chemotherapy within the past 2 years, patient must have a documented serum estradiol level in the post-menopausal range while on an aromatase inhibitor at some time in the past AND the serum estradiol level must be <30 pg/ml during the screening period.
3. Age ≥18 and ≤80 years old.
4. Patient must have recovered from the side effects of previous chemotherapy, surgery, or radiation therapy for early breast cancer.
5. Patient must be able to comprehend and give written informed consent.
6. Ability to read English and complete quality of life questionnaires.

3.3.3 Exclusion criteria

1. History of radiation to the vaginal area
2. Concurrent treatment with any type of oral, injectable or topical form of estrogen or androgen therapy including natural supplements marketed as hormone replacement products
3. Initiation of topical moisturizers (for example, Replens), or herbal or alternative medicines to manage the symptoms of vaginal dryness while on study. Patients

who were previously using these products may continue them with the same usage pattern while on study.

4. Use of vaginal hormonal products (rings, inserts, creams, gels) containing estrogens or androgens within the past 30 days
5. History of an abnormal pap smear within the last 12 months
6. History of endometrial or ovarian cancer
7. Any episode of vaginal bleeding in the past 6 months that has not been evaluated by a gynecological exam and/or pelvic ultrasound
8. History of sexual dysfunction prior to diagnosis of breast cancer (Sexual dysfunction will be defined as loss of libido or inability to achieve orgasm for which patient sought medical attention or which patient felt significantly interfered with quality of life.)
9. Moderate or severe depression for which the patient is receiving ongoing psychological counseling and/or taking antidepressants, **and** for whom, in the investigators opinion may be interfering in the patients sexual function independent of the side effects of breast cancer and aromatase inhibitor use.
10. Use of any investigational agent for breast cancer within 3 weeks of study entry.
11. No liver disfunction or history of an arterial thromboembolic event or venous thrombosis.

3.3.4 Criteria for Discontinuation

Treatment may be discontinued if subject experiences significant vaginal irritation or other side effect thought by the investigators to be related to either of the interventions.

This will be a two stage study. In stage 1, ten patients are enrolled in each arm. If 3 or more of the first 10 patients in an arm fail (i.e. have a "persistently elevated estradiol level" as defined in Section 3.1 Study Design), no more patients are enrolled in that arm and it is concluded that the treatment for that arm is not safe. Patients already enrolled in that arm will be notified and given the option of discontinuing treatment. In stage 2 an additional 25 patients are enrolled in the arms that were not closed after stage 1. A treatment is considered unsafe if a total of 5 or more patients in both stages have failed (i.e. have a "persistently elevated estradiol level" as defined above). Under the null hypothesis that the treatment is unsafe with true probability of failure (i.e. persistent elevation of estradiol above postmenopausal threshold) equal to 25% or greater, the probability of rejecting the null hypothesis falsely (i.e. concluding the treatment is safe when it is not) is 5%. The probability is 98% of concluding that the trial is safe when the true probability of failure is 5% and is 70% when the true probability of failure is 10%.

3.3.5 Procedures for discontinuation

If study treatment is prematurely discontinued prior to the 12 weeks of treatment, patients will be asked to come in for a study termination visit, and the procedures and assessments as described for week 12 in section 3.2 "Study Plan" will be performed.

3.3.6 Replacement Policy

Patients who do not initiate study assigned therapy after signing consent or do not complete the week 4 blood draw for safety evaluation will be considered unevaluable and will be replaced.

4. TREATMENTS

4.1 ESTRING

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. ESTRING has the following dimensions: outer diameter 55 mm; cross-sectional diameter 9 mm; core diameter 2 mm. When placed in the vagina, ESTRING releases estradiol, approximately 7.5 µg/24 hours, in a consistent stable manner over 90 days. Estradiol is chemically described as estra-1,3,5(10)-triene-3,17ß-diol. The molecular formula of estradiol is $C_{18}H_{24}O_2$, and the molecular weight of estradiol is 272.39.

One ESTRING should be inserted into the upper third of the vaginal vault, to be worn continuously for three months.

4.1.1 Pharmacokinetics

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 possibly reducing the induction of several other hepatic proteins. In a Phase I study of 14 postmenopausal women, the insertion of ESTRING (estradiol vaginal ring) rapidly increased serum estradiol (E2) levels attesting to the rapid absorption of estradiol via the vaginal mucosa. 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% lower Cmax, apparently due to reduced systemic absorption via the revitalized vaginal epithelium. The relative systemic exposure from the initial peak of ESTRING accounted for approximately 4% of the total estradiol exposure over the 12 week period.

TABLE 1: PHARMACOKINETIC MEAN ESTIMATES FOLLOWING ESTRING APPLICATION

Estrogen	Cmax (pg/mL)	Css-48 hr (pg/mL)	Css-4w (pg/mL)	Css-12w (pg/mL)
Estradiol (E_2)	63.2[a]	11.2	9.5	8.0
Baseline-adjusted E_2	55.6	3.6	2.0	0.4
Estrone (E_1)	66.3	52.5	43.8	47.0
Baseline-adjusted E_1	20.0	6.2	-2.4	0.8

[a] n=14

The constant and stable release of estradiol from ESTRING was demonstrated in a Phase II study of 166 - 222 post-menopausal women who inserted up to four rings consecutively at three month intervals. Low dose 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. Lower systemic exposure to estradiol and estrone is further supported by serum levels measured during a pivotal Phase III study. In post-menopausal women, mean dose of estradiol systemically absorbed unchanged from ESTRING is ~ 8% [95% CI: 2.8-12.8%] of the daily amount released locally.

4.1.2 Pharmacodynamics

In-vivo, estrogens diffuse through cell membranes, distribute throughout the cell, bind to and activate the estrogen receptors, thereby eliciting their biological effects. Estrogen receptors have been identified in tissues of the reproductive tract, breast, pituitary, hypothalamus, liver and bone of women. ESTRING delivers estradiol constantly at a mean rate of ~7.5 μg/24 hours for a period of up to 90 days. Its use in post-menopausal patients in Phase I and II studies showed no apparent effects on systemic levels of hepatic protein SHBG, or FSH. Lowering of the pretreatment vaginal pH from a mean of 6.0 to a mean of 4.6 (as found in fertile women) over the 12 to 48 week treatment period, and improvements evident in the vaginal mucosal epithelium seen in all studies attest to the local dynamic effects of estrogens.

4.1.3 Special Populations

- **Postmenopausal Women** - Estrogens have been reported to increase the risk of endometrial carcinoma in post menopausal women. Adequate diagnostic measures, including endometrial sampling when indicated, should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding.
- **Pregnancy** - There is no indication for estrogen therapy during pregnancy or during immediate postpartum period. Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the fetus, and possibly other birth defects. Studies of women who received diethylstilbestrol (DES) during pregnancy have shown that female offspring have an increased risk of vaginal adenosis, squamous cell dysplasia of the uterine cervix, and clear cell vaginal cancer later in life; male offspring have an increased risk of urogenital abnormalities and possibly testicular cancer later in life. The 1985 DES Task Force concluded that the use of DES during pregnancy is associated with a subsequent increased risk of breast cancer in the mothers, although a causal relationship remains unproven and the observed level of excess risk is similar to that for a number of other breast cancer risk factors.
- **Breast cancer -** While the majority of studies have not shown an increased risk of breast cancer in women who have ever used estrogen replacement therapy, some have reported a moderately increased risk (relative risks of 1.3 to 2.0) in those taking higher doses or those taking lower doses for prolonged periods of time, especially in excess of ten years. The package for ESTRING states that estrogens

in general are contraindicated in women with a known or suspected carcinoma of the breast or other types of estrogen dependent malignancies.

4.1.4 Geriatric Use

Of the total number of subjects in clinical studies of ESTRING (including subjects treated with ESTRING, placebo, and comparator drug; n=951), 25% were 65 and over, while 4% were 75 and over. No overall differences in safety or effectiveness were observed between these subjects and younger subjects, and other reported clinical experience has not identified differences in responses between the elderly and younger patients, but greater sensitivity of some older individuals cannot be ruled out.

4.1.5 Drug-Drug Interactions

Drug-drug and drug-laboratory interactions have been reported with estrogen administration overall, but were not observed in clinical trials with ESTRING. However, the possibility of the following interactions should be considered when
treating patients with ESTRING:

1. Accelerated prothrombin time, partial thromboplastin time, and platelet aggregation time; increased platelet count; increased factors II, VII antigen, VIII antigen, VIII coagulant activity, IX, X, XII, VII-X complex, II-VII-X complex, and betathromboglobulin; decreased levels of anti-factor Xa and antithrombin III, decreased antithrombin III activity; increased levels of fibrinogen and fibrinogen activity; increased plasminogen antigen and activity.
2. Increased plasma HDL and HDL-2 subfraction concentrations, reduced LDL cholesterol concentration, increased triglycerides levels.

4.1.6 How Supplied

Each ESTRING (estradiol vaginal ring) is individually packaged in a heat-sealed rectangular pouch consisting of three layers, from outside to inside: polyester, aluminum foil, and low density polyethylene, respectively.

NDC 0013-2150-36 ESTRING (estradiol vaginal ring) 2 mg - available in single packs.

4.1.7 Storage

ESTRING should be stored at controlled room temperature 15° to 30° C (59° to 86° F) per recommendation of the package insert.

4.2 Testosterone cream 1% micronized in velvachol

There are a number of different esters of testosterone, including the commonly prescribed injectables of testosterone enanthate and testosterone cypionate, as well other esters such as acetate, propionate, phenylpropionate, isocaproate, caproate, decanoate, and undecanoate. Esterification of testosterone is done in order to improve the solubility of testosterone in oil,

which in turn slows the release of the testosterone from the site at which it enters the body. A number of formulations of androgen replacement have been tested in men, including injections, oral supplementation (either testosterone undecanoate, methyltestosterone, oxandrolone, or DHEA), transdermal testosterone patches, testosterone creams or gels, and subcutaneous testosterone implants. Based on a number of studies, the transdermal route (either patch, cream, or gel) appears to have the most favorable pharmacokinetic properties of available testosterone preparations tested in women.

TESTOSTERONE, MICRONIZED, USP (T1006)

Synonymns: 4-Androsten-17beta-ol-3-one; 17beta-Hydroxy-4-androsten-3-one
($C_{19}H_{28}O_2$: F.W. 288.42)

Testosterone cream will be produced by the UCSF Drug Product Services. The pharmacy has provided us with details of the preparation of the testosterone cream as follows:

The appropriate amount of pure testosterone chemical is weighed to produce a 1% concentration. The powder is levigated with mineral oil, and then this mixture is brought to the desired volume with velvachol.

Velvachol is a water-miscible compounding vehicle in a non-sticky, non-greasy, odorless base that is compatible with a wide variety of chemicals used in topical therapy. Velvachol is stable and neutral in reaction, and washes easily from the hair, skin and clothes. It is physically compatible with acids, bases, strong electrolytes and many other medications commonly applied to the skin. Velvachol is formulated in a hydro-philic emulsion-type base.

4.2.1 Testosterone Cream Absorption and Pharmacokinetics

Extrapolating from the fact that systemic absorption of estrogens is robust after the topical administration of estrogen creams vaginally, absorption of testosterone cream through the vaginal mucosa would be expected to be significant as well, but the pharmacokinetics have not been well described.

4.2.2 Testosterone Cream Storage

The product is dispensed in a plastic opaque tube with an applicator for vaginal administration. The product is stored at room temperature. The expected shelf life is 6 months.

4.3 Doses and treatment regimens

- Testosterone Cream 1% micronized in velvachol - 0.5 gm of cream vaginally 3 times a week for total of 12 weeks of treatment
- ESTRING 2mg ring inserted vaginally once every 12 weeks

4.4 Mechanism of drug storage and destruction

Study drugs will be obtained as a special order from the UCSF Pharmacy (in the case of the ESTRING) or from the UCSF Drug Product Services Laboratory (in the case of the testosterone cream). Due to the limited shelf life of testosterone cream, this will be prepared in

batches of a 10 patient supply. Both the ESTRING and the testosterone cream will be stored under appropriate conditions and dispensed by the investigational pharmacist at the UCSF Mt. Zion Infusion Center Pharmacy. When patients have completed their 12 weeks of study treatment, they will be advised to remove and discard the ESTRING. For patients on the testosterone cream arm, patients will be advised to discard any remaining study drug, or if they choose, to continue using the remaining cream until it gone. Therefore, documentation indicating study drug was destroyed is not applicable to this study.

4.5 Pre-study, concomitant and post-study treatment(s)

Use of vaginal hormonal products (rings, inserts, creams, gels) containing estrogens or androgens within the past 90 days is not permitted.

Treatment with any type of oral, injectable or topical form of estrogen or androgen therapy including natural supplements marketed as hormone replacement products is not permitted during the 12 weeks of study treatment. Initiation or change in usage pattern of moisturizing agent, herbal or alternative medicine to manage the symptoms of vaginal dryness is not permitted during the 12 weeks of treatment.

There are no limitations on post-study treatments.

5. SAFETY MEASUREMENTS AND VARIABLES

5.1 Adverse events

5.1.1 Definitions

Adverse event

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (*e.g.,* nausea, chest pain), signs (*e.g.,* tachycardia, enlarged liver) or the abnormal results of an investigation (*e.g.,* laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

Serious adverse event

A serious adverse event is an AE occurring during any study phase (*e.g.,* run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfils one or more of the following criteria:

- results in death
- is immediately life-threatening
- requires in-patient hospitalization or prolongation of existing hospitalization

- results in persistent or significant disability or incapacity
- is a congenital abnormality or birth defect
- is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

The causality of SAEs (their relationship to study treatment) will be assessed by the investigator(s).

5.1.2 Recording of adverse events

Adverse events will be recorded at week 4 and at the end of the study (week 12 visit). For each AE, the onset, intensity, action taken, and resolution will be recorded. Efforts will be made to assign causality. All events will be evaluated to determine if they represent a SAE. Adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

5.1.3 Reporting of serious adverse events

Investigators and other site personnel will inform the FDA, via a Medwatch form, of any unexpected and possibly study drug related SAE that occurs according to the FDA reporting requirement timelines. A copy of the MedWatch report will be faxed to AstraZeneca at the time the event is reported to the FDA. The investigator will compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

A cover page will accompany the Medwatch form indicating the following:

- Arimidex Investigator Sponsored Study (ISS)
- The IND number assigned by the FDA (in this study, the IND for commercial use of ESTRING will apply)
- The investigator's name and address
- The trial name and AstraZeneca Reference number

Will be sent by way of fax to: 1-800-972-4533, Attention Arimidex ISS Safety Representative

If a non-serious AE becomes serious, this and other relevant follow-up information will also be provided to AstraZeneca and the FDA.

6. DATA MANAGEMENT

Following closure of the study, the investigator will maintain all site study records in a safe and secure location. The records will be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection) and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff.

Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must assure that all reproductions are legible and are a true and accurate copy of the original, and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable backup of these reproductions and that an acceptable quality control process exists for making these reproductions.

The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations; otherwise, the retention period will default to 15 years.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 Description of outcome variables in relation to objectives and hypotheses

The primary outcome variable for determining safety in this study is the number of breast cancer patients being treated with either intravaginal testosterone cream or the ESTRING for a 12 week period who have a persistently elevated serum estradiol level outside the post-menopausal range (as defined in section 3.1).

The test statistic is the number of patients that have a persistently elevated estradiol level above the post-menopausal range as described above. In the medical literature, there are no data to suggest what is “safe” or “unsafe” in terms of postmenopausal breast cancer patients being exposed to levels of estrogen above the postmenopausal range for a short duration of time (such as 12 weeks). No data exist to suggest that short term exposure to levels of estrogen above the post-menopausal range for up to 12 weeks would impact breast cancer recurrences. Therefore, it is fairly arbitrary to conclusively state that a certain rate of patients experiencing a short term increase in estradiol exposure with either of the treatment interventions (ESTRING or testosterone cream) is safe or unsafe. Taking this into consideration, we will consider each intervention “safe” if no more than 25% of patients in that arm have persistently elevated serum estradiol levels above the postmenopausal range after exposure to either of the study interventions.

The trial is designed to test the null hypothesis that a treatment is unsafe (25% or more of patients have a persistently elevated estradiol above the postmenopausal range) at a 5% level of significance. The alternate hypothesis is that a treatment is safe (i.e. fewer than 25% of patients have a persistently elevated estradiol above the postmenopausal range). Each treatment arm is tested separately. The trial is designed to have 98% power of concluding that a treatment is safe if the true failure rate is 5% and 70% power when the true failure rate is 10%.

7.2 Interim Analysis / Stopping Rule

This will be a two stage study. In stage 1, ten patients are enrolled in each arm. If 3 or more of the first 10 patients in an arm fail (i.e. have a "persistently elevated estradiol level" as defined in Section 3.1 Study Design), no more patients are enrolled in that arm and it is concluded that the treatment for that arm is not safe. Patients already enrolled in that arm will be notified and given the option of discontinuing treatment. In stage 2 an additional 25 patients are enrolled in the arms that were not closed after stage 1. A treatment is considered unsafe if a total of 5 or more patients in both stages have failed (i.e. have a "persistently elevated estradiol level" as defined above). Under the null hypothesis that the treatment is unsafe with true probability of failure (i.e. persistent elevation of estradiol above postmenopausal threshold) equal to 25% or greater, the probability of rejecting the null hypothesis falsely (i.e. concluding the treatment is safe when it is not) is 5%. Figure 1 shows the operating characteristic using this two stage design.

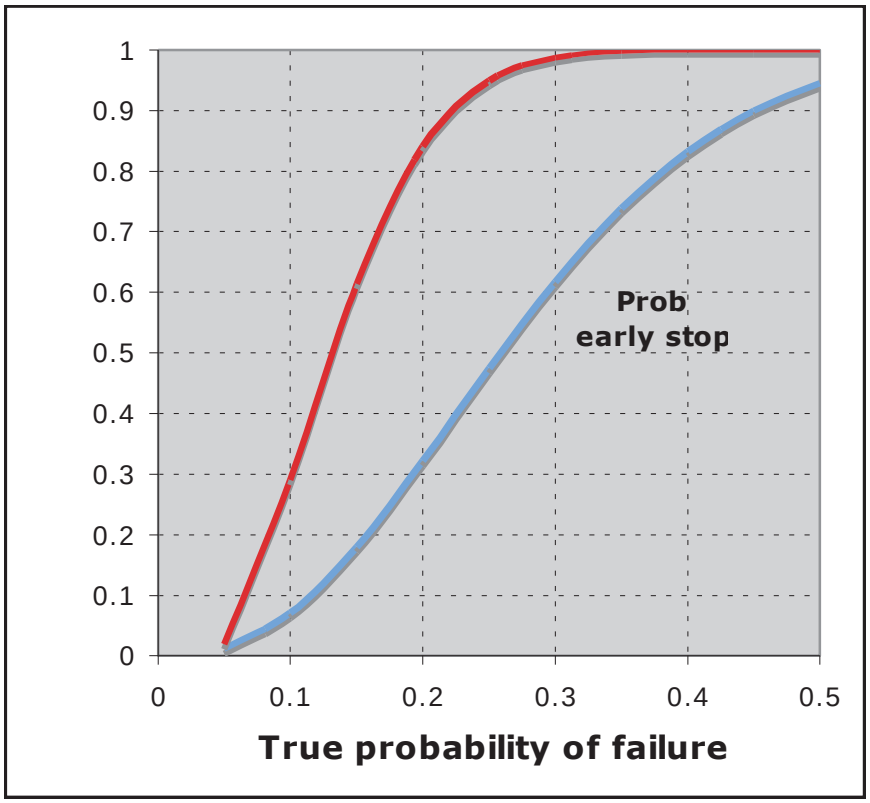

Figure 1. Operating characterisic for two-stage safety trial.

7.3 Secondary Outcomes Statistical Analysis

A secondary outcome variable in this study is the change in the scores on the CARES Sexual Dysfunction Subscale from baseline to week 12 within each patient treatment group. The ESTRING and testosterone cream treatment groups will be analyzed independently. Another secondary outcome variable will be the difference in the change in CARES Sexual Dysfunction Subscale from baseline to week 12 between the ESTRING and testosterone cream treatment groups.

Based on results from previous studies investigating menopausal symptoms and sexual dysfunction in breast cancer survivors published by Ganz (11, 39), mean scores on the CARES Sexual Dysfunction Subscale (SDS) was 1.35±1.13 for one group and 1.53±1.17 for the other group (on a scale on 0-4). Within patient correlations were not published in either of these studies, but we would expect it to be 0.8 or greater. The formula for the sd when comparing 2 correlated measurements is: sd of difference = sqrt(2*sd^2*(1 - cor)).

Based on a standard deviation of 1.17 from a study of different patients, a table for the sd(diff) as a function of the correlation is:

corr	sd(diff)
0.7	0.91
0.8	0.74
0.9	0.52

With 35 evaluable patients in each treatment group (ESTRING and testosterone cream), we will have 90% power to detect an improvement of 0.41 when the correlation is 0.8, based on a two-sided paired t-test at 5% level of significance. This translates to a reduction from 1.53 to 1.12 for treated patients.

Using a two-sample t-test, and with 35 evaluable patients in each treatment group, we would have 80% power to detect a difference of 0.57 in the change scores (e.g. a reduction of 0.1 in one group vs. a reduction of 0.67 in the other group). Currently, there is no published research to suggest an expected effect size for these interventions. However, after carrying out this trial we will have estimates for the reduction for each treatment type and could use these estimates to plan a larger study to determine if one intervention is actually superior to the other.

7.4 Analysis of serum estradiol and testosterone levels

Paired t-tests will be used to compare estradiol samples sent to the standard clinical laboratory utilized by UCSF (Quest Diagnostics) and those samples sent to a research lab in England that has reported the use of an ultrasensitive assay to detect very small changes in estradiol levels in postmenopausal women. With 35 samples we will have 90% power to detect a 0.564 sd difference between the two systems of measurements. We will also measure the correlation to assess the agreement between these two measurements. The data for one method will be plotted against the other method to determine whether the relationship between the two is linear over the range of values.

7.5 Other Statistical Analyses

Baseline characteristics (age, stage of disease, initial estradiol, etc.) will be compared between the two study arms using t-tests for continuous measures and chi-square contingency table analyses for categorical variables. To minimize the effects of multiple testing, no formal analyses of patients' patterns of response over time (baseline, 4 and 12 weeks) are planned. Data will be plotted for each patient; any apparent patterns will be noted for testing in subsequent trials.

7.6 Data and safety monitoring plan

One of the principle investigators will meet with a trial coordinator on a weekly basis to review AEs, complete reporting forms, and determine if AEs were treatment related, disease related or unknown. Adverse events and serious adverse events will be reported monthly in the UCSF Breast Cancer Site Committee. This committee consists of a minimum of three medical oncologists specializing in the care of breast cancer, a radiation oncologist, a statistician, a breast cancer surgeon and several basic scientists with expertise in cancer biology. During this meeting, an ongoing account of AEs and SAEs will be reviewed and any unexpected toxicities will be discussed. The group will then address whether the AEs, SAEs, or unexpected toxicities justify early closure of the study.

8. STUDY MANAGEMENT

8.1 Audits and inspections

Authorized representatives of AstraZeneca, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the centre to perform audits or inspections, including source data verification. The purpose of an AstraZeneca audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements. The investigator should contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at his or her centre.

9. ETHICS

9.1 Ethics review

The final study protocol, including the final version of the Written Informed Consent Form, will be approved by the UCSF Committee on Human Research (CHR) prior to patient enrollment. The investigator must submit written approval to AstraZeneca before he or she can enroll any subject into the study.

The principal investigator is responsible for informing the CHR of any amendment to the protocol in accordance with local requirements. In addition, the CHR must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the CHR annually. Progress reports and notifications of serious unexpected adverse drug reactions will be provided to the CHR according to local regulations and guidelines. The principal investigator is also responsible for providing the CHR with reports of any serious adverse drug reactions from any other study conducted with the investigational product. AstraZeneca will provide this information to the principal investigator.

If modifications are made to the protocol or informed consent document according to local requirements, the new version has to be approved by AstraZeneca.

9.2 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements.

9.3 Written informed consent

It is the responsibility of the investigator to ensure that each patient reads, understands, and signs a document of informed consent before the subject can participate in the study. The contents and process of obtaining informed consent should be in accordance with all applicable regulatory requirements.

The subject's signed and dated informed consent(s) must be obtained before conducting any procedure specifically for the study. The subject will receive a copy of the signed and dated form, and the original will be retained in the site's study records. It is the responsibility of the investigator or his/her designee to inform the patient that participation in the study is entirely voluntary, and that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

If modifications are made to the protocol or informed consent according to local requirements, the new version will be approved by AstraZeneca.

9.4 Subject data protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), the Written Informed Consent Form must include a subject authorization to release medical information to AstraZeneca and/or allow AstraZeneca, a regulatory authority, or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

10. EMERGENCY PROCEDURES

10.1 Procedures in case of medical emergency

This protocol does not involve treatments or interventions that are likely to result in a medical emergency, the principal investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study.

10.2 Procedures in case of pregnancy

All patients enrolled in this study will be confirmed to be post-menopausal prior to study entry, and therefore pregnancy will not be a possible event.

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Appendix A.

CARES Sexual Interest Four-Item Subscale

How much does each item apply to you in the past 4 weeks?

(0 = not at all…to….4 = very much)

I do not feel sexually attractive.
I do not think my partner(s) finds me sexually attractive.
I am not interested in having sex.
I do not think my partner(s) is interested in having sex with me.

CARES Sexual Dysfunction Four-Item Subscale

How much does each item apply to you in the past 4 weeks?

(0 = not at all…to….4 = very much)

I find that frequency of sexual intercourse has decreased.
I have difficulty becoming sexually aroused.
I have difficulty getting lubricated.
I have difficulty reaching orgasm.

Sexual Satisfaction One Item Measure

Overall, how satisfactory to you is your sexual relationship with your partner?

1= Extremely unsatisfactory
2 = Moderately unsatisfactory
3 = Slightly unsatisfactory
4 = Slightly satisfactory
5 = Moderately satisfactory
6= Extremely satisfactory

Appendix B. Description of the 3-point vaginal epithelium score

A number of vaginal changes occur with loss of estrogen. There is loss of the normal vaginal rugae and the resulatant thin epithelium often has visible blood vessels. Petechiae relates to visually apparent spontaneous hemorrhage of these blood vessels within the vaginal epithelium. Friability relates to thin dry vaginal tissues that bleed easily to the touch. The color changes from pinkish to a pale white. Loss of lubrication occurs and the epithelium appears dry. A number of studies evaluating vaginal atrophy have used 3 -5 point scales to assess vaginal health indices. These assessments are not typically validated but allow for objective measurements by a clinician. We have chosen a common 3 point scale to assess the degree of atrophy with numeric values of 0 for none, 1 for mild and 2 for moderate or severe changes. Composite scores at baseline will then be compared to those at study termination.

Descriptive Assessment of Vaginal Mucosa

	Vaginal atrophy score			
Signs of atrophy	None*	Mild	Moderate	Severe
Rugae	Normal number and depth	Reduced rugae	Rare rugae	Smooth vagina
Pallor	Normal pink	Light pink	Very pale	White or deep red
Petechiae	None	Bleeds on scraping	Bleeds on contact	Clearly seen
Mucosal thinning (elasticity)	Normal	Decreased	None	Stenosis
Dryness	Normal lubrication	Slightly decreased	Minimal lubrication	Dry

* Normal subject.

References of studies that have used similar 3-5 point vaginal assessment scales.

Eisberg et al. Endometrial and Vaginal Effects of low-dose estradiol delivered by vaginal ring or vaginal tablet. Climacteric 2005;8:83-92

Bachmann GA, Notelovitz M, Kelly SJ, et al. Long-term non-hormonal treatment of vaginal dryness. Clin Pract Sexuality 1992; 8:3-8

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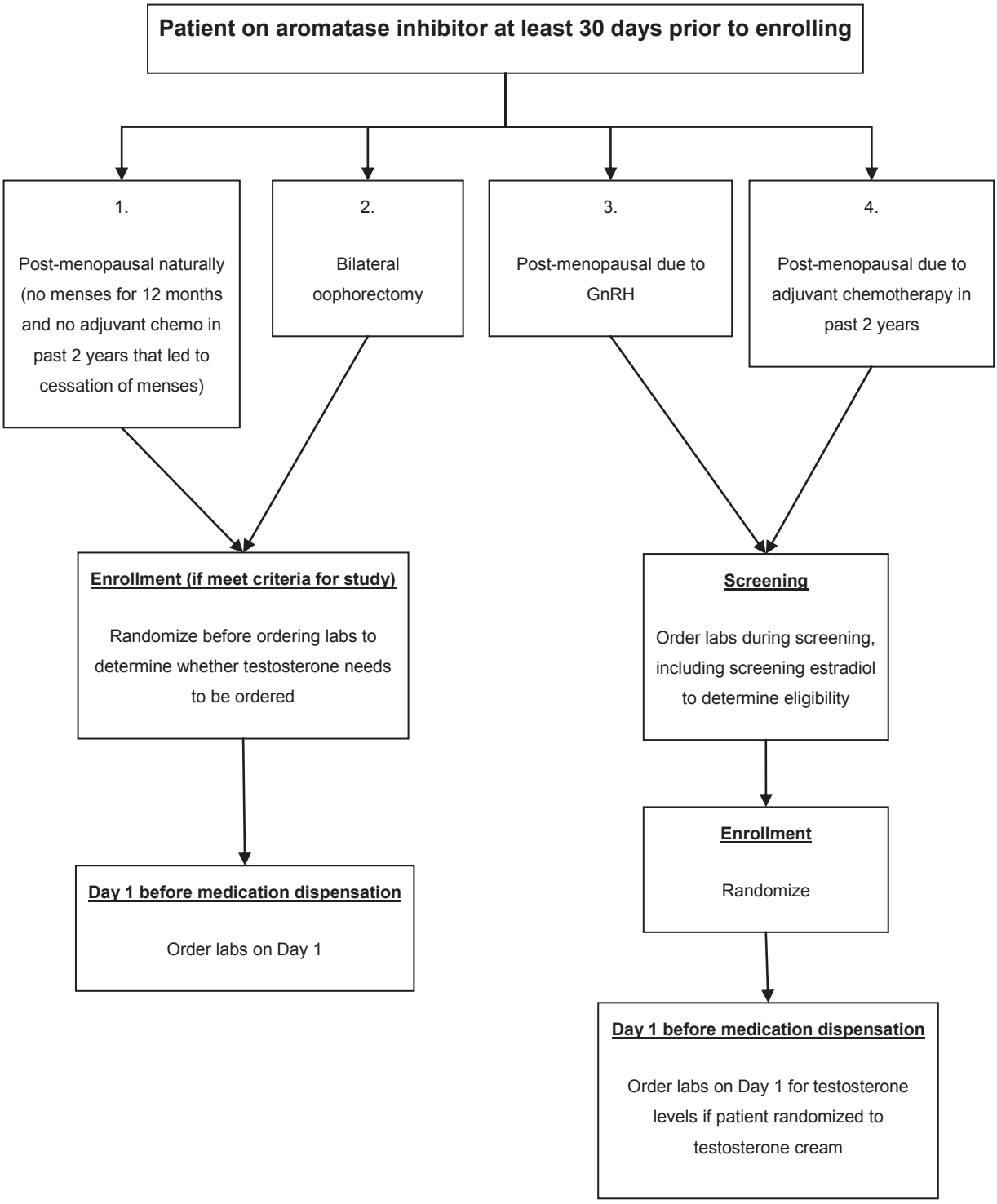
Appendix C.


Patient on aromatase inhibitor at least 30 days prior to enrolling
1.
Post-menopausal naturally (no menses for 12 months and no adjuvant chemo in past 2 years that led to cessation of menses)
2.
Bilateral oophorectomy
3.
Post-menopausal due to GnRH
4.
Post-menopausal due to adjuvant chemotherapy in past 2 years
Enrollment (if meet criteria for study)
Randomize before ordering labs to determine whether testosterone needs to be ordered
Screening
Order labs during screening, including screening estradiol to determine eligibility
Day 1 before medication dispensation
Order labs on Day 1
Enrollment
Randomize
Day 1 before medication dispensation
Order labs on Day 1 for testosterone levels if patient randomized to testosterone cream

APPENDIX D.: Blood Specimen Collection and Handling Instructions for Research serum estradiol assay

At baseline and Week 4, one sample will be collected for standard hormone testing at UCSF or Quest Diagnostic® labs (routine lab test), and a second sample will be collected for a specialized low-level estradiol assay (research lab test).

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- The lab slip should specify that an extra sample of blood is to be collected for the study.
- At least 3-4 ml of blood is needed to create the research serum sample that will be sent for low-level estradiol assay.
- The study personnel responsible for obtaining the research sample from the lab should label the red top vacutainer tube with the patient's identification (patient number and patient initials), protocol number (067519), date and time of blood draw (dd-MMM-yyyy format for the date (i.e. 01-JAN-2003) and 24:00 hour clock format for the time).
- The **research lab sample** will be collected by the lab technician in a red top vacutainer at the time the standard hormone blood sample is collected using standard venipuncture techniques.

Serum Collection Tube:

1. 4-6 mL red top vacutainer tube

Specimen Transfer Tube:

1. Non-sterile polypropylene specimen tube (cryovial) with screw cap.
2. Freezer resistant specimen label to fit the cryovial.

Serum Collection and Shipping Instructions

1. Draw 3-4 mL of blood (yielding ≥0.5 mL of serum) into a red top vacutainer tube at each designated specimen timepoint using standard venipuncture techniques.
2. After collection, the study personnel will take the sample to the centrifuge.
3. Allow the blood to clot upright at room temperature for 30 minutes.
4. Centrifuge the sample for approximately 15 minutes.
5. Completely fill out the label with the corresponding patient's identification (patient ID number and patient initials), protocol number (067519), date and time of blood draw (dd-MMM-yyyy format for the date (i.e. 01-JAN-2003) and 24:00 hour clock format for the time) and affix it to one of the room temperature polypropylene specimen tubes.
    - A freezer resistant label should be used so that water or freezing temperatures do not destroy the label since samples must remain frozen until shipment

6. Draw off the supernatant and pipette 0.5mL – 1.5 mL of the serum into the properly labeled polypropylene specimen tube.
7. Freeze the samples at or below -80°C until ready for shipment. The freezer location is: Tissue Core freezer #5441 in room N155 located at 2340 Sutter St., San Francisco, first floor.

Shipping Serum Samples

1. Research personnel will follow the "*UCSF Guide to Shipping with Dry Ice*" to package and ship the samples internationally using dry ice. Instructions are found on the UCSF Office of Environmental Health and Safety website: http://www.ehs.ucsf.edu/Manuals/SDIM/SDIM.pdf

   An inventory list all of the samples being sent must be included in each shipment. A copy of the shipping list should remain at the site. Prior to shipment, send email to Dr. Liz Folkerd at Elizabeth.Folkerd@icr.ac.uk alerting her to the impending shipment of the samples, and attach an electronic copy of the inventory list of samples being sent.

2. To prevent damage to the frozen samples, care should be taken to ship the samples on a Monday, or Tuesday AND avoid shipping one or two days prior to a public holiday either at the country of origin or the country of destination.

3. The shipping address is:
   Dr Liz Folkerd
   The Academic Department of Biochemistry
   The Royal Marsden NHS Trust
   Fulham Road
   London
   SW3 6JJ
   UK