Serum Estradiol Levels In Postmenopausal Women with Breast Cancer Receiving Adjuvant Aromatase Inhibitors and Vaginal Estrogen

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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IRB Protocol

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Duration: 24 weeks

Background: Atrophic vaginitis is a significant complaint in 40% of postmenopausal women. While non-hormonal therapies are available for the treatment of many of the sequelae of menopause, estrogen has been shown to be the most effective treatment option for menopause induced urogenital atrophy. However, systemic estrogen has traditionally been contraindicated in women with breast cancer or other estrogen dependent cancers because it may stimulate any remaining microscopic foci of cancer or be a factor in the occurrence of a second primary. Therapies directed at reducing breast cancer risk usually act by blocking estrogen. In recent years, local vaginal estrogen with known minimal absorption has emerged as a possible treatment option for such patients. Anecdotal evidence suggests that breast cancer patients have positive experiences with vaginal 17-β estradiol, which is offered to them only after failing other local therapies such as lubricants.

Aromatase inhibitors (AI's) block the peripheral conversion of androgens to estrogen, resulting in sub-physiologic levels of estrogen that may lead to more profound urogenital atrophy. Original studies evaluating vaginal 17-β estradiol in postmenopausal women found only a transient increase in estradiol levels with return to baseline within 12 weeks. However, a recent report (n=6) performed by Kendall et al. demonstrated elevation in estradiol levels in 5 out of 6 women on aromatase inhibitors after two weeks of treatment with vaginal 17-β estradiol. After twelve weeks of therapy, estradiol levels decreased, but returned to pre-treatment levels in only 2 out of 6 women. The small sample size renders it difficult to reach definitive conclusions, but it does underscore the need to evaluate concurrent use of AI's and vaginal 17-β estradiol.

Study Objective:
The primary specific aim is to determine the change in estradiol and FSH from baseline to twelve weeks in postmenopausal women receiving adjuvant aromatase inhibitors during treatment with low dose vaginal 10 μg 17- β estradiol. A secondary specific aim is to compare the rise in estradiol in women on letrozole vs. anastrozole during treatment with low dose vaginal 10 μg 17- β estradiol to see if there are differences between aromatase inhibitors. Another secondary specific aim is to describe patterns of estradiol and FSH levels over the twenty-four week study period. A third secondary aim is to compare the patient's FSFI score at baseline, weeks 12 and 24.

Patient Population: 60 postmenopausal breast cancer patients who have completed their primary treatment for breast cancer with the exception of endocrine therapy who are currently being treated with adjuvant aromatase inhibitors with no evidence of disease. 60 women are being recruited with the goal of obtaining 48 evaluable patients.

Study Design: This study is a prospective longitudinal pilot study measuring serum estradiol and follicle stimulating hormone (FSH) levels in 60 postmenopausal breast cancer patients currently receiving treatment with adjuvant aromatase inhibitors (30 being treated with letrozole and 30 with anastrozole) who are initiated on vaginal estrogen for symptoms of urogenital atrophy. After a patient elects to participate and provides informed consent, a blood sample will be obtained to measure baseline estradiol and FSH levels. Patients will then receive a prescription

for vaginal 10 µg 17- β estradiol. We hypothesize that low dose vaginal 17-β estradiol is a potentially safe and effective intervention, which does not cause an elevation in serum estradiol levels at 12 weeks in postmenopausal breast cancer patients on aromatase inhibitor therapy. In addition to studying the primary endpoint of estradiol levels, we will also study FSH, and FSFI/menopause symptom checklist as secondary endpoints.

All subjects will receive vaginal 10 µg 17- β estradiol tablets. The primary endpoint will be change in estradiol and FSH from baseline to 12 weeks. Each subject will serve as their own control. Serum estradiol and follicle stimulating hormone (FSH) will be monitored closely from 0-24 weeks. Estradiol and FSH levels will be measured at baseline, and subsequently at weeks 2, 7, 12, 18 and 24 after commencing therapy with vaginal 10 µg 17- β estradiol. The bloodwork will take less than one hour to perform and will be drawn +/-72 hours from the date the patient is due for bloodwork. Baseline bloodwork will be drawn within 14 days of study enrollment prior to the initiation of vaginal 10 µg 17- β estradiol. Patients will be encouraged to come for their research bloodwork 12-24 hours after the insertion of the vaginal 10 µg 17- β estradiol. Patients will record in their diary the time of their last vaginal 10 µg 17- β estradiol tablet insertion. Along with the laboratory measurements of estradiol and FSH, the FSFI and a menopause symptom checklist will be administered at baseline, and the 12 and 24 week time points +/-72 hours**.** The baseline FSFI and menopause symptom checklist will be completed within 14 days of study enrollment prior to the initiation of vaginal 10 µg 17- β estradiol. The FSFI will take approximately 10 minutes to complete and the menopause quality of life check list questionnaire takes approximately 3 minutes to complete.

Both the patients and the investigators will be blinded to the results of the estradiol and FSH assays for the 24 weeks of study therapy. Results will be unblinded after accrual is complete and all patients have completed the 24 weeks of planned therapy. At week 24, patients can choose to remain on vaginal 10 µg 17- β estradiol or discontinue the treatment. Patient's estradiol levels may continue to be monitored for the duration of their treatment with vaginal 10 µg 17- β estradiol at her physician's discretion and depending on the findings of this study. We will continue to collect this data if the patient is being followed at MSKCC. Patients can choose to remain on it for as long as they desire.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

SPECIFIC AIMS:

Primary Specific Aim:

- To determine the change in estradiol and FSH from baseline to twelve weeks in postmenopausal women receiving adjuvant aromatase inhibitors during treatment with low dose vaginal 10 µg 17- β estradiol.

Secondary Specific Aims:

- To compare the rise in estradiol in women on letrozole vs. anastrozole during treatment with low dose vaginal 10 µg 17- β estradiol to see if there are differences between aromatase inhibitors.
- To describe patterns of estradiol and FSH levels over the twenty-four week study period.

- To compare the patient's FSFI scores and menopause quality of life questionnaire at baseline, week 12 and week 24

3.0 BACKGROUND AND RATIONALE

Many commonly used treatments for breast cancer, including adjuvant chemotherapy and endocrine therapy, either induce menopause or increase the frequency of urogenital complaints including vaginal dryness, irritation, pruritus, dyspareunia, urinary frequency and urinary incontinence. Aromatase inhibitors (AIs) are used to treat postmenopausal patients with both early stage and metastatic disease. AIs block the peripheral conversion of androgens to estrogen, resulting in sub-physiologic levels of estrogen that may lead to more profound urogenital atrophy. Estrogen has been shown to be the most effective agent for treatment of menopause induced urogenital atrophy. However, breast cancer patients are discouraged from using systemic estrogens because they may reduce the effectiveness of adjuvant hormonal therapy and may increase the risk of disease recurrence or new primaries. Hormone replacement therapy (HRT) has been shown to increase recurrence rates in patients with hormone sensitive breast cancer.[1] Vaginal creams or suppositories are considered and often given to these women with breast cancer, but the safety of topical estrogens in this population is unknown.

Low dose vaginal 17-β estradiol was shown to be highly effective in treating menopausal urogenital atrophy in women without cancer.[2] Vaginal 17-β estradiol was designed to relieve symptoms of urogenital hypoestrogenism with minimal systemic absorption and improved ease of usage compared to other vaginal estrogen formulations. In a study comparing 17-β estradiol to vaginal estrogen cream, both methods were equivalent for relief of atrophic vaginitis symptoms, but patients using vaginal 17-β estradiol had less systemic estrogen absorption, less endometrial proliferation and greater overall satisfaction.[3] In another study, vaginal 17-β estradiol caused vaginal maturation, which gradually diminished absorption and systemic "spill over" of estradiol.[4] In postmenopausal women after fourteen days of treatment, maturation of the vaginal epithelium occurred and absorption of estradiol subsequently declined significantly. FSH levels were unchanged during treatment with the 17-β estradiol. When systemic estradiol levels increase, FSH levels decrease correspondingly. Given the results of these studies, it was felt vaginal 17-β estradiol was efficacious and safe even for women trying to avoid estrogens. Recently a lower dose, 10 mcg 17-β estradiol, was FDA approved for postmenopausal women and is now readily available.

Despite the prevalence of urogenital symptoms in women with breast cancer, treatments have not been well studied in this population and are limited to over the counter lubricants, moisturizers, and dilator use with minimal impact. Vaginal 17-β estradiol was initially considered to be efficacious and safe even for women trying to avoid estrogens, and became widely prescribed to patients with breast cancer including women on endocrine therapy. Studies evaluating vaginal 17-β estradiol in postmenopausal women without breast cancer found only a transient increase in estradiol levels with return to baseline within twelve weeks. However, a recent prospective small observational study (n=6) performed by Kendall et al. demonstrated elevation in estradiol levels in 5 out of 6 women on aromatase inhibitors after two weeks of treatment with 25 mcg 17-β estradiol.[5] After twelve weeks of therapy, estradiol levels decreased, but returned to pre-treatment levels in only 2 out of 6 women, which calls into question the safety of low dose 17-β estradiol in women with breast cancer. . The concern is that using low-dose vaginal estrogens

may negate or diminish the effect of AIs. However, the small sample size renders it difficult to reach definitive conclusions, but it does underscore the need to perform a rigorous, adequately sized clinical trial to evaluate concurrent use of aromatase inhibitors and low dose 17-β estradiol. While it is clear that lowering estradiol levels as much as possible is ideal in preventing recurrences, the absolute estradiol level to minimize risk is unknown.

While the study suggests that this transient increase in estradiol may be of concern in this population, there is no clear evidence that a transient rise in estradiol causes any detrimental effects in patients with breast cancer. How much, if any, of these topical estrogens are absorbed through the mucous membranes of the vagina into the circulation is not known and the impact of low dose estradiol absorption on breast cancer outcomes is also unknown. Furthermore, the absorption will wane as the mucus membranes regenerate under estrogen exposure, which explains the return to baseline after 12 weeks noted in general population studies. In addition, since the vaginal mucosa of women using AI's has been even more depleted of estrogen exposure, it intuitively makes sense that a return to baseline will take longer. Therefore, this study will follow women for 24 weeks to see if longer therapy impacts estradiol level and systemic absorption. Finally, it is not known if the absorption of 17-β estradiol is different in the breast cancer population. It is therefore critical that we obtain further information regarding the safety of 17-β estradiol, an apparently efficacious treatment, in patients with breast cancer being treated with AIs. Since the risk/benefit of 17-β estradiol has not been rigorously evaluated and the lower 10mcg is now available, it was felt that evaluating the lowest possible dose for efficacy is the logical first step. We hypothesize that the systemic absorption of estrogen might be less with the 10mcg 17-β estradiol than with the 25mcg 17-β estradiol.

The third generation AIs (anastrozole, letrozole, exemestane, vorozole) are the most potent and specific AIs, leading to improved tolerability and efficacy compared to prior generation AIs[6]. However, there appears to be some variation between the third generation AIs. Clinical data shows that letrozole more effectively suppresses all plasma estrogen fractions, including estradiol, when compared to anastrozole. Geisler et al. showed that with respect to both tissue estrogen and plasma estrogen, letrozole consistently suppressed the estradiol level below the levels recorded for anastrozole[7]. The average percentage of suppression for plasma estradiol during treatment with letrozole was 95.2% vs 92.8% with anastrozole, which was statistically significant with a p=0.018. However, in an open randomized trial of second line endocrine therapy in patients with advanced breast cancer, there was no statistically significant difference in the median time to progression in patients treated with letrozole vs anastrozole[8]. Although letrozole did have a statistically significant superior overall response rate 19.1% vs 12.3% with anastrozole with a p=0.013. Therefore, this study will also compare changes in serum estradiol levels in women with breast cancer being treated with letrozole vs anastrozole during treatment with vaginal 17-β estradiol. It is unclear if it will take a longer time for estradiol levels to normalize in patients on letrozole given the more effective suppression. Patients who are currently on exemestane will be excluded from this study because metabolites of exemestane interfere with the estradiol assay causing elevations in estradiol measurements.

AIs are a commonly used endocrine therapy that causes vaginal dryness.[6] One component of sexual functioning, vaginal dryness, is an important predictor of sexual health in this population.[9] Sexual dysfunction is a side effect of both chemotherapy and endocrine therapies, causing a

substantial burden on women with breast cancer.[10-11] Sexual functioning affects quality of life in women during their treatment and survivorship.[12] The Female Sexual Function Index (FSFI) is a validated instrument, which is an overall measure of female sexual dysfunction.[13] It is based on six domains: desire, arousal, lubrication, orgasm, satisfaction and pain. In the study demonstrating validity and reliability of the FSFI, an analysis of sensitivity and specificity yielded a cut score of 26.55 for the identification of women with sexual dysfunction[14] Therefore, we will report the prevalence of sexual dysfunction in this population as the percentage of women with FSFI scores less than or equal to 26.

The menopause SCL checklist is an instrument devised by the National Surgical Adjuvant Breast Project for the use in the P-1 and P-2 breast cancer chemoprevention trials.[15] It contains 36 items querying a range of physical and psychological symptoms, as well as symptoms associated with estrogen deprivation. This instrument was successfully administered as part of a baseline QOL assessment to over 9700 participants in the NSABP P1 trial. Estrogen levels may influence mood and quality of life. It takes approximately 3 minutes to complete.

Atrophic vaginitis in breast cancer survivors is prevalent and its management is complex. It is therefore critical that we obtain further information regarding the safety of 17-β estradiol, an apparently efficacious treatment, in patients with breast cancer treated with AIs. This pilot intervention study of 10 µg 17-β estradiol will inform future investigations. A 10 µg 17-β estradiol tablet was recently FDA approved and is now commercially available. The 10mcg vaginal 17-β estradiol dose has never been studied in women with breast cancer. This will be the first study evaluating 10mcg vaginal 17-β estradiol in women with breast cancer. In postmenopausal women without breast cancer, a randomized controlled trial was performed comparing the efficacy of 25mcg vaginal 17-β estradiol vs. 10mcg vaginal 17-β estradiol vs. placebo.[16] Both the 10mcg and 25mcg vaginal 17-β estradiol were effective in the treatment of atrophic vaginitis. However, there was greater improvement and relief of vaginal symptoms and more systemic absorption with the 25mcg vaginal 17-β estradiol. Depending on the results from this study, our next study may be a randomized trial evaluating the 10µg vs 25µg dose of vaginal 17-β estradiol.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This study is a prospective longitudinal pilot study measuring serum estradiol levels in 60 breast cancer patients in order to obtain 48 evaluable patients currently receiving treatments with adjuvant aromatase inhibitors. The goal is to have 30 patients on letrozole and 30 on anastrozole who are initiated on vaginal estrogen for symptoms of urogenital atrophy. Eligible women (see eligibility criteria section 6.0) seen in either breast medicine or gynecology clinic at MSKCC will be counseled on the use of low dose vaginal estrogens for symptomatic relief. A member of the clinical research staff will invite patients interested in using vaginal estrogens to participate in the study. After a patient elects to participate and provides informed consent, we will obtain serum to measure baseline estradiol and follicle stimulating hormone (FSH) levels. Patients will also complete the FSFI questionnaire. Patients will then receive a prescription for 10mcg vaginal 17-β estradiol.

All subjects will receive 10mcg vaginal 17-β estradiol tablets. The primary endpoint will be change in estradiol and FSH from baseline to 12 weeks. Each subject will serve as her own control. We will monitor serum estradiol and follicle stimulating hormone (FSH) levels closely from 0-24 weeks. We will measure serum estradiol and FSH levels at baseline, and subsequently at weeks 2, 7, 12, 18 and 24 after commencing therapy with 10mcg vaginal 17-β estradiol. The bloodwork will take less than one hour to perform and will be drawn +/- 72hrs from the date the patient is due for bloodwork. Baseline bloodwork will be drawn within 14 days of study enrollment prior to the initiation of vaginal 10 µg 17- β estradiol. Patients will be encouraged to come for their research bloodwork 12-24 hours after the insertion of the vaginal 10 µg 17- β estradiol tablets. Patients will record in their diary the time of their last vaginal 10 µg 17- β estradiol tablet insertion. Since we do not know the clinical significance of transient estradiol elevation in these patients, both the patients and the investigators will be blinded to the results of these assays for the 24 weeks of study therapy. This will also help avoid patient and investigator bias. Results will be unblinded after accrual is complete and all patients have completed the 24 weeks of planned therapy. At week 24, all patients go off study, but patients can choose to remain on vaginal 17-β estradiol or discontinue the treatment. Patient's estradiol levels may continue to be monitored for the duration of her treatment with vaginal 17-β estradiol at her physician's discretion and depending on the findings of this study. We will continue to collect this data if the patient is being followed at MSKCC. Patients can choose to remain on it for as long as they desire.

Use of conventional estradiol assays is not appropriate in this patient group due to inaccuracies in measuring serum estradiol levels <25 pmol/l. Therefore, serum estradiol levels will be measured using a more sensitive radioimmunoassay, which can accurately quantify the low levels of estradiol seen in postmenopausal women being treated with aromatase inhibitors. The assay will be done by a commercially available RIA kit from Diagnostic Systems Laboratories (Beckman Coulter Company). This test is specifically formulated as an Ultra-Sensitive estradiol assay. The assay measures estradiol in pg/ml and the assay sensitivity is 2.2 pg/ml. We will run the estradiol assays in batch mode depending on the accrual rate for the specimens. Assaying serial specimens in the same run will eliminate inter-assay variation. Specimen degradation and freeze thawing are not a problem since estradiol is a steroidal hormone and extremely stable in serum/plasma.

The FSFI (Female Sexual Function Index) is an overall measure of sexual function and is based on six domains: desire, arousal, lubrication, orgasm, satisfaction and pain.[13] The menopause SCL checklist is an instrument devised by the National Surgical Adjuvant Breast Project, which contains 36 items querying a range of physical and psychological symptoms, as well as symptoms associated with estrogen deprivation We will administer the FSFI survey questionnaire and menopause symptom checklist via either paper or wireless tablet computers in private areas of waiting rooms at the MSKCC 64th street Breast Center and gyn 53rd street clinic at baseline, week 12 and week 24 +/-72hrs from the date the patient is due for the FSFI questionnaire and menopause symptom checklist. The FSFI will take approximately 10 minutes to complete and the menopause symptom

checklist takes approximately 3 minutes to complete. The paper or computers will be brought to enrolled patients by the study RSA at the designated visit dates (baseline, week 12, week 24) +/-72hrs. The baseline FSFI/menopause symptom checklist will be completed within 14 days of study enrollment prior to the initiation of vaginal 10 μg 17-β estradiol. Each patient will have a unique, secure username and password for entering information into the online survey. The computers, survey, and online security will be overseen by the MSKCC institutional Web Survey Core, which is under the direction of Dr. Ethan Basch, who is an investigator on this protocol. The survey platform and Web Core server configuration have previously undergone MSKCC security and privacy review and approval, and the data will be stored on a secure server at the MSKCC New Jersey Data Center with capability for data transfer to CRDB. The survey will be programmed and maintained by Marwan Shouery, Senior Programmer for the Web Survey Core. The MSKCC Web Survey Core was developed for protocols like this one, which require secure, private administration of surveys, which are ideally administered electronically.

If a patient discontinues vaginal 17-β estradiol, we will gather information regarding her reason(s) for discontinuation and we will record this information in the medical record. If a patient fails to return for assessment during the defined intervals, one of the investigators will contact her and gather information regarding her reason(s) for not returning. If she is still using vaginal 17-β estradiol, we will ask her to return to have her estradiol level checked.

If at any time a patient develops recurrent disease (e.g. a new breast primary, locoregional recurrence, or distant recurrence) she will be taken off study and referred for alternative therapy. If a study participant develops recurrent disease while on Vagifem, a social worker will be available at the MSKCC breast medicine outpatient facility to debrief the patient. Should a patient develop unacceptable toxicity, defined as any Vagifem related toxicity greater than or equal to a grade three toxicity, she will also be removed from the study.

4.2 Intervention

All study participants will receive 10mcg 17-β estradiol tablets. At the initial medicine or gynecology visit, we will instruct each patient to insert one 10mcg 17-β estradiol tablet into her vagina at bedtime for two weeks as the initiation dose. After the initial two-week period ends, the dosing will decrease to one tablet vaginally two times per week for the subsequent 22 weeks for maintenance. A member of the clinical research staff will explain the proper method of treatment administration using a standardized script. Patients can also refer to the package insert during the initial visit for further clarification. Patients will also be asked to keep a medication diary. In it they will record the date and time each estrogen tablet is used. They will also record any symptoms or side effects they experience. They will be required to bring completed diaries to their appointments.

Timepoints for FSH and estradiol bloodwork and FSFI survey:

Weeks	FSH	Estradiol	FSFI/Menopause Symptom Checklist
0	X	X	X
2	X	X	
7	X	X	
12	X	X	X
18	X	X	
24	X	X	X

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

Vaginal 17-β estradiol is an FDA approved medication for the treatment of atrophic vaginitis. Each 17-β estradiol vaginal tablet contains 10 micrograms of estradiol. Vaginal administration circumvents first pass metabolism that occurs with oral estrogens, and the levels of estradiol and estrone are within postmenopausal range after a 12 week course of vaginal 17-β estradiol in postmenopausal women who do not have breast cancer.[17]

Reported adverse events (%17-β estradiol vaginal vs placebo) in a placebo controlled trial where 91 patients received 17-β estradiol vaginal and 47 patients received placebo included headache (9 vs 6), abdominal pain (7 vs 4), upper respiratory tract infection (5 vs 4), genital moniliasis (5 vs 2) and back pain (7 vs 6).[17] Adverse events (%) reported in an open label study of 80 patients included genital pruritus (6), headache (10), and upper respiratory tract infection (11). Other adverse events that occurred in less than 5% of patients included allergy, bronchitis, dyspepsia, hematuria, hot flashes, insomnia, pain, sinusitis, vaginal discomfort, and vaginitis although a causal relationship to 17-β estradiol has not been established.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

The following eligibility criteria will be used:

6.1 Subject Inclusion Criteria

- History of breast cancer, stages I-III with pathology confirmed at MSKCC
- Women who have completed all of their primary treatment (surgery, radiation therapy, adjuvant chemotherapy) with the exception of endocrine therapy and currently have no clinical evidence of disease.
- Women who are currently on aromatase inhibitors for at least three months--either letrozole or anastrozole

- Women with symptomatic urogenital atrophy: vaginal dryness, irritation, pruritus, dyspareunia, urinary frequency and/or urinary incontinence
- Menopausal at study entry defined as:
    - Bilateral salpingo-oophorectomy independent of age
    - If natural menopause, age ≥ 50 with cessation of menses for at least 12 months
    - If menopause induced by chemotherapy, age ≥ 50 with no menstrual period at least 12 months after chemotherapy finished
- At least 18 years of age
- Able to participate in the informed consent process
- Gynecology examination within six months
- Able to read/speak English.

6.2 Subject Exclusion Criteria

- Inability to give informed consent
- Vaginal bleeding of unknown etiology within 12 months of study entry
- History of prior vaginal 17-β estradiol or other topical estrogen use within the past six months

7.0 RECRUITMENT PLAN

Women at breast medicine oncology clinics and gynecology clinics at Memorial Sloan-Kettering Cancer Center (MSKCC) will be identified by their treating physicians as potential research subjects. If the investigator is a member of the treatment team, s/he will discuss the study with the patient. Otherwise the patient will be referred to an investigator.

During the initial conversation between the investigator/research staff and the patient; the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. Specifically, patients will be asked about symptoms related to urogenital atrophy. The investigator/research staff may also review portions of her medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes. If the woman agrees to study participation, a consenting individual (listed below in section 15.1) will obtain informed consent from the patient until a goal of 60 eligible patients are entered into the study in order to have 48 evaluable patients.[18]

In most cases, the initial contact with the prospective subject will be conducted either by the treatment team, investigator or the research staff working in consultation with the treatment team. The recruitment process outlined presents no more than minimal risk to the privacy of the patients who are screened and minimal PHI will be maintained as part of a screening log. For these reasons, we seek a *(partial)* limited waiver of authorization for the purposes of (1) reviewing medical records to identify potential research subjects and obtain information

relevant to the enrollment process; (2) conversing with patients regarding possible enrollment; (3) handling of PHI contained within those records and provided by the potential subjects; and (4) maintaining information in a screening log of patients approached (if applicable).

8.0 PRETREATMENT EVALUATION

After informed consent is established, subjects will have a blood sample drawn for a baseline estradiol and FSH level and complete a baseline FSFI. Baseline bloodwork and the baseline FSFI will be drawn within 14 days of study enrollment prior to the initiation of vaginal 10 µg 17- β estradiol. During the initial visit, we will perform a history and physical examination to confirm that the subject has no evidence of disease. A gynecological exam will also be perfomed if the patient's last examination was more than six months ago.

9.0 TREATMENT/INTERVENTION PLAN

At the initial medicine or gynecology visit, we will instruct patients to insert one estradiol tablet into their vagina at bedtime for two weeks and then one tablet twice weekly for to the subsequent twenty-two weeks. Treatment will begin within two weeks of the initial visit after the baseline bloodwork has been drawn and the baseline FSFI/Menopause symptom checklist is completed. A member of the clinical research staff will explain the proper method of treatment administration using a standardized script. Patients can also refer to the package insert for further clarification. We will instruct patients not to use other forms of transvaginal estrogens or vaginal moisturizers while actively participating in the study to avoid biasing of the results. Water based lubricants will be allowed.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

All subjects will receive vaginal 10 µg 17- β estradiol tablets. Each subject will serve as their own control. Serum estradiol and follicle stimulating hormone (FSH) will be monitored closely from 0-24 weeks. Serum estradiol and FSH levels will be measured at baseline, and subsequently at weeks 2, 7, 12, 18 and week 24 after commencing therapy with vaginal 10 µg 17- β estradiol. The bloodwork will take less than one hour and will be drawn +/- 72hrs from the date the patient is due for bloodwork. Baseline bloodwork will be drawn within 14 days of study enrollment prior to the initiation of vaginal 10 µg 17- β estradiol. Patients will be encouraged to come for their research bloodwork 12-24 hours after the insertion of the vaginal 10 µg 17- β estradiol tablets. Patients will record in their diary the time of their last vaginal 10 µg 17- β estradiol tablet insertion. Both the patients and the investigators will be blinded to the results of these assays for the 24 weeks of study therapy. Results will be unblinded after accrual is complete and all patients have completed the 24 weeks of planned therapy. At week 24, patients can choose to remain on vaginal 10 µg 17- β estradiol or discontinue the treatment. Patient's estradiol levels may continue to be monitored for the duration of their treatment with vaginal 10 µg 17- β estradiol at her physician's discretion and depending on the findings of this study. We will continue to collect this data. Patients can choose to remain on it for as long as they desire.

Patients will complete the FSFI survey and menopause symptom checklist at baseline, week 12 and week 24. The FSFI will take ten minutes to complete and the menopause symptom checklist

will take three minutes to complete. Both will be administered +/- 72hrs from the date the patient is due for the survey. The baseline FSFI/ menopause symptom checklist will be completed within 14 days of study enrollment prior to the initiation of vaginal 10 μg 17- β estradiol. Patients will also be asked to keep a medication diary. In it they will record the date and time each estrogen tablet is used. They will also record any symptoms or side effects they experience. They will be required to bring completed diaries to their appointments. If a patient discontinues vaginal 10 μg 17- β estradiol, information will be gathered regarding her reason(s) for discontinuation and this information will be recorded in the medical record. If a patient fails to return for assessment during the defined intervals, she will be contacted by one of the investigators and information will be gathered regarding her reason(s) for not returning. If she is still using vaginal 10 μg 17- β estradiol, she will be asked to return to have her estradiol level checked.

11.0 TOXICITIES/SIDE EFFECTS

Because serum estrogen levels with low dose vaginal 17β-estradiol are within postmenopausal range, the potential risks associated with systemic estrogen use are not expected to occur.[19] A recent double-blind, randomized study of vaginal 25 μg 17 β-estradiol vs 10μg 17 β-estradiol in postmenopausal women shows that after 12 weeks of therapy for atrophic vaginitis, women did not have accumulations of circulating estradiol.[4]

As previously stated (see section 5.0), adverse events reported with 17 β-estradiol vaginal vs placebo in a controlled trial included headache (9% vs 6%), abdominal pain (7% vs 4%), upper respiratory tract infection (5% vs 4%), genital moniliasis (5% vs 2%), and back pain (7% vs 6%).[17] In an open label study of 80 patients, adverse events included genital pruritus (6%), headache (10%), and upper respiratory tract infection (11%). Other adverse events that occurred in less than 5% of patients included allergy, bronchitis, dyspepsia, hematuria, hot flashes, insomnia, pain, sinusitis, vaginal discomfort, and vaginitis, although causal relationships cannot be established.

The benefits include the advancement of knowledge relating to the treatment of urogenital symptoms in the breast cancer patient with possible benefit for both current and future patients.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Primary Specific Aim:

- To determine the change in estradiol and FSH from baseline to twelve weeks in postmenopausal women receiving adjuvant aromatase inhibitors during treatment with low dose vaginal 10 μg 17- β estradiol.

Secondary Specific Aims:

- To compare the rise in estradiol in women on letrozole vs. anastrozole during treatment with low dose vaginal 10 μg 17- β estradiol to see if there are differences between aromatase inhibitors.
- To describe patterns of estradiol and FSH levels over the twenty-four week study period.
- To compare the patient's FSFI scores at baseline, week 12 and week 24

Both FSH and estradiol levels will be measured in the clinical chemistry laboratory at MSKCC. Conventional FSH assays will be used. However, use of conventional estradiol assays is not appropriate due to inaccuracies in measuring serum estradiol levels <25 pmol/l.

The study will be called "VAG" with a unique five digit identification number for each patient (for example VAG-123456). The specimens will be anonymized and no name will be needed. Each time a specimen is received for an individual, the same identifier (for example VAG-12345) will be used. The first digit after VAG will indicate the site where the subject was consented. (1 for Gynecology at 53rd Street , 2 for Breast at 66th Street, 3 for Commack, 4 for Basking Ridge, 5 for Rockville Center, 6 for Sleepy Hollow.) Only the date and comment (for example - baseline, 2 weeks, 7 weeks, etc) will be specific for the time relative to treatment. The specimen will be sent to the lab with a paper requisition since the panel will not be ordered in OMS. The specimens will be accessioned, aliquoted, tested and the results will be available on the hospital computer that is not accessible to the clinicians running the study (results are blinded).

The FSH will be run in real time and the estradiol will be stored frozen until assay. Stability of the specimen is not a problem. Estradiol will be assayed in a batch mode and the turnaround time is to be determined – depending on the rate of accrual. The FSH will be performed on the Siemens Centaur XP (an automated immunoassay analyzer system). At present we are planning to perform the ultrasensitive estradiol by a manual RIA procedure. Use of conventional estradiol assays is not appropriate in this patient group due to inaccuracies in measuring serum estradiol levels <25 pmol/l. Therefore, serum estradiol levels will be measured using a more sensitive radioimmunoassay, which can accurately quantify the low levels of estradiol seen in postmenopausal women being treated with aromatase inhibitors. The assay will be done by a commercially available RIA kit from Diagnostic Systems Laboratories (Beckman Coulter Company). This test is specifically formulated as an Ultra-Sensitive estradiol assay. The assay measures estradiol in pg/ml and the assay sensitivity is 2.2 pg/ml. The estradiol assays will be run in batch mode depending on the accrual rate for the specimens. Assaying serial specimens in the same run will eliminate inter-assay variation. Specimen degradation and freeze thawing are not a problem since estradiol is a steroidal hormone and extremely stable in serum/plasma.

There is an automated method under development on the Beckman Access automated analyzer that is "For Research Only" and if this assay becomes available and proves reliable, it would be a better and faster alternative. However, this method is still under development and not yet proven. The required specimen is serum (speckled Red top tube). The specimen should be centrifuged and the speckled tube sent to the Lab. No refrigeration is necessary. Ann Dnistrian will be responsible for supervising the tests.

13.0 CRITERIA FOR REMOVAL FROM STUDY

- If at any time the patient develops recurrent or progressive disease (e.g. a new breast primary, loco-regional or distant recurrence) she will be taken off study and referred for conventional therapy.

- If at any time the patient develops unacceptable toxicity, defined as any Vagifem related toxicity greater than or equal to a grade three toxicity, she will be removed from study.
- If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (i.e. a change in diagnosis), the patient will be removed from the study.

14.0 BIOSTATISTICS

This is a prospective longitudinal pilot study, and the targeted patient population is postmenopausal women with breast cancer being treated with adjuvant aromatase inhibitors who are initiated on vaginal 17β-estradiol to relieve symptoms of atrophic vaginitis. These women have completed primary breast cancer treatment including surgery, radiation therapy, and chemotherapy with the exception of hormonal therapy and should have no evidence of disease. . One common symptom of breast cancer therapy is atrophic vaginitis. Recent literature has suggested that women with breast cancer who use estradiol vaginal tablets for the treatment of atrophic vaginitis have had spikes in serum estradiol. This is a potentially dangerous suggestion since breast tumors are often fueled by hormones. The published studies are observational and based on very small series of patients. The aim of this study is to prospectively evaluate this trend in a larger series of breast cancer patients who use vaginal estradiol tablets.

Serum estradiol levels and FSH levels will be assessed at baseline, and weeks 2, 7, 12, 18 and 24 during treatment with vaginal estradiol tablets. The bloodwork will take less than one hour to perform and will be drawn +/- 72hrs from the date the patient is due for bloodwork. Baseline bloodwork will be drawn within 14 days of study enrollment prior to the initiation of vaginal 10 µg 17- β estradiol. Patients will be encouraged to come for their research bloodwork 12-24 hours after the insertion of the vaginal 10 µg 17- β estradiol tablets. Patients will record in their diary the time of their last vaginal 10 µg 17- β estradiol tablet insertion. FSFI (Female Sexual Function Index) is an overall measure of sexual function and is based on six domains: desire, arousal, lubrication, orgasm, satisfaction and pain.[13] Along with the laboratory measurements of estradiol and FSH, the FSFI survey questionnaire and menopause symptom checklist will be administered at baseline, and the 12 and 24 week time points. The FSFI will take ten minutes and the menopause symptom checklist will take three miinutes to complete. Bothwill be administered +/- 72hrs from the date the patient is due for the survey. The baseline FSFI and the menopause symptom checklist will be completed within 14 days of study enrollment prior to the initiation of vaginal 10 µg 17- β estradiol.

The primary analysis will focus on analyzing the change in estradiol level from baseline to 12 weeks using a two-sided paired t-test. We plan to recruit sixty eligible women, with the expectation that 48 (80%) will complete the study and be compliant. We plan to recruit 30 women who have been on anastrozole therapy and30 women who have been on letrozole therapy. Patients who drop out due to self-withdrawal, recurrence or noncompliance will be eliminated from the primary analysis but will be examined closely. Patients are evaluable if they have at least baseline and week 12 values.

The primary analysis will be a two-sided paired t-test to evaluate whether there was a change in estradiol levels from baseline to 12 weeks. For a sample size of 48, we will be able to detect a difference of .41 or higher on a standard unit scale with 80% power and 5% type I error using a paired t-test. To translate this into the scale commonly used to report estradiol, we examine a series of 6 breast cancer patients published by Kendall et al (2006). In Kendall et al, estradiol levels ranged from close to 0 to 5 pmol/l before starting vaginal estradiol, to close to 0 to 25 at 7 weeks. Based on these data, we conservatively estimate the standard deviation of the difference to be 7 (roughly, range/4). Thus, for a sample size of 48 we will be able to detect an absolute difference in estradiol of 2.88 pmol/l with 80% power and 5% type I error.

In addition to the main analysis described above, there are several secondary analyses planned for the FSH and FSFI/menopause checklist endpoints. Two-sided paired t-tests will be performed to evaluate change in FSH levels from baseline to 12 weeks and FSFI from baseline to 12 weeks and baseline to 24 weeks. To adjust for covariates of interest, such as type of aromatase inhibitor, an ANCOVA will be fit to the data. Trends of estradiol levels, FSH levels, and FSFI/menopause symptoms over the course of the study for all available timepoints will also be examined using graphical methods and repeated measures models. The graphs will illustrate patients and drug-specific short- and long term variations in estradiol (primary endpoint), FSH, and FSFI/menopause symptoms (secondary endpoints). It is hypothesized that estradiol levels will increase until approximately week 12 and then return to baseline after the vaginal walls reepithelialize. It is also hypothesized that FSH will remain constant throughout the 24 weeks and the FSFI/some menopausal symptoms will show improvement from baseline to both 12 and 24 weeks. To bring data from all these timepoints together, we will fit a repeated measures model[20] and if needed adjust our model to allow the time-effect to be modeled nonparametrically.[21] Modeling the time-effect nonparametrically may be needed if estradiol trends over time, for example, do not have a simple parametric form such as a straight line or a quadratic curve. Model parameters and graphs of the raw data will help us identify where there are increases, decreases, or plateaus in estradiol, FSH, and FSFI over the study period. All these models can be fit in standard statistical software like SAS for Windows.

As in any prospective study, we expect missing data on a subject and item level. This is a small study, and thus, we do not plan to do any imputation. However, we will evaluate the degree of missingness in the final study by calculating attrition rates and the percent of missing values for a given variable. We will also examine the patient characteristics of the patients who have missing values. All analyses will be interpreted with missing data issues in mind.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain written informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.
All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol study team.

The data collected for this study will be entered into a secured database (Clinical Research Database, CRDB) at Memorial Sloan-Kettering Cancer Center. Source documentation will be available to support the computerized patient record.

We will administer the FSFI survey questionnaire and menopause symptom checklist via either paper or wireless tablet computers in private areas of waiting rooms at the MSKCC 64th street Breast Center or gyn 53rd street clinic at baseline, week 12 and week 24. The FSFI will take ten minutes to complete the menopausal symptom checklist will take three minutes to complete. Both will be administered +/- 72hrs from the date the patient is due for the survey. The baseline FSFI/menopausal symptom checklist will be completed within 14 days of study enrollment prior to the initiation of vaginal 10 μg 17- β estradiol. The paper/computers will be brought to enrolled patients by the study RSA at the designated visit dates (baseline, week 12, week 24) +/-72 hours. Each patient will have a unique, secure username and password for entering information into the online survey. The computers, survey, and online security will be overseen by the MSKCC institutional Web Survey Core, which is under the direction of Dr. Ethan Basch, who is an investigator on this protocol. The survey platform and Web Core server configuration have previously undergone MSKCC security and privacy review and approval, and the data will be stored on a secure server at the MSKCC New Jersey Data Center with capability for data transfer to CRDB. The survey will be programmed and maintained by Marwan Shouery, Senior Programmer for the Web Survey Core. The MSKCC Web Survey Core was developed for protocols like this one, which require secure, private administration of surveys, which are ideally administered electronically.

16.1 Quality Assurance

Registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study and potential problems will be brought to the attention of the study team for discussion and action.

Random-Sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year and more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: http://mskweb2.mskcc.org/irb/index.htm.

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA,) departmental procedures for quality control, and two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for it's level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.0 PROTECTION OF HUMAN SUBJECTS

Prior to the enrollment of each patient, the risks, benefits and objectives of the study will be reviewed with the participant, including a discussion of the possible toxicities and side effects. Financial costs and burdens of the trial will be reviewed, including a detailed discussion of the tests that will be the financial responsibility of the study and the low dose vaginal 17-ß estradiol cost, which will be the financial responsibility of the patient. Blood work measuring estradiol and follicle stimulating hormone level at baseline and subsequently weeks 2, 7, 12, 18 and 24 will be provided at no cost to the patient. Every effort will be made to keep study records private. Neither the patient's name nor anything else that could identify the patient will be used in any reports or publications that result from this study. Trained staff at Memorial Hospital and the Institutional Review Board at Memorial Hospital may review medical records if necessary. The patient may terminate participation in the study at any time during the trial.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use

and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org containing the following information:

Fields populated from the CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following information:
  - A explanation of how the AE was handled
  - A description of the subject's condition
  - Indication if the subject remains on the study
  - If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

- The nature and objectives, potential risks and benefits of the intended study.

- The length of study and likely follow-up required
- Alternatives to the proposed study. (This will include standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
- The name of the investigator(s) responsible for the protocol.
- The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information.

In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 Appendix

Appendix A: FSFI Survey

Appendix B: Patient Diary

Appendix C: Patient Instructions

Appendix D: Patient Calendar

Appendix E:Menopause Symptom Checklist