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Protocol #: STUDY 00002440

PROTOCOL TITLE

Pilot Study of the Effect of Duavee® on Benign Breast Tissue Proliferation in Peri or Post-Menopausal Women at Moderate Risk for Development of Breast Cancer

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IND: **FDA IND Exempt (10 April 2015)**

Agent(s)/Supplier: Duavee® (Bazedoxifene/conjugated estrogens) provided by Pfizer/Wyeth Pharmaceuticals, Inc.

Protocol Version:	**Revision or Amendment #**	**Date**	**Approval**
	Version 1.0c	24 June 2016	7 July 2016
	Version 1.0b	25 April 2016	4 May 2016
	Version 1.0a	4 March 2016	10 March 2016
	Version 1.0	6 July 2015	10 July 2015

SCHEMA

Pilot Study of the Effect of Duavee® on Benign Breast Tissue Proliferation
in Peri or Post-menopausal Women
at Moderate risk for Development of Breast Cancer

Population

Peri- or post-menopausal women ≤65 with a uterus and BMI < 36 kg/m^2 who are not taking systemic hormone replacement therapy, who exhibit vasomotor symptoms, and who are at moderate risk of developing breast cancer based on any of the following: 1st or 2nd degree relative with breast cancer, estimated mammographic breast density 25% or higher , prior biopsy showing proliferative breast disease, multiple prior biopsies, or by having either a 5-year risk Gail Risk (as calculated by the NCI Breast Cancer Risk Assessment Tool) or 10-year risk by the IBIS Breast Cancer Risk Evaluation Tool of 2X or more that of the average for the age group.

Women at very high risk such as those with a BRCA1/2 mutation or LCIS are **not** eligible.

Screening/Eligibility

Normal mammogram within 6 months of aspiration
If performed at KUMC, then eligible for optional volumetric (Volpara) and area (Cumulus) assessment of density
RPFNA yielding >500 cells on the Ki-67 and/or cytology slide;
Ki-67 ≤4% positivity (500 or more cells)

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Baseline Study Visit

Fasting blood draw to archive for serum SHBG, FSH, estradiol, testosterone, progesterone (progesterone for peri-menopausal only), chemistry profile, cytokine panel, and bazedoxifene levels. If peri-menopausal, negative pregnancy test required
DEXA Scan for bone density and body composition; Medical history and physical; Height/weight
29 item validated Menopause Quality of Life Intervention Questionnaire and hot flash assessment

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Total of 40 participants will receive 6-8 months of open-label Duavee® one tablet daily

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End of Study (6-8 months)

Brief physical, height/weight,
29 item validated Menopause Quality of Life Intervention Questionnaire and hot flash assessment. Study Agent Stopped; Study Agent Returned; Compliance Calculated

Procedures

Mammogram for volumetric and area density (if baseline at KUMC); DEXA; RPFNA
Fasting blood draw to archive for serum SHBG, FSH estradiol, testosterone, progesterone (peri-menopausal only), chemistry profile, cytokine panel, and bazedoxifene levels

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LIST OF ABBREVIATIONS

ADH	Atypical Ductal Hyperplasia
AE	Adverse Event
AH	Atypical Hyperplasia
AI	Aromatase Inhibitor
AUC	Area Under the Curve
BMD	Bone Mineral Density
BMI	Body Mass Index
CBC	Complete Blood Count
CBE	Clinical Breast Exam
CRF	Case Report Form
CSA	Clinical Supply Agreement
CTA	Clinical Trials Agreement
CTCAE	Common Terminology Criteria for Adverse Events
DARF	Drug Accountability Record Form
DCF	Data Collection Form
DCIS	Ductal Carcinoma In Situ
DEXA	Dual-Energy X-ray Absorptiometry
DSMC	Data Safety and Monitoring Committee
EGF	Epithelial Growth Factor
EGFR	Epithelial Growth Factor Receptor
EH	Epithelial Hyperplasia
ELISA	Enzyme-Linked Immunosorbent Assays
ER	Estrogen Receptor
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
HRT	Hormone Replacement Therapy
HSC	Human Subjects Committee
IGF-1	Insulin-like Growth Factor-1
IGFBP-3	Insulin-like Growth Factor Binding Protein - 3
IGFR	Insulin-like Growth Factor Receptor
IRB	Institutional Review Board
KUMC	University of Kansas Medical Center
LCIS	Lobular Carcinoma In Situ
LDL	Low Density Lipid
LH	Luteinizing Hormone
NCI	National Cancer Institute
NSAID	Non-Steroidal Anti-Inflammatory Drug
PgR	Progesterone Receptor
PI	Principal Investigator
PID	Participant Identification number
QNS	Quantity Not Sufficient
qRT-PCR	Quantitative Real Time – Polymerase Chain Reaction
RIA	Radioimmunoassay
RPFNA	Random Periareolar Fine Needle Aspiration
SAE	Serious Adverse Event
SERM	Selective Estrogen Receptor Modulator
SHBG	Sex Hormone Binding Globulin

1. OBJECTIVES

Duavee® is tissue specific estrogen complex of bazedoxifene plus conjugated estrogen which is FDA approved for relief of menopausal symptoms and prevention of osteoporosis in women with a uterus who have not been diagnosed with estrogen dependent neoplasia. The overall purpose of this research is to demonstrate in a preliminary fashion that despite reduction in menopausal symptoms, (Duavee®) does not increase and may decrease proliferation in benign breast tissue in a cohort of peri- or post-menopausal women at moderately increased risk for breast cancer. If this pilot shows rapid accrual, good retention, and lack of significant increase in the risk biomarker Ki-67 in benign breast tissue, a larger prevention trial is envisioned in the Southwest Oncology Group

1.1 Primary Feasibility Objective

To determine feasibility for a larger clinical trial in women at moderately increased risk we will:
1.1.1 Determine if accrual is brisk as demonstrated by accrual of at least 20 moderate risk women in the initial 6 months (3.5 entrants per month) in a single institution;
1.1.2 Determine if there is a 15% or lower dropout rate over the 6 month intervention; and
1.1.3 Determine if 20% or fewer women with paired RPFNA specimens exhibit a meaningful increase in benign breast cell proliferation (Ki-67) where meaningful increase is defined as an increase in Ki-67 to 2% or higher in individuals with initial Ki-67< 1% or a doubling of Ki-67 in individuals with initial Ki-67 of 1% or higher.

1.2 Secondary Exploratory Objectives

A number of analyses will be conducted in an effort to document and descript the effects of Duavee® on a variety of variables that are actual or potential risk biomarkers for development of breast cancer.

- Assess benign breast tissue for decrease in Ki-67 in women with Ki-67 of 1% or higher
- Assess changes in benign breast tissue estrogen responsive genes by RTqPCR
- Assess changes in benign breast tissue peptides and phosphopeptides.
- Assess changes in serum bioavailable hormones and cytokines
- Assess change in bazedoxifene serum levels as a function of BMI, percent body fat, and compliance (by tablet count)
- Document change if any in body composition (DEXA), and waist circumference
- Document change if any in volumetric breast density (Volpara) and compare to % dense area (Cumulus)

2. BACKGROUND

2.1 The Scope of the Problem

Vasomotor and other menopausal symptoms are a significant problem impacting quality of life for up to 75% of women undergoing natural menopause transition usually between age 45 and 55 and generally last about 5 years [Freeman, 2011]. Approximately 1/3 of women have severe enough symptoms to prompt them to seek medical attention. These women and those who develop vasomotor symptoms early in transition are likely to be those who are bothered with these symptoms for 10 or more years [Freeman, 2011; Freeman, 2014]. Hormone replacement therapy, either estrogen alone for women without a uterus or estrogen and progesterone for women with a uterus, is the most effective means of relief but concerns about increasing the risk for breast cancer make providers reluctant to provide prescriptions for hormone replacement therapy for even average risk women much less those at increased risk. Reluctance is largely based on results of the Women's Health Initiative study in which an average of 5 years of combined conjugated estrogen 0.625 mg plus a progestin resulted in a 24% increase in breast cancer risk overall and a 34% increase in risk for women beginning it in their 50's (HR 1.34; 0.82-2.19) [Manson 2013; Anderson 2012]. No increase in risk of breast cancer was observed for conjugated estrogen 0.625 mg/day alone [Manson 2013; Anderson 2012].

At least 25% of women 45 and older are at increased risk for breast cancer because of family, reproductive, or personal health history. In addition to counseling for lifestyle modifications (normal weight, exercise, reduce alcohol intake), peri- and post-menopausal women 35 and older at moderately or higher increased risk may be counseled to take an anti-estrogen to reduce their risk [Visvanathan 2013]. Overall only 4% of women eligible for antihormonal primary prevention therapy agree to take it although it can reduce the risk of breast cancer by 40% or more [Fisher 1998; Vogel 2006; Ropka 2010; Goss 2011; Cuzick 2013; Cuzick 2014].

Given that medications such as tamoxifen and aromatase inhibitors often increase vasomotor symptoms and are not associated with a survival advantage in the primary prevention setting [Visvanathan 2013; Goss 2011; Cuzick 2014], it is likely that an even lower percentage of women currently having difficulty with hot-flashes will agree to take anti-estrogens as primary prevention therapy. The development of tissue selective estrogen complexes combining estrogen (for hot flashes and bone health) plus a selective estrogen receptor modulator (SERM) with breast and uterine antagonist activity presents a potential solution to this conundrum for women with both vasomotor symptoms and an increased risk for development of breast cancer.

2.2 Rationale for Present Proposal

In clinical trials of bazedoxifene plus conjugated estrogen (now marketed as Duavee®) versus placebo, relief of hot flashes and improvement of both hip and lumbar spine bone density was accomplished without development of uterine hyperplasia, increase in breast tenderness, or increase in mammographic breast density [Harvey 2013; Pinkerton 2013; Pinkerton 2014a; Pinkerton 2014b; Pinkerton 2014c; Abraham 2014; Komm 2014]. Contra-indications to use of Duavee® include estrogen dependent neoplasia (such as breast, ovarian, and endometrial cancer), predisposition to or prior thromboembolism, hepatic impairment, and abnormal uterine bleeding. There is no explicit label warning about women who may be at increased risk for breast cancer [Duavee® Prescribing Information, 2014].

Preclinical studies in MCF-7 breast cancer cells suggests that bazedoxifene down-regulates ER alpha and decreases cyclin D1 as well as cell growth [Lewis-Wambi 2011].

In non-human primates, 6 months of bazedoxifene or bazedoxifene combined with conjugated estrogen was associated with reduced Ki-67 compared to conjugated estrogens alone in normal mammary tissue [Ethun 2012]. Bazedoxifene combined with conjugated estrogen still functions as an estrogen antagonist in the macaque monkey uterus with reduction of Estrogen Receptor alpha (ER alpha) related genes GREB1 and pS2 (Trefoil Factor 1) [Ethun 2013]. There was little effect on lipids or coronary or peripheral atherosclerosis for bazedoxifene alone but bazedoxifene partially antagonized the protective effects of conjugated estrogens on coronary and peripheral but not cerebral/carotid atherosclerosis [Clarkson 2014].

There is no FDA approved chemopreventive agent for breast cancer risk reduction which reduces menopause symptoms, in fact the SERMs tamoxifen (for pre and postmenopausal women) and raloxifene (for postmenopausal women), as well as aromatase inhibitors (for postmenopausal women), all increase menopause symptoms [Visvanathan 2013]. If bazedoxifene and conjugated estrogens were actually found to reduce proliferation in benign breast tissue similar to what has been observed in animal models, then it should be explored in placebo-controlled Phase IIB trials for change in tissue proliferation and in Phase III trials for change in cancer incidence. The combination of menopause symptom relief and breast cancer risk reduction would be a major advance for women in early menopause. Even if breast cell proliferation did not decrease but was at least stable (not increased) after Duavee®, this would be a major advance as women at increased risk for breast cancer (and their physicians) would likely be more comfortable with its use.

2.3 Proliferation in Hyperplastic Benign Breast Tissue as a Response Endpoint

Women with diagnostic biopsy evidence of proliferative breast disease following an abnormal finding on physical exam or mammogram are at increased risk for breast cancer ranging from ~ 2 fold for hyperplasia without atypia to a 4-5 fold increase in relative risk for atypical ductal or lobular hyperplasia [Dupont 1985; Tavessoli 1990; Drystad 2015; Hartmann 2014]. However, women age 50-69 who simply have a diagnostic breast biopsy without evidence of proliferative breast disease or family history may also have a 20% up to a 2 fold increase in the risk for breast cancer [Drystad 2015; Castells 2015]. Proliferation as measured by detection of mitotic index and/or detection of nuclear associated proliferation antigens such as Ki-67 (MIB-l) progressively increases from normal to hyperplasia to atypical hyperplasia and DCIS [Shrestha 1992; Mommers 1999; Allred 1998].

In normal pre-menopausal breast epithelium, proliferation (Ki-67) correlates with systemic levels of progesterone, and is lowest in the follicular phase (0.2-0.7%) and highest in the luteal phase (~2%) [Soderqvist 1997; Shoker 1999]. Ki-67 labeling averages 0-1% in normal postmenopausal epithelium [Shoker 1999]. In diagnostic biopsies from proliferative breast disease Ki-67 has been reported to vary between 1 and 6% [Shaaban 2002; Allred 1998].

A Ki-67 labeling index of 2% or higher in usual duct hyperplasia (cross sectional study from Shaaban) or atypical hyperplasia (Mayo prospective cohort study Santisteban) is associated with increased risk of breast cancer compared with women with hyperplasia or atypical hyperplasia with lower levels of proliferation [Shaaban 2002; Santisteban 2010]. We have used random periareolar fine needle aspiration to obtain benign breast tissue from women at increased risk for breast cancer for risk stratification and to obtain tissue for Phase II prevention

trials for over 2 decades [Fabian 2000, 2002, 2005, 2007, 2010, 2013]. Similar to diagnostic biopsy series, [Dupont 1985; Hartmann 2014] women with cytologic evidence of hyperplasia with atypia have an increased risk of DCIS and invasive cancer as well as a higher proliferative rate [Fabian 2000; Khan 2005]. We observed a median Ki-67 labeling index of 1.4% in RPFNA samples exhibiting hyperplasia with or without atypia from high-risk post-menopausal women in whom there were sufficient cells for Ki-67 to be attempted [Khan 2005]. Inter- and intra-observer variation in interpretation of staining results was low with a Cronbach's alpha of 0.99 [Khan 2005].

A number of antihormonal treatments effective in breast cancer treatment and prevention have been shown to reduce proliferation indices in benign and cancerous breast epithelial cells by 17-50% relative to baseline. [Clarke 1993; Chang 2000; Dowsett 2001; Dowsett 2005; Decensi 2003]. Reduction in proliferation (Ki-67) at two weeks and 12 weeks in the neoadjuvant IMPACT Trial (tamoxifen, anastrozole, combination) was positively associated with disease free survival [Dowsett 2005]. Tamoxifen has been shown to reduce proliferation (Ki-67) in benign breast tissue of postmenopausal women [de Lima 2003]. In a single arm pilot trial six months of the aromatase inhibitor letrozole reduced proliferation by 66% in benign tissue from high-risk women despite continuing hormone replacement [Fabian 2007].

Ki-67 is quantitative, exhibits low intra- and inter-observer variance, and is favorably modulated in benign breast tissue with agents known to be effective in primary and secondary breast cancer prevention. Consequently, change in proliferation as measured by Ki-67 immunohistochemistry is often used as a primary response endpoint in Phase II and pilot prevention studies of premenopausal women and postmenopausal women on hormone replacement therapy [Fabian 2005; Fabian 2007]. We and others have shown that random periareolar fine needle aspiration is accepted by high risk women and is a reproducible means to sample breast tissue [Fabian 2000; Ibarra-Drendall 2009]. However, given the extent of lobular involution in definitively postmenopausal women not on hormone replacement therapy, a sizable number of women may need to be screened by RPFNA to detect those with a Ki-67 of 1-2% or higher, making reduction in Ki-67 an impractical endpoint for primary prevention trials in definitively postmenopausal women not on hormone replacement. Ki-67 has not been adequately studied in moderate to high risk peri-menopausal women < 60 having menopausal symptoms in order to determine the proportion of women with a Ki-67 of >1% in their RPFNA specimen.

2.4 Other Risk and Mechanism of Action Biomarkers in Benign Breast Tissue Likely to be Modulated by Duavee®

Sampling of benign breast tissue also provides the opportunity to assess fresh frozen tissue for assessment of change in gene or protein expression to better define mechanism of action. We will assess a number of estrogen response genes including ER alpha, progesterone receptor, pS2 (Trefoil factor 1) cMYC, GREB-1, amphiregulin, known to be increased by estrogen and antagonized by bazedoxifene in mice benign and malignant mammary tissue [Song 2012]. Several additional genes known to be increased at the mRNA level by estrogen such as amphiregulin, FOS, and JAK2 will also be assessed by RTqPCR [Chang 2010; Fabian 2010; Phillips 2013; Ibarra-Drendall 2012; Fabian 2013]. Reverse phase protein arrays will also be used to identify changes in proteins and phosphoproteins in pathways important in breast carcinogenesis [Akbani 2014; Meric-Bernstam 2014; Fabian2013].

2.5 Mammographic Breast Density as Risk and Response Biomarker

Mammographic density which is composed histologically of ductal and stromal tissue, and fluid is recognized as a risk factor for breast cancer particularly when corrected for age and Body Mass Index (BMI) [Boyd 1993; Boyd 1995; Boyd 1998; Byrne 1995; Warwick 2014]. Although absolute dense area along with % dense area are both risk biomarkers, % density appears to be the stronger risk biomarker [Pettersson 2014]. In a recent analysis, Boyd et al. suggest that women with a high density in the highest tertile of mammographic density corrected for BMI have a risk of 2.63x that of women in the lowest tertile (0-20% density), and women in the mid tertile (20-41%) a risk of 2.15X that of women in the lowest tertile [Boyd 2014]. Given these updated results it is reasonable to include women with an estimated breast density of 25% or higher in the risk criteria for consideration of participation in this study.

Change in mammographic density is validated as a surrogate response biomarkers for trials involving the selective estrogen receptor tamoxifen which is known to reduce risk for breast cancer by 40% or more [Cuzick 2011; Cuzick 2013]. However, change in breast density is not modulated by raloxifene, a selective estrogen receptor modulator known to reduce risk for breast cancer in postmenopausal women almost to the same extent as tamoxifen [Martino 2004; Freedman 2001; Pearman 2010]. Nor is density modulated with exemestane, an aromatase inhibitor that is shown to reduce breast cancer risk by 65%. [Cigler 2011; Goss 2011]. Weight reduction may actually increase percent dense area [Fabian 2013; Boyd 1997]. Breast density, even with computer assisted calculations, is subjective and pre and post intervention assessments should be performed by the same individual at the same setting with the rater blinded as to which image was baseline [Stone 2003]. In addition there are a number of technical factors that can introduce error when change is being evaluated including variation in type of imaging, positioning and degree of compression**.**

Breast density (% dense area) was not significantly altered compared to controls over 24 months in a centrally read retrospective analysis of digital mammograms from the SMART 1 study which compared bazedoxifene + 0.45 mg CE or 0.625 mg CE vs placebo to placebo or 60 mg of raloxifene. (Mean reductions of 0.05-.41%). In a prospective cohort of over 900 women in the SMART 5 study ages 40-65 with a mean baseline density of ~25% there was no change in centrally read digital mammographic density over a 12 month period comparing 0.45 or 0.625 mg CE + 20 mg bazedoxifene vs placebo (both declined by ~ 0.3 %). A comparator of CE + medoxyprogesterone acetate increased by 1.66% [Pinkerton 2013]. Automated volumetric mammographic breast density assessments appear to have less variation than computer assisted operator density assessments [Eng 2014; Alonzo-Proulx 2015; Brand 2014; Ekpo 2014]. Automatic Volpara volumetric density measurements utilized in this study will be compared to the Cumulus % dense area to explore whether small changes in density with 6 months of Duavee® may be detected with the volumetric method.

2.6 Serum risk Biomarkers as Response Endpoints

Higher endogenous bioavailable estradiol and testosterone levels have long been associated with breast cancer risk in postmenopausal women [Toniolo 1994; Pearce 1989; Tworoger 2014; Verkasalo 2001; Missmer 2006; Farhat 2011]. Several serum biomarkers associated with metabolism are known to be risk biomarkers for proliferative breast disease and breast cancer in postmenopausal women including serum insulin, markers of insulin resistance such as HOMA-IR, adiponectin, leptin: adiponectin ratio [Fabian 2012; Catsburg 2014; Gunter 2009; Hernandez 2014; Vona-Davis 2007]. Lipocalin 2 (Lcn2) and retinol binding protein 4 (RBP4) are

also increased in insulin resistance. Lipocalin 2 has been shown to promote breast cancer development and progression primarily through activation of the estrogen receptor in preclinical models [Guo 2012; Yang 2009; Shi 2008]. An increase in the serum ratio of retinol binding protein to STA6 is oncogenic and is mediated through the actions of JAK2 and Stat 3 and 5 at the cellular level [Berry 2014]. Treatment of ovariectomized mice with conjugated estrogen (CE), bazedoxifene (BZA) or the combination vs control suggests that both CEE and BZA alone or in combination have favorable effects on leptin, adiponectin, Lcn2, and RBP4. The most dramatic effects appear to be for the leptin: adiponectin ratio (reduced by over 10 fold in the ovariectomized mouse) [Kim 2014]. Ovariectomized mice subjected to 10-12 weeks of diets producing obesity showed attenuation of weight gain, fat mass gain, and measures of insulin resistance when treated with BZA and CE [Barrera 2014].

3. SUMMARY OF STUDY PLAN

Peri- or post-menopausal women meeting the risk and medical eligibility criteria (see below) will undergo random periareolar fine needle aspiration (RPFNA) under the umbrella screening protocol HSC 4601. Women with at least 500 cells on their cytomorphology and/or Ki-67 slide, but with Ki-67 expression less than 4% will be eligible to proceed to the intervention phase of this study. At a baseline on-study visit, women will have a physical exam, DEXA for body composition, complete a 29 item Menopause Quality of Life Intervention Questionnaire [Lewis 2005] and hot flash assessment, have fasting blood obtained for biomarkers as well as to assure reasonable normal organ function. Key symptoms assessed at baseline will be hot flashes. Forty women will be entered on the treatment portion of the trial and will take Duavee® one table daily. Drug will be supplied by Pfizer. After 6-8 months of study agent, biomarkers will be reassessed. Mammogram, DEXA for body composition, RPFNA, and blood draw will be repeated. A complete side effects assessment will be performed. Entry of 40 women should allow 36 women to complete the study and be evaluable for the primary endpoint. Trial accrual is anticipated to take 6 months with completion of clinical aspects of the study by 12 months. Since this is not a blinded study, analysis of feasibility and changes in Ki-67 can take place immediately.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria for Screening RPFNA

Note that the screening RPFNA may be conducted under a separate IRB-approved research protocol with its own consent form. Potential participants are not required to make a decision regarding participation in the Duavee® trial until after receipt of the results of their RPFNA. However, if a woman is undergoing an RPFNA expressly for participation for this protocol, the following should be kept in mind for eligibility

4.1.1 Women with vasomotor symptoms with a uterus who are postmenopausal (no menstrual period for 12 months) or in late menopause transition (no period for 3 months and elevated FSH) [Sherman 2005].

4.1.2 Age ≤65

4.1.3 BMI: <36 kg/m^2

4.1.4 Breast Imaging: Class I-III mammogram within 6 months of RPFNA. If Class 0 or 4, must be resolved with additional procedures. If breast imaging pre and post study is performed at KUMC, then volumetric assessment by Volpara software will be performed.

4.1.5 If previously on oral contraceptives or hormone replacement, off for 8 weeks or more prior to baseline RPFNA. The exception is low dose vaginal hormones (estring, vagifem, or 0.5 gram or less of conjugated estrogen vaginal cream twice weekly or less often). These vaginal preparations may be continued at the same dose.

4.1.6 Risk Factors/Level. Moderate risk of developing breast cancer based on either by having at least one of following:

- First or second degree relative with breast cancer age 60 or younger;
- Prior breast biopsy;
- Prior RPFNA atypia;
- Estimated Mammographic Density of 25% or higher;
- Gail 5-year risk of >1.7% (as calculated by the NCI Breast Cancer Risk Assessment Tool) or a 5 year Gail Risk of 2X that for age group; and/or
- IBIS Breast Cancer Risk Evaluation (http://www.ems-trials.org/riskevaluator/) [Tyrer–Cuzick 2004]. 10-year relative risk of >2X that for the population for age group.

4.2 Exclusion Criteria for Screening RPFNA

4.2.1 Risk: A prior biopsy showing LCIS, DCIS, or invasive breast cancer, or individual with BRCA1/2 deleterious mutation.

4.2.2 Medical Conditions:

- Have a predisposition to or prior history of thromboembolism, deep venous thrombosis, pulmonary embolism, or stroke
- History of renal or liver disease

- Prior ovarian or endometrial cancer
- Stopped or started hormone replacement within 8 weeks
- Any other condition or intercurrent illness that in the opinion of the investigator makes the woman a poor candidate for RPFNA.

4.2.3 Medications

- Current anticoagulant use (must discontinue for 3 weeks prior to FNA)
- Taking systemic hormones within two months (eight weeks) prior to screening RPFNA.
- Taken tamoxifen, raloxifene, or an aromatase inhibitor within the past 6 months
- Participation on any chemoprevention trial within 6 months

4.3 Inclusion Criteria for Intervention Phase

4.3.1 RPFNA Results: An RPFNA performed less than six months prior to enrollment on study that exhibits at least 500 cells on the cytology or Ki-67 slide, and a Ki-67 positivity measured on at least 500 cells that is less than 4%.

4.3.2 Baseline chemistry profile showing reasonably normal renal and hepatic function. Creatinine <2.0, Bilirubin < 2.5, and albumin > 3.4

4.3.3 Willingness to comply with study procedures.

- Willing to have a blood draw for serum to archive bioavailable estradiol, SHBG, FSH, and bazedoxifene levels as well as a chemistry profile to ensure reasonable normal organ function at baseline and 6 month visits (approximately four tubes of blood collected)
- Willing to have a DEXA scan for body composition and waist measurement at baseline and 6-8 months.
- Willing to have a repeat mammogram and RPFNA at 6-8 months following initiation of study drug
- Willing to undergo a history, physical, vitals and breast exam at baseline and 6 month visits
- Willing to be contacted by the trial coordinator at months 1 and 3 during the 6 month study period.
- Willing to complete a 29 item validated Menopause Quality of Life Intervention Questionnaire and hot flash assessment at baseline and 6 month visits
- Willing to sign and able to understand separate consents for the RPFNAs and for study participation

4.4 Exclusion Criteria for Study Intervention

4.4.1 RPFNA Criteria:

- Cells suspicious for malignancy

4.4.2 Medical: Intercurrent illness which makes potential participant unsuitable for study or started hormone replacement therapy between RPFNA and enrollment on study.

4.5 Inclusion of Women and Minorities

All women who meet eligibility criteria and members of all races and ethnic groups are eligible for this trial.

4.6 Recruitment and Retention Plan

Potential participants will be screened in the Breast Cancer Prevention Center at the University of Kansas Medical Center (KUMC), Westwood, Kansas. All potential participants will be involved in the High Risk Breast Program at KUMC. The duration of accrual is expected to take 6 months, accruing 6.7 participants per month.

Study participants will be contacted by phone at month 1 and month 3 during the months between clinic visits in order to insure active participation and to collect adverse event, dosing and other pertinent information. It is expected that the dropout rate will be less than 10%.

5. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis.

5.1 Dose Regimen and Dose Groups

Open label Bazedoxifene (20 mg) plus conjugated estrogen (0.45 mg) marketed as Duavee® for six months will be given to all trial participants. There will be no randomization as this is a one arm trial. All participants will be given eight months (two months of overage) of Duavee® to allow for scheduling study procedures.

5.2 Duavee® Administration

The KUMC Investigational Pharmacy will be responsible for receiving, inventory and dispensing of study agent.

A study number will be assigned to each participant and provided to the investigational pharmacy. Study agent will be dispensed with the study number and initials of each participant. Eight months of Duavee® will be dispensed. The study coordinator will then give the study agent to the participant with instructions to take one tablet at approximately the same time of day in conjunction with intake of food and to keep track of missed doses or lost tablets.

5.3 Concomitant Medications

Study participants are strongly encouraged to continue any routine medications throughout the duration of the study to avoid confounding adverse events due to starting and stopping other medications while on study. Participants are not allowed to start tamoxifen, raloxifene, anastrozole, letrozole or any other SERM or aromatase inhibitor (or other chemopreventive) while participating on this trial.

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (*e.g.*, biopsy) should also be included.

5.4 Dose Modification

Dose modifications will not be allowed on this study. Because study agent is only available in a single dose tablet, no dose modification option is available. If a participant is experiencing adverse events or is having a medical procedure that requires a brief ***suspension*** of Duavee®, this must be noted in the CRF on the dose suspension page. At the discretion of the PI, participants may be removed from participation for significant dose suspension time periods.

Participants with grade 2 adverse events considered to be related to study agent will have the agent suspended until the toxicity has resolved. If the adverse event resolves within one month, the agent will then be resumed. If toxicity again occurs, and is thought to be agent-related, the agent will be permanently stopped.

Participants who experience grade 3 toxicity will have the study agent suspended until the adverse event resolves. If the investigator is reasonably certain that the toxicity is not due to the agent, the agent may be re-instituted after resolution of the adverse event.

Development of a grade 4 adverse event will require suspending the study agent. If the investigator feels the event is not study agent related, then it may be re-instituted at full dose once the adverse event is resolved.

Examples of grade 3 or 4 adverse events which would require permanent study agent discontinuation and withdrawal from the trial include development of breast or any other type of cancer (other than skin) that requires definitive treatment; deep venous thrombosis; symptomatic ovarian cysts requiring hospitalization or narcotic pain management. Examples of grade 3 or 4 adverse events requiring temporary discontinuation and possible re-institution include bronchitis/pneumonia, broken bones, nausea-vomiting due to influenza, and rash of unknown cause.

5.5 Adherence/Compliance

Participants that maintain 70% compliance throughout the duration of the trial will be considered “compliant”. Compliance will be measured through tablet counts conducted by the trial coordinator and recorded in the CRF.

6. PHARMACEUTICAL INFORMATION

Duavee® is the combination of 0.45 mg of conjugated estrogen and 20 mg of bazedoxifene and was approved for relief of menopausal symptoms and prevention of postmenopausal osteoporosis in women with a uterus in October 2013. Contraindications listed in prescribing information include undiagnosed abnormal uterine bleeding, past history of breast cancer, current estrogen-dependent neoplasia, active or history of venous or arterial thromboembolism, hypersensitivity to estrogen or bazedoxifene, known hepatic impairment or disease, known protein C, protein S, or anti-thrombin deficiency or other thromboembolic disorders, or pregnancy and nursing.

6.1 Duavee® Provider

Duavee® will be provided for this study by Pfizer, Inc., the manufacturer.

6.2 Pharmacology

6.2.1 Absorption: Both bazedoxifene and conjugated estrogen are well absorbed from the gastrointestinal tract. Bio-availability of bazedoxifene is 6%. Steady state concentrations are obtained by day 10. At day 10 steady state C_{max} for the estrogen component is 2.6 ng/ml estrone and 6.9 ng/ml bazedoxifene with T_{max} after a single dose of 6.5 and 2.5 hours respectively in postmenopausal women.

6.2.2 Food Effect: Duavee® may be taken without regard to meals but a high fat meal increased the AUC by 25%.

6.2.3 Distribution: The estrogen component is bound to SHBG and albumin and is widely distributed. Bazedoxifene is also highly distributed and circulates highly bound (97-98%) to plasma proteins but not SHBG. A radio-ligand assay has been developed for bazedoxifene [Komm 2005 Endocrinology, Harris 2002]

6.2.4 Metabolism: The estrogen component is metabolized similarly to endogenous estrogen with conversion reversibly to estrone which can be converted to 17 B estradiol or estriol, or estrone sulfate. Bazedoxifene is metabolized via glucuronidation with little cytochrome P450 mediated metabolism. The concentration of the 5-glucuronidated metabolite in the circulation is 10x that of the parent compound.

6.2.5 Excretion: Total estrone is eliminated primarily in the urine with a half-life of 17 hours. Bazedoxifene is eliminated via biliary excretion and feces with <1% in urine. The elimination half-life of bazedoxifene is 30 hours. It is likely that it undergoes an extensive entero-hepatic circulation and that some drugs might interfere with elimination. However, commonly used antibiotics, NSAIDs, and antacids had minimal tested effect on Bazedoxifene concentrations with increases in AUC of Bazedoxifene of 6-7% with co-administration of ibuprofen, atorvastatin, and aluminum and magnesium hydroxide and decease of bazedoxifene by 15% with azithromycin.

6.3 Mutagenesis, Carcinogenesis and Fertility

There is no evidence that bazedoxifene is mutagenic or genotoxic. In female rats and mice there is evidence of increase in benign ovarian, and ovarian granulosa cell tumors. Estrous cycles and fertility is impaired in female rats.

6.4 Pfizer Sponsored Clinical Studies

6.4.1 Relief of Menopausal Symptoms in Women: a 12 week double blind placebo controlled trial in women age 42-62 (mean 53) who had moderate to severe hot-flashes showed a reduction in mean number of hot flashes of 5.9 per day compared to a reduction of -2.8 for the placebo by 4 weeks as well as a reduction in severity.

6.4.2 Uterine Side Effects in Women: The incidence of endometrial hyperplasia was < 1 % and spotting showed little difference from placebo.

6.4.3 Bone Density: Mean % change in BMD with Duavee® at 24 months was + 1.72 vs -1.90 for placebo in women menopausal for 1-5 years.

6.5 Special Considerations

6.5.1 Hepatic or Renal Impairment: Duavee® is contraindicated in women with hepatic impairment (AUC of bazedoxifene increases by 100 % in moderate and over 300 % in severe hepatic impairment) and has not been evaluated in women with renal impairment. Women with significant hepatic or renal abnormalities will not be eligible for this study.

6.5.2 Women with high BMI: a 17% reduction in bazedoxifene is predicted in women with a BMI < 27 kg/m^2 compared those with < 27 kg/m^2. Reduction in bazedoxifene levels may be associated with increased risk of uterine hyperplasia in women using the Duavee® long term but it is unlikely to be an issue in our short term trial. Nonetheless we will restrict eligibility to women with a BMI of < 36 kg/m^2.

6.5.3 Advanced Age: No difference in safety or effectiveness were noted in the small number of women tested between 65-74 in clinical trials with Duavee® and Duavee® has not been tested in women over 75. Due to concerns about obtaining adequate amounts of breast tissue by RPFNA we will limit study entry to those 65 and younger.

6.6 Availability and Agent Distribution

Duavee® comes from Pfizer as tablets containing 20 mg of bazedoxifene and 0.45 mg of CE**.** 240 tablets containing 20 mg of bazedoxifene and 0.45 mg of CE will be dispensed at entry of the participant onto the study so as to provide an adequate number of tablets for 8 months. The 8 months is based on the planned 6 months of administration, plus two additional months if required to accommodate scheduling of procedures.

6.7 Agent Accountability

The Protocol Lead Investigator (Fabian) is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to the KUMC Investigational Pharmacy. Include on receipt record from whom the agent was received

and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant.

6.8 Packaging and Labels

Duavee® will be supplied by Pfizer in a form ready for dispensing. For dispensing, each container will be labeled with a one-part label identifying study specific information, such as Study title, HSC protocol number, subject ID number, dosing instructions, recommended storage conditions, and a caution statement indicating that the agent is limited by United States law to investigational use only and the agent should be kept out of reach of children.

6.9 Storage

Study agent will be stored in the controlled access Investigational Pharmacy, at room temperature.

6.10 Registration/Randomization

There is no randomization for this open-label, single arm study. Registration will be in an enrollment log managed by the study coordinator, with matching participant study numbers and initials in the Investigational Pharmacy at KUMC.

All participants signing consent forms will be assigned a Study ID number, have the Registration CRF completed, and be entered into the CRIS database.

6.11 Blinding and Unblinding Methods

Not applicable.

6.12 Agent Destruction/Disposal

At the completion of investigation, all unused study agent will be returned to the PI for return to the KUMC Investigational Pharmacy and destroyed in accordance with KUMC investigational Pharmacy regulations.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

SCHEDULE OF EVENTS

Evaluation/ Procedure	Pre-Study/ Screening RPFNA	Baseline	Contact End of Month 1 & 3	Month 6	Follow Up Phone Contact
Mammogram	X			X	
RPFNA for cytomorphology, Ki-67, RTqPCR, proteomics	X			X	
Fasting blood archived for SHBG, estradiol, testosterone, progesterone, FSH, adipocytokines, and Bazedoxifene levels		X		X	
Assess Eligibility	X	X			
Informed Consent		X			
Medical History		X			
Physical Exam/CBE		X			
Vital Signs/ Height and Weight, BMI, waist measurement		X		X	
29 item validated Menopause Quality of Life Intervention Questionnaire and hot flash assessment		X		X	
Blood Chemistry		X		X	
DEXA body composition and waist measurement		X		X	
Symptom Assessments		X	X	X	X
Concomitant Medications		X	X	X	X
Pregnancy test if peri-menopausal		X			
Dispense Study Agent		X			
Collect Study Agent				X	
Adverse Events			X	X	X
Telephone or Email Contact			X		X

7.2 Baseline Testing/Pre-study Evaluation

7.2.1 Pre-study Eligibility RPFNA

Women who potentially meet medical eligibility criteria for trial and are interested will undergo a pre-study screening visit. Women may take vitamin K 10 mg/day for 3 days prior to the aspiration to help prevent hematoma formation but this is not required.

RPFNA will be performed under local anesthesia from both breasts (two sites on each breast) and all cells pooled from both breasts (one breast in the case of prior DCIS or invasive cancer) into a single 15 cc tube containing modified Cytolyt™ (9 cc Cytolyt™ and 1 cc 10% neutral buffered formalin). After cleansing the breast with betadine and alcohol swabs, the breast is anesthetized with epinephrine and lidocaine using a tuberculin syringe. After the surface has been anesthetized, epinephrine and lidocaine are injected deeper into the breast at two sites, approximately at the 10 and 2 o'clock locations. Then, 5-6 needle passes are made into the anesthetic puncture sites to withdraw epithelial cells lining the ductal tree.

After the procedure, participants will have ice packs placed on the breast for 10-15 minutes to control swelling and bleeding. Participants are then wrapped with gauze cling wrap to create pressure on the puncture sites. A snug bra is also strongly recommended. Participants are advised to limit strenuous physical activity over the next 24 hours and to report any pain or signs of infection.

Participants are offered Ativan 1 mg prior to the procedure for anxiety, and analgesics after the procedure in order to control any pain that may occur. These medications are optional. Participants should also be instructed to eat a full breakfast the morning of the RPFNA, to abstain from vitamin E, NSAIDS and fish oil products for three weeks prior to RPFNA, and if planning on taking Ativan, to have someone with them to drive them to and from the clinic.

7.2.2 Pre-study risk assessment

- Gail Risk assessment: A 5-year projected probability of breast cancer development ("Gail risk") will be calculated using the NCI website algorithm (http://www.cancer.gov/bcrisktoolmobile). The risk is expressed as relative to the average risk for women in the same age-group, based on values calculated from the Surveillance, Epidemiology, and End Results (SEER) Program provided by the NCI (http://srab.cancer.gov/devcan/)
- IBIS Risk assessment: a 10 year The IBIS Risk Assessment will be performed. The IBIS Breast Cancer Risk Evaluation Tool can be found (http://www.ems-trials.org/riskevaluator/).
- Mammographic Density Estimation: Mammographic Density will be estimated from the pre-study digital mammogram left cranial caudal view. Note that a digital mammogram must have been performed within 6 months of RPFNA.

7.2.3 Baseline Studies

7.2.3.1 Brief Physical and Medical History

All women will have a brief physical (including clinical breast exam) performed by a study-associated clinician. This may be performed at the time of the RPFNA. All abnormal findings will be recorded on the physical source document, including duration and severity. Height and weight, BMI, and waist measurement will also be recorded. Women will have a medical history collected by study personnel, including event dates, duration, and severity.

7.2.3.2 DEXA Scan

Dual energy x-ray absorptiometry (DEXA; Lunar Corp.) will be performed within 90 days prior to study initiation and at the completion of study participation to analyze change in body fat mass, fat free mass, and percent body fat. DEXA uses very low X-ray doses (0.02 mrem), that is, less than several hours of background exposure, and is able to detect changes in body composition on the order of 1.6-3.8%. DEXA will also be used to estimate visceral adipose composition. All calculations use GE Prodigy 9.1 software.

7.2.3.3 Bloodwork

Fasting (10-12 hours) blood will be drawn for

- Archived serum for FSH, testosterone, estradiol, SHBG, and for perimenopausal women progesterone, as well as adipocytokines and bazedoxifene levels.
- Chemistry profile will also be drawn and processed real time at the KUMC Westwood lab to ensure reasonable normal organ function. The chemistry profile will be approved by the PI or study associated Nurse Practitioner prior to dispensing of study agent.

7.2.3.4 Baseline symptom assessment

A baseline symptom assessment will be made, including such relevant conditions as hot flashes and other vasomotor symptoms. A 29 Item validated Menopause Quality of Life Intervention Questionnaire will be utilized at baseline and end of study. We will also utilize the hot flash scoring system developed by Dr. Charles Loprinzi at Mayo Clinic [Loprinzi 2002]. Adverse events will be recorded using NCI common terminology criteria for adverse events version CTCAE 4.0.

7.2.3.5 Negative Pregnancy Test

For peri-menopausal women with most recent menstrual period less than 12 months, a negative pregnancy test is required prior to dispensing study agent. This may be either blood or urine based.

7.3 Evaluations During Study Intervention

Participants will be contacted after 1 and 3 months by the study coordinator by telephone or e-mail as the subject prefers to review dosing and adverse events. These will be recorded in the source documents and subsequently entered onto CRFs.

Women who report grade 3-4 adverse events (other than menopausal symptoms) will be evaluated in person to fully assess adverse events; and will be followed at intervals deemed necessary by the investigator until the adverse event has resolved.

7.4 Evaluation at Completion of Intervention

The off-study fine needle aspiration is scheduled to occur after 6 months of study agent. However, aspiration may be delayed for scheduling purposes and study agent may be continued for up to two additional months, until the aspiration is performed. If this occurs, then studies associated with the repeat aspiration and completion of study will be conducted at that time, not at a calendar six months.

Participants will have a mammogram, DEXA, RPFNA, and a brief physical (including clinical breast exam) performed by a study-associated clinician. An off study mammogram will be obtained at KUMC paid for by the study if it is not time for the regular screening mammogram. All abnormal findings will be recorded on the physical source document, including duration and severity. Vital signs, height, weight, BMI, and waist measurement will also be recorded.

The collection of fasting blood for biomarker assay should be done same day if at all possible and will be performed prior to the RPFNA. Women will eat prior to having the RPFNA performed

When the participant has completed taking study agent, any remaining supply will be returned to the Investigational Pharmacy and the number of tablets returned will be counted for assessment of compliance.

7.5 Post-intervention Follow-up Period

Approximately four weeks after last consumption of study agent, the participant will be contacted by the trial coordinator to assess adverse events and answer any remaining questions.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

Prior to formal analysis, there are two aspects of a feasibility trial that are based on simple numbers. If we cannot accrue 40 eligible women within a reasonable time (no more than 12 months) and/or if more than 15% of enrolled participants do not complete the study, then it would not be practical to propose future studies.

For the Specific Aim of documenting that Duavee® does not cause a meaningful increase in proliferation, only participants for whom paired specimens evaluable for Ki-67 will be included. An increase in Ki-67 will be defined by one of two scenarios: a) from a baseline Ki-67 <1% to a Ki-67 >2%; or b) for a baseline Ki-67 >1%, at least a doubling of the baseline value.

8.2 Secondary Endpoints

For the second Specific Aim (Does Duavee® cause a reduction in Ki-67?), we will consider only participants having baseline Ki-67 >1%; and thus the potential to experience a decrease.

Any remaining assessments of modulation due to Duavee® would be considered exploratory for purposes of informing future applications and trial designs. This would include change in breast cytomorphology index score; changes in gene expression (specifically, pS2, PGR, ER alpha, GREB-1, Amphiregulin, SDF-1, cyclin D1) by RTqPCR; changes in levels of ER alpha and cyclin D1 and possibly other proteins and phosphoproteins by proteomics; change in serum bioavailable hormones, adipocytokines, and change in menopause specific quality of life. For these analyses, all participants with paired pre-study and post-study data will be included.

8.3 Off Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, medical contraindication, or physician determination. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

8.4 Off Study Criteria

Participants may go ‘off-study’ for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/serious adverse event, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death, or participant wishes. If a participant goes off-study for any other reason, details will be recorded.

8.5 Study Termination

A frequency of greater than grade 2 adverse events (exclusive of hot flashes and menstrual irregularities) that exceeds 20% will be cause for consideration of stopping the trial; unless there is adequate information to suggest that the adverse events are not related to the study agent.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Ki-67 and Cytomorphology

Tissue for Ki-67, cytomorphology (4/5 of aspirate), are placed in a modified Cytolyt solution as indicated below and from ~ 1/5th of the aspiration placed immediately in PBS and is immediately frozen in liquid nitrogen for gene and protein expression studies.

9.1.1 Ki-67 Primary Endpoint

Cells are acquired by RPFNA following informed consent. Cells are pooled in a 15 cc conical tube containing 10 cc of formalin modified Cytolyt™ and processed via a liquid based thin layer cell preparation system (ThinPrep™ 2000, Cytyc Corporation). A minimum of two slides will be made: at least one each for cytology and one for Ki-67. Total epithelial cells will be delineated with Mayer's hematoxylin counterstain and proliferating cells with a MIB-1 (DAKO M7240) antibody for Ki-67. We have adapted a staining technique using high temperature antigen retrieval [Khan 2005].

A DAKO automatic stainer will be used for all stains except hematoxylin to insure staining consistency. Ki-67 is visualized as punctate brown dots in the nucleus. Manual counting will be performed by two laboratory technicians employed and scoring assessed by two laboratory technicians. The scores will consist of number of cells assessed, and number of positive cells for Ki-67. Hyperplastic ducts with the highest concentration of MIB-1 positively staining cells will be selected for analysis and 500 total ductal cells will be scored. Numbers of nuclei expressing Ki-67 will be expressed as a percentage of total numbers of cells counted. If hyperplasia is not present in the follow-up specimen at 6 months, non-proliferative areas will be assessed. Cell clusters assessed are viewed with a digital imaging system and representative images archived. A separate score for each technician and a consensus score will be recorded. The consensus score will be utilized for analysis of the primary endpoint.

9.1.2 Cytomorphology

Cytologic assessment of all screened and on-study participants will be performed by Dr. Carola Zalles, the designated cytopathologist for this study. She will provide a traditional characterization (QNS, normal, apocrine metaplasia, epithelial hyperplasia, or hyperplasia with atypia) and a semi-quantitative cytology index score (modified Masood score [Masood 1990])

9.2 Serum Assays

Blood will be drawn fasting for adipocytokines and hormones. All baseline and end of study serum assays will be run together along with pooled serum to control for batch variation. Adipocytokines are run using customized Luminex kits in the breast cancer prevention laboratory. Estradiol and testosterone will either be performed in the Breast Cancer Prevention laboratory or, alternatively, at the Ligand Assay and Core Analysis Laboratory at the University of Virginia (UVA). The lower limit of detection for estradiol at the Ligand Lab is 1.5 pg/ml and the intraassay variation is 4.3%. SHBG is assayed by an immunometric assay using the Immulite analyzer (Diagnostic Products Corp), with a lower limit of detection 0.2 nmol/L and intraassay variation of 2.4%.

9.3 Exploratory Molecular Markers

Depending upon funds availability, exploratory studies may be conducted of molecular markers from archived frozen tissue stored at -80°C. This includes RTqPCR for estrogen inducible gene transcripts and a proteomics panel.

If residual cellular material is available, cells may be processed to slides and processed for evaluation of ER expression by immunocytochemistry. ER expression will be assessed with ID5 antibody from Dako diluted at 1:100 and applied for 30 minutes. The ER protocol has been optimized for fine needle aspirate specimens processed as ThinPrep slides [Petroff 2006]. This protocol involves use of low temperature (90°C) retrieval for two minutes with a 0.2X nuclear decloaker (Biocare) and use of a 0.01% glucose oxidase blocking reagent for 30 minutes at 37°C. ER expression will be hand scored by two readers utilizing 100 cells from a hyperplastic duct exhibiting ER staining. The proportion of cells staining at each intensity (0-4+) will be assessed. The proportion of cells staining at each intensity is then multiplied by the intensity to achieve proportion/intensity sub-scores. The weighted intensity score (IS) is computed by adding together the proportion/ intensity sub-scores. Under this system, scores can theoretically range from 0-4. However, for benign hyperplastic cells which average 30% ER expression with 2+ staining, our median intensity score is approximately 0.6.

10. SPECIMEN MANAGEMENT

10.1 Specimen Processing and Laboratories

10.1.1 RPFNA

RPFNA is performed in the Breast Cancer Prevention Center as outlined above. Cytolyt/formalin fixed specimen is placed on a rocker and frozen specimen is kept in liquid nitrogen prior to transport (few hours) and processing in the Breast Cancer Prevention Laboratory. The KUMC Breast Cancer Prevention Center Laboratory, under the direction of Carol Fabian MD. Is located on the first floor of the Cancer Research Building with a total of 1597 square feet plus 400 square feet of office space. The laboratories are fully equipped for cell culture, the processing and assessment of cytology and immunocytochemistry slides, multiple serum and tissue assays (ELISA's RIAs), laser microdissection, RT-qPCR, and various other molecular biology techniques. Staff located in the administrative/laboratory unit include an experienced cytology technician, a masters prepared biologist, and an additional technician experienced in multiple molecular techniques.

10.1.2 Blood Based Assays

Initial processing for blood tests occurs in the Breast Cancer Prevention Center where we have all necessary equipment for phlebotomy, and initial processing including a refrigerated centrifuge and immediate access to a -80°C freezer.

Blood for the chemistry profile will be drawn in a mint gel clot tube performed by a current CLIA certified clinical laboratory. Generally the clinical laboratory at the Cancer Center Clinical Laboratory at the Westwood campus.

Hormones: Two full gel clot tubes are allowed to clot at room temperature for thirty minutes, then spun at 3500 rpm for 15 minutes. 1-1.5 ml aliquots are placed into four polypropylene cryovials. Tubes are then stored in the -80°C freezer until all specimens from a participant can be analyzed in the same batch.

Adipocytokine panel: 1 red serum tube (no gel stopper) is allowed to clot at room temperature for thirty minutes, then centrifuged at 4,100 RPM for 5 minutes at 4°C. Place 1-1.5 ml aliquots into three 2 ml cryovials. Tubes are then stored in the -80°C freezer until all specimens from a participant can be analyzed in the same batch.

Blood for Bazedoxifene Levels: 1 EDTA plasma tube is obtained. Centrifuge at 3000 RPM for 10 minutes. Place 1ml aliquots into two 2 ml cryovials. Tubes are then stored in the -80°C freezer until all specimens from a participant can be analyzed in the same batch**.**

10.3 Shipping instructions

Samples to be assayed at the University of Virginia will be shipped on dry ice.

11. REPORTING ADVERSE EVENTS

DEFINITION: An adverse event (AE) is any untoward medical occurrence in a study participant. An AE does not necessarily have a causal relationship with the treatment or study participant. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes any death that may occur while a participant is on a study.

11.1 Adverse Events

11.1.1 Reportable Adverse Events

All adverse events that occur after the informed consent is signed must be recorded on the adverse event CRF whether or not related to study agent.

11.1.2 AE Data Elements

- AE reported date
- AE Verbatim Term
- CTCAE Term (v 4.0)
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a Serious Adverse Event (SAE)
- Action taken with the study agent
- Outcome of the event
- If participant discontinues trial because of AE
- Comments

11.1.3 Severity of AEs

Identify the adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov.

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to their impact on the participant's ability to perform daily activities as follows:

Grade	Severity	Description
1	Mild	Barely noticeable, does not influence functioning Causing no limitations of usual activities
2	Moderate	Makes participant uncomfortable, influences functioning Causing some limitations of usual activities
3	Severe	Severe discomfort, treatment needed Severe and undesirable, causing inability to carry out usual activities

4	Life threatening	Immediate risk of death Life threatening or disabling
5	Fatal	Causes death of the participant

11.1.4 Assessment of relationship of AE to treatment

The possibility that the adverse event is related to study drug will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

11.2 Serious Adverse Events

11.2.1 Definition

ICH Guideline E2A and Fed. Reg. 62, Oct. 7, 1997 define serious adverse events as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).*
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner.

11.2.2 Reporting Serious Adverse Events to

As per institutional procedures, SAEs will be reported to the University of Kansas Cancer Center Data Safety and Monitoring Committee and to the Human Subjects Committee (KUMC's IRB)

Elements to be reported may include

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Protocol number
- Title of protocol
- Description of the SAE, including attribution to drug and expectedness

All SAE's will be followed until there is resolution or stability based on the PI's judgment.

12. STUDY MONITORING

12.1 Data Management

Investigator/staff will also keep a log of potentially eligible individuals screened for study and if not entered the primary reason they declined participation.

Eligibility checklist and all clinical data capture form (including current medications) will be completed by the PI or clinical staff. Laboratory reports and data will be kept in the participant specific study binders as well. Co-existent disease and adverse events will be coded using the NCI CTCAE, version 4.0.

Source documents will consist of reports (mammogram reports, laboratory reports, etc.), and forms created specifically for this study that will capture information such as physical exam, phone contact information, adverse events, concomitant medications, demographics, etc. Source document files/chart or electronic medical records must be kept available for monitoring and throughout data analysis.

All participants will be registered in the Comprehensive Research Information System (CRIS) managed by the Department of Biostatistics and the Cancer Center's Biostatistics and Informatics Shared Resource. Questionnaires will be completed online by participants for direct entry into REDCAP. Where this is not possible, paper forms will be used and will be directly photocopied (assuring that no personal identifiers such as name or medical record number are retained) and these will be sent for data entry. All data forms will be stored in subject specific binders in the data office. All CRFs and questionnaires will be identified by participant initials and study ID number only. Data from laboratory and other research assessments will be entered into appropriate databases for eventual relay to the biostatistician for merging together with clinical information collected in REDCAP for statistical analyses, including quality control checks.

All data will be audited and verified prior to being considered final. When the database has been declared to be complete and accurate, the database will be locked and sent for statistical analysis.

12.2 Data and Safety Monitoring Committee

This study will be monitored by the University of Kansas Cancer Center Data Safety and Monitoring Committee, as per institutional guidelines. There is a quarterly review of subject recruitment, subject retention, adverse events, and serious adverse events.

12.3 Sponsor Monitoring

Any required clinical monitoring by the provider of the study agent will be accommodated and specific procedures incorporated into the protocol.

12.4 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.), as well as IRB records and other regulatory documentation will be retained

by the Protocol Lead Investigator (Dr. Carol Fabian) in a secure storage facility in compliance with HIPAA, OHRP, FDA regulations and guidelines.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The experimental design for the study is an open label, single arm pilot study of Duavee® as a prevention drug for breast cancer in high risk peri- or post-menopausal women at high risk for development of breast cancer, with Ki-67 positivity of <4%. Participants will receive Duavee® one tablet daily for six months. No randomization or stratification is involved. The primary endpoint is demonstration of feasibility for subsequent trials of Duavee® using a series of pre-set criteria having to do with accrual rate, completion, and lack of evidence for an increase in breast epithelial cell proliferation. A secondary endpoint will examine the specific potential for Duavee® to actually reduce cell proliferation and thus function as a breast cancer chemopreventive agent. A series of exploratory endpoints will document clinical efficacy (relief of vasomotor symptoms) and will examine modulation of other quantitative biomarkers potentially related to breast cancer development.

13.2 Primary Feasibility Endpoints

The primary endpoint overall is an assessment of feasibility that will inform the decision about and the design of subsequent trials. This will be based on considerations of 1) ease of accrual of the target population; 2) acceptance and tolerability of the intervention. Basically. If either fail, then it would indicate that future trials with this approach are not feasible to consider

For the first aspect of accrual, a criterion has been set at 20 participants accrued in 6 months (starting from when the trial is actually open to enrollment). If the accrual rate is slower than this, it would be impractical to conduct a similar trial at a single institution. While total accrual will be used for this initial criterion, the relative ease of accrual into the two cohorts (Cohort A, Ki-67<1%; Cohort B, 1% ≤ Ki-67 < 4%) will be taken into consideration. Certainly it is anticipated that accrual to Cohort A will be easier and faster, but if accrual to Cohort B is not at least half the overall rate (i.e., 20 participants per 12 months) then it would indicate that a trial requiring measureable Ki-67 >1% would not be a feasible option. For this assessment, accrual is considered as meeting all eligibility criteria, signing an informed consent, and receiving the first dose of study agent. This number will then be used for consideration of the second feasibility aspect.

The second aspect of feasibility is retention of participants on trial, completion of the planned 6 months of intervention, and acquisition of repeat RPFNA specimen (plus blood, mammogram, etc.) for assessment of modulation in quantitative biomarkers. A criterion has been set at 85% completion overall. While the ultimate goal is paired samples for comparison of biomarkers (for sample size and power calculation purposes), a high incidence of early problems with participant compliance in general (adverse events, complaints about the study agent or the schedule, etc.) would serve as an indication that the intervention would not be suitable for future trials.

The above criteria are intended to inform consideration of future biomarker trials with Duavee®; not to provide conclusive proof of principle. Thus, pre-set values for “success” or “failure” have been established; rather than a formal approach of statistical analysis and sample size power calculations. The total number of 40 participants represents a reasonable number for a pilot trial. Given our experience with similar biomarker trials using a variety of SERMs or an AI, this size

trial will readily document efficiency in accrual and identify problems with participant acceptance (compliance, retention, completion).

13.3 Primary Quantitative Endpoint

The primary quantitative (but secondary overall) endpoint addresses the specific question that Duavee® might produce the deleterious effect of increasing breast cell proliferation. While there is no evidence for this (and the FDA does not consider this a risk to be included in labelling) it is still a perceptual concern by women and providers. Thus, an assessment will be made of the frequency of participants that exhibit a likely clinically meaningful increase in Ki-67. This will be defined differently for the two cohorts, but both will contribute to the analysis of potential increase. For Cohort A (initial Ki-67 < 1%), a meaningful increase is defined as any value of 2% or greater on the off-study aspiration. For Cohort B (initial Ki-67 $\geq$1% but <4%), a meaningful increase is defined as a doubling or greater of the initial rate (i.e., a repeat aspiration value of at least 2%). Historically, for women in our High Risk Breast Clinic similar to those being enrolled in this study, approximately an 11% incidence of Ki-67 increasing is observed in the absence of any intervention. Therefore, with a minimum (per feasibility endpoint #2) of 34 paired specimens in which to assess change, an observed frequency of 10% or less would provide assurance that the true rate does not exceed 20% (95% confidence interval). Conversely, an observed frequency as high as 20% could still accommodate a true rate of 10%. Thus, as long as the observed frequency is ≤20%, subsequent trials could be designed with a placebo-control arm. Only for an observed frequency >20% would it be impractical to design future biomarker trials.

13.4 Secondary Exploratory Endpoints

This study is not specifically powered to address the objectives of estimating favorable change in quantitative biomarker endpoints that may be related to risk for development of breast cancer.

The most critical biomarker from a breast cancer prevention standpoint is that of cell proliferation, specifically a reduction in value for participants whose baseline specimen exhibited sufficient Ki-67 staining (1%) that there is the potential for a decrease). Thus, this analysis will be limited to those participants in Cohort B. Also this parameter will be analyzed first and separately from all other biomarkers. Descriptive statistics will be used to summarize the values of Ki-67 (percent of cells staining positive) in the baseline specimen, the 6-month (off-study) specimen, and the change (both absolute and relative) from baseline to off-study. For statistical analysis, the Wilcoxon signed-rank test will be used to accommodate the expected small sample size and to avoid any assumptions regarding normal distributions. Only participants who provide paired specimens evaluable for Ki-67 will be included in this analysis. However, for off-study specimens where the cell yield is so low (<100 cells per slide) that assessment of Ki-67 is not even possible, an anti-proliferative effect can be inferred. Thus, a value of 0.5% will be imputed as a reasonable compromise between imputing a value of 0% (no evidence of any proliferation) and a value of 1% (e.g., only 1 positive cell out of 100 counted). Since a non-parametric approach is used, the exact imputed value has minimal impact given that the criterion for inclusion is a baseline value of 1%. For this primary evaluation of modulation of a quantitative measure, $p<0.05$ will be considered the criterion for statistical significance.

Should there be adequate, paired specimens, further exploration of this primary endpoint variable, relative change in Ki-67, will be accomplished by developing an appropriate model and testing covariates. If necessary, an appropriate transformation will be used in this regression.

For these possible analyses, as for the exploratory analyses below, no adjustment will be made for multiple comparisons, but the results will be interpreted with caution.

Secondary to analysis of Ki-67, other variables will be assessed that represent a wide variety of cellular, molecular, and physical factors that have been proposed to have some level of connection with the process of breast cancer development. Categorized by the "specimen" being assessed, the variables are:

Participant
- Mammographic breast density
- Weight and BMI,
- DEXA body composition and waist measurement

Blood
- Hormones
- Adipocytokines
- Bazedoxifene levels

RPFNA
- Cytomorphology (categorical)
- Peptides and phosphopeptides (proteomics)
- Gene expression (RT-qPCR)

Descriptive statistics will be used to summarize the values at baseline (prior to start of intervention), at 6 months (after completion of intervention), and the change (both absolute and relative) from baseline to off-study. Medians, ranges, and interquartile ranges will be employed for all variables, along with mean and standard deviation for variables shown to be normally distributed. As appropriate, 95% confidence intervals will also be computed.

It should be noted that these variables are anticipated to be assessed for all participants that provide paired specimens, and thus the total should be close to 40. This provides additional opportunity to achieve normal distributes and to conduct exploratory correlational analyses. Thus paired t-test will be used for normally distributed values (initial or after appropriate transform), but nonparametric tests such as the Wilcoxon signed rank test will be used when necessary

Since these analyses are considered as exploratory, no adjustments will be made for multiple comparisons and $p<0.05$ will be considered the criterion for statistical significance. However, caution will be employed in the interpretation and presentation of the results.

Lastly, an assessment of menopause symptom relief in this cohort will be conducted using a 29 item validated Menopause Quality of Life Intervention Questionnaire and hot flash assessment. The same statistical methods as above will be employed. The intent is not statistically significant "proof" of efficacy as Duavee® is already FDA-approved for this indication.

13.5 Reporting and Exclusions

All participants who receive any Duavee® will be analyzed for adverse events. For biomarker modulation, all participants that have evaluable pre-study and post-study specimens and assessments will be included in the analysis,

Compliance will be determined by tablet counts, which will be done at the end of treatment. Participants that maintain 70% compliance throughout the duration of the trial will be considered "compliant". Compliance, in terms of the number of tablets missed and percent of participants compliant, will be summarized with descriptive statistics. Supporting tests for the analyses will

be performed including only these participants who are compliant and have taken Duavee® for at least 3 months and have taken Duavee® within 2 weeks prior to the post-study aspiration.

13.6 Interim Analysis

There will be no formal interim analysis of the endpoints of the study. Particularly, the quantitative endpoints (other than cytomorphology and Ki-67) are routinely batch-processed at the completion of study with pre- and post-intervention samples assayed together. However, it is possible that the feasibility endpoints could be evaluated early. For example, if six participants drop out of the study early, one would not require enrolment of the full planned 40 participants to conclude that the drop-out rate of 15% was unacceptably high. If it becomes obvious that the trial will not demonstrate feasibility, then accrual to the trial can be terminated early.

Safety data will be reviewed quarterly by the KUMC KUCC Data Safety and Monitoring Committee.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Investigator will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations.

14.2 Other Required Documents

- Signed and dated current (within two years) Curriculum Vitae or biosketch for all investigators listed on the Form FDA 1572.
- Current medical licenses for all investigators listed on Form FDA 1572.
- Lab certification (e.g., CLIA, CAP) and lab normal ranges for all labs listed on Form FDA.
- IRB Membership list/letter from IRB.
- Documentation of training in “Protection of Human Research Subjects” for all investigators listed on the FDA Form 1572.

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Principal Investigator must obtain written approval to conduct the study from the University of Kansas Medical Center’s Human Subjects Committee (IRB). Should changes to the study become necessary, amended protocol must be approved by the KUMC HSC prior to implementation.

14.4 Informed Consent

An informed consent will be signed by every participant that undergoes an RPFNA. This informed consent is used in conjunction with the KUMC HSC# 4601 study, which is the High Risk Protocol. If a participant is interested and eligible for study participation, she will then be given a copy of the study-specific informed consent document.

All potential study participants will be given a copy of the KUMC HSC-approved study-specific Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, she will be asked to sign the study-specific Informed Consent document. The study agent will not be released to a participant who has not signed the study-specific Informed Consent document. Participants who refuse to participate or who withdraw from the study will be treated without prejudice.

As part of the informed consent, participants will allow the use of blood samples and tissues collected for this study to be used for further research purposes.

The study-specific informed consent document must be reviewed and approved by the KUMC HSC prior to study initiation. Any subsequent changes to the study-specific informed consent must be approved by the KUMC HSC prior to implementation.

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15. FINANCING, EXPENSES, AND/OR INSURANCE

All laboratory tests and clinic visits required specifically for study participation will be done at no cost to the participant or her insurance company.

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