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Synopsis

This study will use a 2 x 2 randomized design with all 100 post- menopausal subjects being randomized to metformin, placebo, placebo and intensive lifestyle intervention, or metformin and intensive lifestyle intervention. Treatments will last 4 months. Participants will be assessed at baseline and end of treatment with endometrial sampling to see if there are changes in the biomarkers.

Protocol Sections

1.0 Objectives

1.1 Primary Objectives

1.1.1 The primary objective of this study is to evaluate the independent effects of metformin and a diet and physical activity intervention on potential surrogate endpoint biomarkers (SEBs) of the endometrium in obese post-menopausal women. The SEBs includes the panel of genes listed in Table 1 in Section 2.6 and Ki-67 biomarker.

1.1.2 Other biomarkers will be examined, including

1.1.2.1 A panel of genes (PCNA, IGF-IR, RALDH2, sFRP4, survivin, and EIG121) relevant to estrogen-dependent endometrial proliferation, hyperplasia and cancer using Q-PCR.

1.1.2.2 Biomarkers specific to the effect of metformin treatment (phospho-AMPKa, phospho-ACC, phospho-mTOR and phospho-S6 ribosomal protein).

1.2 Endpoints

1.2.1 The primary endpoint biomarker that will be investigated is proliferation Ki-67.

1.2.2 Secondary endpoints include:

1.2.2.1 Changes in serum levels of estradiol, estrone, testosterone, DHEA-S, sex hormone binding globulin (SHBG), adiponectin, glucose (fasting), HbA1c, insulin (fasting) and insulin-like growth factor-1 (IGF-1).

1.2.2.2 Changes in diet, physical activity, body composition (BMI, waist and hip circumference, and measurements from the DEXA scan), quality of life, and psychosocial determinants of behavior change will be examined in this sample of obese, post-menopausal women taking metformin or receiving a diet and physical activity intervention.

2.0 Background

2.1 Obesity and endometrial cancer

Numerous epidemiologic studies have demonstrated that obesity is strongly associated with an increased risk of endometrial cancer. While an average woman has a 3% lifetime risk of endometrial cancer, obese women have a 9-10% lifetime risk of endometrial cancer.[1] In addition, obese women account for almost 50% of all endometrial cancers.[1] In a recent review of diet and cancer by the American Institute for Cancer Research and World Cancer Research Fund (WCRF), authors stated that the evidence relating body mass index and cancer is strongest for endometrial cancer, but also is compelling for breast, colon, kidney and gallbladder cancer.[2]

There are a number of ways in which obesity has been hypothesized to increase risk of endometrial cancer. Obese women have increased bioavailability of adrenal steroids, increased peripheral conversion in adipose tissues of the adrenal steroids to estrone, and increased bioavailability of free estrogens due to decreased sex hormone binding globulin. This “hyper-estrogenic state” is believed to result in increased endometrial cell mitosis, leading to endometrial hyperplasia, a pre- malignant lesion, and to endometrial cancer. However, increased levels of circulating endogenous estrogen cannot completely explain the association of obesity and endometrial cancer. While some clinical studies show a correlation between higher plasma levels of endogenous estrogens or androgens in women with endometrial cancer versus controls, others do not.[3-13] Epidemiologic studies addressing this question also yield conflicting results. In a large study by Potischman et al, the authors found that body mass index remained a significant risk factor for the development of endometrial cancer even after controlling for endogenous estrogen. They concluded that high levels of endogenous estrogens were not sufficient to explain the increased risk of obesity to endometrial cancer.[11] Another prospective multicenter study demonstrated that the risk of endometrial cancer associated with endogenous estrogen levels in postmenopausal women remained significant after adjusting for BMI.[13]

What other mechanisms could explain the association of obesity to the development of endometrial cancer? Are there other mechanisms that explain why obesity isassociated with a number of other cancers? The American Cancer Society has recently highlighted the increased risk of a number of cancers in obese women. In particular, cancers of hormonally regulated organs, including endometrium, breast and ovary, have been shown in epidemiologic studies to be associated with obesity.[14]

Other cancers including gallbladder, colon and prostate are also seen more frequently in the obese.[14] Is there an underlying metabolic cause for this increased cancer risk?

Hyperinsulinemia and the insulin resistant state are closely associated with obesity, and may contribute in a meaningful way to increase the risk of cancer in an obese person. Recently, adiponectin, an adipocyte- derived protein highly correlated with hyperinsulinemia, has been shown to be associated with endometrial cancer, independent of body mass index.[15, 16] One study has demonstrated that this association between adiponectin and endometrial cancer may be stronger in postmenopausal women than premenopausal women.[16] Studies in patients with diabetes have demonstrated varying effects of metformin on serum levels of adiponectin.[17, 18]

Furthermore, since insulin has been shown to potentiate estrogen-regulated gene expression, obese women in particular may be at increased risk for estrogen associated cancers, including endometrial cancer. Epidemiologically, a number of studies have shown a modest association of diabetes with endometrial cancer risk.[19-24] Interestingly, in three studies the most significant risks were seen in women who were both obese and diabetic.[19, 22, 24] A study by Troisi et al examined insulin levels in women with endometrial cancer compared to controls in order to determine if elevated insulin levels could explain the association of obesity and endometrial cancer.[25] While elevated serum insulin levels, as measured by C-protein, were associated with increased risk of endometrial cancer, elevation of serum insulin levels *alone* could not account for the association of obesity and endometrial cancer. In this study we will address the hypothesis that obesity associated hyperinsulinemia and insulin resistance, in combination with hyperestrogenism, contributes to the increased risk of endometrial cancer.

2.2 Metformin, mechanism of action and possible role in the prevention of cancer

While metformin was first described more than 50 years ago, the exact molecular mechanism of action for this drug has only recently been determined.[26] Metformin activates AMP-activated protein kinase (AMPK) via phosphorylation by the LKB1 tumor suppressor.[27, 28] AMPK is typically activated in response to metabolic stress, and results in inhibition of ATP-consuming pathways, such as cholesterol synthesis, fatty acid synthesis and protein translation.[29, 30] Importantly, AMPK inhibits mTOR via phosphorylation and activation of tuberous sclerosis complex 2 (TSC2). TSC1/TSC2 forms the TSC complex that negatively regulates mTOR signaling.[31]

Recent clinical studies suggest that metformin may be associated with a decreased risk of cancer. In an observational cohort study of diabetics performed by Libby et al, incident cancer was diagnosed among 7.3% of 4,085 metformin users compared with 11.6% of 4,085 comparators ($p<0.001$).[32] In a recent case-control study performed at MD Anderson, Li et al reported that diabetic patients who had taken metformin had a significantly lower risk of pancreatic cancer compared to those who had not taken metformin.[33] Jiralerspong et al reported that among diabetic women with breast

cancer undergoing neoadjuvant chemotherapy, those individuals who were on metformin had a higher pathologic complete response rate than those who were on other anti-diabetic medication.[34] The results of these studies are provocative, and suggest that metformin may have a role in both the prevention and treatment of multiple cancers.

The signaling pathways activated by metformin treatment suggest a promising role for metformin as an anti-cancer agent in endometrial cancer, as well as other cancers that have activated PIK3CA/AKT/mTOR pathways. Metformin treatment in estradiol treated Balb/c mice resulted in significantly decreased density of endometrial glands, decreased immunohistochemical expression of mTOR signaling and decreased proliferation indices as measured by PCNA expression.[35] Ben Sahra et al investigated the effects of metformin on human prostate cancer cells (LNCaP) and in an *in vivo* xenograft mouse model. Their studies identified that in LNCaP cancer cells metformin activated AMPK and inhibited proliferation by blocking the cell cycle in G0/G1 phase, which was accompanied by decreased cyclin D1, decreased phosphorylation of Rb and increased p27Kip1 expression. Metformin treatment of an LNCaP tumor bearing xenograft model resulted in a significant reduction in tumor growth.[36] Schneider et al. investigated the effect of metformin in high-fat diet fed hamsters; a model of pancreatic cancer. They identified that 50% of the high-fat diet fed hamsters develop pancreatic tumors; whereas metformin treated high-fat diet fed hamsters did not develop tumors.[37] Additionally, administration of metformin to LKB1 hypomorphic PTEN+/- mice increased AMPK phosphorylation and significantly delayed tumor onset with 100% of the control group developing tumors of the liver, spleen and intestine by 4 months of age and the metformin treated group developing tumors by 5 months of age.[38] Taken together, these studies suggest that metformin may prevent or significantly delay tumor progression as a consequence of AMPK activation.

The effect of metformin on the endometrium has been examined in women with polycystic ovarian syndrome. In a small, open-label study of metformin versus rosiglitazone, endometrial histology was evaluated at baseline and following a 3- month treatment period in 16 women with PCOS. Two women with simple hyperplasia were treated with metformin and experienced regression during treatment providing preliminary evidence that metformin may have beneficial effects on the endometrium.[39] The results of this study, in addition to our studies in rodents, provide evidence that metformin has a direct effect on the endometrium.

2.3 Diet and exercise linked to hyperinsulinemia and insulin resistance

The landmark Diabetes Prevention Program (DPP) trial compared a lifestyle intervention or metformin to metformin placebo. The intensive portion of the lifestyle intervention was composed of 16 face-to-face sessions with a lifestyle coach in which participants were assisted with increasing their physical activity, reducing their caloric intake, and reducing weight by at least 7%. The trial demonstrated that both a lifestyle intervention and metformin reduced incidence of diabetes, with larger effects for the lifestyle intervention [22]. **Particularly notable given the relationship between**

endometrial cancer and adiponectin was that both interventions were associated with increases in adiponectin, with the effects of the lifestyle intervention being approximately 3.5 times larger than those for the metformin group [23]. Lifestyle intervention and metformin were also associated with reductions in metabolic syndrome [24] and (C-reactive protein) CRP in women, the reduction in CRP was related to weight loss [25]. The effects of the lifestyle intervention on adiponection may be related primarily to weight loss; a small study found that reduced calorie diet with or without exercise increased circulating adiponectin, but that exercise alone and exercise plus reduced calorie diet enhanced expression of adiponectin receptors in skeletal muscle and adipose tissue [26]. Increases in adiponection also have been found in response to weight loss after bariatric surgery [27]. Interestingly, in a recent report on the association of bariatric surgery and cancer incidence, the disease most profoundly affected was uterine cancer, primarily EndoCa [28].

We are intrigued by the possibility that both pharmacologic intervention with metformin and a lifestyle behavior intervention, both of which reduce insulin resistance, might also have a direct chemopreventive effect of EC. Currently, options for EC chemoprevention include use of the oral contraceptive or use of progestins.[40] Both have limitations in an obese cohort. Oral contraceptives (OCP) are contraindicated in morbidly obese individuals due to an increase in rate of thrombo-embolic events. In addition, obese women frequently have hypertension or hypercholesterolemia, which are also considered contraindications to OCP use. Progestins have similar contraindications and compound obesity by stimulating appetite. While weight loss and exercise are obvious solutions to decreasing the risk of EC in obese individuals, exploration of alternative approaches is necessary. The novelty of these chemoprevention/behavioral prevention strategy is twofold. First, it proposes to treat an underlying metabolic syndrome. Rather than focusing on the prevention of a single cancer, this strategy may have not only had an overall benefit for the cardiovascular health of the obese woman but may also decrease the risk of endometrial, pancreatic and perhaps other cancers associated with obesity and insulin resistance.[33] Second, it exploits the newly identified function of metformin as a drug that demonstrates direct anti-proliferative effects on endometrial (and other) cells via activation of AMPK and inhibition of mTOR.

2.4 Rationale and definition of cohort

Endometrial cancer has well-defined risk cohorts. Epidemiologic studies have consistently shown that women who are overweight are at increased risk of endometrial cancer, and women who are very overweight are at very increased risk. Brinton et al conducted a large case control study and found that women who were greater than 200 lbs. had a relative risk of 7.2 compared with women less than 125 lbs.[41] In order to maximize risk for our cohort, we will include women who fulfill standard criteria for obesity grade I and above, which is defined as body mass index (BMI) greater than or equal to 30 kg/m^2. An upper age limit of 65 has been set. We chose not to include pre-menopausal women in this study because of the confounding problem of irregular cycling and varying estrogen levels in pre- menopausal obese, insulin resistant women (i.e. women with polycystic ovary

syndrome). We also chose not to include women who fulfill criteria for diabetes, since additional agents, other than metformin, may be necessary to control their diabetes.

2.5 Safety issues of metformin, as chemoprevention for endometrial cancer in a cohort of obese women

Metformin hydrochloride (Glucophage, *Bristol-Myers Squibb*) is a biguanide antihyperglycemic agent. It corrects Type II diabetes mellitus by altering glucose production and absorption, in contrast to sulfonylureas, which stimulate insulin secretion. Specifically, metformin improves insulin sensitivity by increasing peripheral glucose and utilization, decreasing intestinal absorption of glucose and suppressing glucose production by the liver, thus lowering serum insulin levels.

We believe that metformin is not only safe in this population, but also beneficial.

2.5.1 A randomized phase III study with metformin was conducted in this same population i.e.: insulin resistant but non-diabetic patients (the Diabetes Prevention Program Randomized Trial NEJM 2002 (6)346, p393), and demonstrated that metformin was well tolerated in a population of insulin resistant, non-diabetic individuals (and decreased the incidence of diabetes).[42] This study demonstrates the safety of using this drug in this population and also suggests that we can potentially dovetail the cardiovascular and metabolic health benefit of the drug with chemoprevention.

2.5.2 Metformin does not increase secretion of insulin and therefore does not cause hypoglycemia**.**

2.5.3 Use of metformin in insulin resistant but non-Type II diabetics also has precedence in the treatment of women with polycystic ovarian syndrome, or PCOS. Clinicians use metformin or thiazolidinediones, insulin-sensitizing agents, in young PCOS women, whose underlying metabolic problem is insulin-resistance. Interesting, treatment for their insulin resistance results in normalization of their pituitary/reproductive hormone axis and subsequently results in ovulation and regular periods.[43, 44]

2.6 Biomarkers as surrogate endpoints

Endpoints for chemoprevention studies can either be development of cancer, or a surrogate for the development of cancer. In this study we will use surrogate biomarkers as the primary endpoint.

Identification of appropriate biomarkers is essential for this study. An ideal biomarker is a histological or molecular "red flag" that precedes the onset of cancer. Ideal surrogate endpoint biomarkers are quantitative and are modulated by

chemopreventive agents.[45] In our prior endometrial cancer chemoprevention study of women with HNPCC, we evaluated a panel of 30 biomarkers thought to be relevant in endometrial carcinogenesis. From that study, we have narrowed down the field to a subset of 6 validated, estrogen-regulated genes and a proliferation marker Ki-67.[46]

The following is a summary of the analyses to be performed on the endometrial biopsies at baseline and following four months of hormonal therapy:

- **Proliferation index Ki-67**
- **Histology**
- **Quantitative PCR transcript analysis**

Table 1: Estrogen-dependent panel of genes for PCR transcript analysis

Gene	Marker / Pathway
Proliferating Cell Nuclear Antigen (PCNA)	Proliferation marker
Insulin-like Growth Factor Receptor (IGF-IR)	IGF Pathway
Retinaldehyde dehydrogenase 2 (RALDH2)	Retinoid Pathway
Secreted Frizzled-Related Protein 4 (sFRP4)	Wnt Pathway
Survivin	Apoptosis
Estrogen-inducted gene (EIG121)	Novel gene of unknown function

Biomarkers associated with metformin function and downstream signaling includes:

- phospho-AMPKa
- phospho-ACC
- phospho-mTOR
- phospho-S6 ribosomal protein.

We will examine pre- and post- treatment endometrial biopsies for histology and proliferation using Ki-67. These are standard surrogate endpoints used in cancer chemoprevention trials. In addition, our group has identified a set, or panel of genes expression assays relevant to estrogen-dependent endometrial proliferation, hyperplasia and cancer. The set of genes, listed in Table 1 above will also be examined using real-time PCR in the pre- and post- treatment biopsies.

The Ki-67 as well as the biomarkers associated with metformin action analysis will be performed by the MD Anderson Core Histology lab and read by Dr. Broaddus in the Pathology Core. Q-PCR analyses will be performed by Dr. David Loose, PI of the Biomarkers core. Dr. Loose has been interested in identifying genes in human endometrium whose expression is altered by hormone replacement therapy (HRT). Over the last 5 years he has used several techniques including differential display, microchip array screening, and real-time quantitative PCR to find such genes. The tools he has developed are directly applicable to the investigation of surrogate endpoint biomarkers (SEBs) in HNPCC patients treated with estrogens or estrogens plus

progestins.

2.7 Description of Proposed Involvement of Human Subjects

This study will be conducted at UT-MD Anderson Cancer Center, with recruitment focused on 100 subjects. Women >/=50 to </=65 years old, with BMI $\geq$ 30 kg/m^2, fasting blood glucose $\leq$ 126 mg/dl, and hyperinsulinemia, but not frankly diabetic are eligible. Those women who demonstrate fasting blood glucose greater than 126 mg/dl will not be referred for the study, and will instead be referred to contact an Endocrinologist from their insurance provider for management of diabetes. Women of any race or ethnicity who meet the eligibility criteria will be enrolled in the study. Based on our participant base, we anticipate the minority inclusion to be: 58% White and Non-Hispanic; 30% Hispanic; and 12% African-American.

Hyperinsulinemia will be determined by using the fasting insulin and fasting glucose levels to calculate the QUICK I, an index of insulin resistance. QUICK I = 1/ [log fasting insulin (μU/ml) + log fasting glucose (mg/dL)]. Values below and equal to 0.357 will be used to identify women who are insulin resistant. [47-49].

3.0 Background Drug Information

Metformin hydrochloride is a biguanide antihyperglycemic agent. It corrects Type II diabetes mellitus by correcting insulin resistance, in contrast to sulfonylureas, which stimulate insulin secretion. Specifically, metformin improves insulin sensitivity by increasing peripheral glucose and utilization, decreases intestinal absorption of glucose and suppress glucose production by the liver. Metformin does not increase secretion of insulin and therefore does not cause hypoglycemia**.** In both animal models and in humans, metformin decreases plasma glucose.

3.1 Agent

Metformin hydrochloride (Greenpark Pharmacy)

3.2 How supplied

Each capsule contains 425 mg of metformin hydrochloride that will be used in this study.

3.3 Dose groups

Metformin hydrochloride 425 mg capsule(s) vs. no treatment. See below for monthly dose and duration of Metformin hydrochloride.

3.4 Dose and Duration of exposure

Table 2: Monthly Dosage of Metformin or Placebo capsule(s)

	Week 1	Week 2	Week 3	Week 4
1st Month	One capsule daily	One capsule 2X daily	One capsule 3X daily	Two capsules 2X daily
2-4 Month	Two capsules 2X daily	Two capsules 2X daily	Two capsules 2X daily	Two capsules 2X daily

- Orally for 120 days (plus or minus 10 days).
- The dose of metformin hydrochloride will be in 425 mg capsule(s) by mouth, the dose used in The Diabetes Prevention Program Randomized Trials.[50]

3.5 Half life

Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life of approximately 6.2 hours. In blood, the elimination half-life is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution.

3.6 Administration

Metformin hydrochloride is self-administered.

3.7 Side effects

Diarrhea
Nausea/ Vomiting
Flatulence
Abdominal discomfort
Asthenia Ingestion
Weight gain Edema
Headache Heart
failure Lactic
Acidosis

3.8 Contraindications

3.9.1 Renal disease or renal dysfunction (e.g., as suggested by serum creatinine levels ≥ 1.4 mg/dL [females] or abnormal creatinine clearance) which may also result from conditions such as cardiovascular collapse (shock), acute myocardial infarction, and septicemia.

3.9.2 Congestive heart failure requiring pharmacologic treatment.

3.9.3 Known hypersensitivity to Metformin hydrochloride.

3.9.4 Acute or chronic metabolic acidosis, including diabetic ketoacidosis, with or without coma. Diabetic ketoacidosis should be treated with insulin. This will be assessed based on symptoms reported and reviewed with research staff during monthly toxicity screening visits.

3.9.5 Metformin will require a two day hold if given certain contrast medicines before X-rays, CT scans, MRI, and other procedures.

3.9 Use during pregnancy

Studies have shown that the drug does not exert teratogenic or embryocidal effects on animals, but there are no controlled studies in women.

3.10 Manufacturer and Supplier

Greenpark Pharmacy

4.0 Participant Eligibility

4.1 Inclusion Criteria

4.1.1 For this study, only women will be enrolled
4.1.2 Body Mass Index (BMI) $\geq$ 30 kg/m^2
4.1.3 Not frankly diabetic, as measured by a fasting blood glucose $\leq$126 mg/dL.
4.1.4 Demonstrate hyperinsulinemia with a QUICK I value </=0.357.
4.1.5 Age >/= 50 and </= 65
4.1.6 Zubrod Performance Scale 0-1
4.1.7 Hemoglobin >/= 10 g/dl
4.1.8 TSH 0.27 – 4.20 µIU/mL
4.1.9 Menopause as defined as no menses for 1 year and / or FSH $\geq$ 25.8 mIU/ml.
4.1.10 Must be able to read, write, and speak English.
4.1.11 Must have a Primary Care Provider (PCP).

4.2 Exclusion Criteria

4.2.1 Prior hysterectomy or endometrial ablation.
4.2.2 ALT $\geq$ 2.0x Upper Limit of Normal (ULN)
4.2.3 Serum creatinine $\geq$ 1.4 mg/dl

4.2.4 Triglycerides (fasting) ≥ 400
4.2.5 Known inability to participate in the ongoing appointments for the four months of the study and scheduled follow-up tests.
4.2.6 Significant medical or psychiatric history which would make the participant a poor protocol candidate, in the opinion of the principal investigator, for any aspect of study participation including metformin, unsupervised exercise program or dietary behavior change.
4.2.7 Participant reported history of congestive heart failure
4.2.8 Prior treatment with Metformin
4.2.9 Currently being treated for diabetes or meeting criteria for new diagnosis of diabetes.
4.2.10 Known allergy to Metformin or other biguanide (Proguanil).
4.2.11 Use of Aromatose Inhibitors, GNRH-agonists i.e.Lupron, Zoladex within the last 6 months
4.2.12 Use of SERMS (selective estrogen receptor modulators) in the past 6 months, including Tamoxifen and Raloxifen
4.2.13 Hormone replacement therapy within the last 6 months
4.2.14 Women who have been treated with chemotherapy for prior malignant disease or currently have an untreated malignancy other than non-melanoma skin cancer
4.2.15 Participants who have had prior radiation to the pelvis

5.0 Design and Methods

5.1 Study Design

For the proposed study, post- menopausal women with a BMI ≥ 30 kg/m^2 who demonstrate insulin resistance will be recruited from the Harris Health Hospital (Lyndon B. Johnson General Hospital - LBJ), community, and from the employee pool at MD Anderson Cancer Center using various outlets as advertisement. Project LEAP fliers will be posted at the LBJ for recruitment purposes only. All consenting, study visits, and study procedures will be done at the MD Anderson Cancer Center.

Participants will be assessed for their eligibility, first during a **Screening Call**, in which interested women will call the contact number and be screened using the **Screening Script**.

- Screening Call
  - Participants are screened using screening script. Once preliminary eligibility has been determined, then their information is forwarded to the study research nurse.
  - Study research nurse will:
    - Obtain reported medical history and medication history and schedule for Screening Visit 1.

- Explain the informed consent process. Participant may review the informed consent information electronically by email, by telephone, or in-person.
- Instruct participant on fasting and explain the blood tests to be collected on Visit 1 (See Table 5: Study Parameters for the list of lab tests to be collected on Screening Visit 1.)
- Provide driving directions to MD Anderson Cancer Center.
- Answer any relevant questions or concerns of the study.
- Initiate a New Patient (participant) Intake form by phone and send to Mays Clinic Gynecologic Oncology Business Center. Instruct participant that she will register in-person at the Mays Clinic Gynecologic Oncology Business Center on Screening Visit 1 day to obtain Medical Record Number (MRN).

5.2 Screening Visit 1

- Screening Visit 1 includes:

a. Registration at Mays Clinic Gynecologic Oncology Business Center (MCGOBC) to obtain a Medical Record Number (MRN).

b. Confirm that participant has fasted that day. Review previous information discussed by phone, and answer questions as needed.

c. Participant to meet with study Gynecologist briefly at MCGOBC for obtaining consent and discussing any concerns of the study. Once consent is attained then participant will be scheduled for laboratory tests to be collected during the same day (see Table 5: Study Parameters for the list of laboratory test to be collected for Screening Visit 1).

d. Pre-registration completed in CORe (See section 5.6 for Registration Process).

e. Provide participant education:

i. Endometrial biopsy (education packet that lists pre- and post- procedure instructions and contact information). See Appendix L for Patient Education Packet on Endometrial Biopsy (EMB).

ii. Taking NSAIDs (Non-Steroidal Anti-inflammatory Drugs) orally such as ibuprofen or Motrin or Advil if no prior allergies and available over the counter.

1. Pre-biopsy: 600 mg of NSAIDs once approximately 1 hour before EMB.
2. Post-biopsy: 600 mg of NSAIDs every 6 hours as needed for cramps or pain.

iii. Participants who are unable to take ibuprofen / Motrin / Advil and have no allergies can also take acetaminophen (Tylenol) over the counter as an alternative NSAID.

1. Pre-biopsy: 325 mg acetaminophen take once approximately 1 hour before EMB
2. Post-biopsy: 325 mg of acetaminophen every 6 hours as needed for cramps or pain

At the Screening Visit 1, participants will be asked to complete one 24-hour dietary recall during the screening visit, using NCI's online ASA-24 system. Anytime between the Screening Visit 2 and 7 days post biopsy, the participant will complete two additional 24-hour dietary recalls (one on a weekday and 1 on a weekend). These additional recalls can be completed by phone or using their own home computer. Participants will also be given the survey packet to be completed before they return for the Screening Visit 2. Both the 24-hour diet recall and survey packet are described in detail below. All participants will be given an accelerometer (to obtain objective measure of physical activity), shown how to use it, and asked to wear it for one week between the screening visit and
7 days post-biopsy. They will return the accelerometer at the Screening Visit 2. If they are not able to return it at the second visit, they may return it by mail (prepaid mailer will be provided) or at their first intervention session (if randomized to receive the lifestyle intervention).

5.2.1 24-hour Dietary Recall

The Automated Self-administered 24-hour Dietary Recall (ASA24) will be used to document the participant's food intake for a total of 24 hours. Three recalls will be obtained at baseline and at 4 months, at least one of which will be for a weekend day. The overview of this questionnaire can be located at http://riskfactor.cancer.gov/tools/instruments/asa24. The participants will be given a designated url which will provide them access to the online questionnaire. If the participant does not have computer or internet access, the recall will be performed over the phone with the assistance of the Research Coordinator or Research Dietitian. To assist participants, the ASA24 utilizes graphics, pictures (to help approximate portion size), an animated guide, and audio cues and languages, which aid populations with low literacy [60]. The foods selected by the participants are from the USDA's Food and Nutrient Database for Dietary Studies' (FNDDS) most up-to-date database. Participants will begin completing these assessments approximately one week before their Screening Visit 2 and at four-month BRTC visits, with the third assessment completed within 1 week of the second visit/4-month visits.

5.2.2 Godin Leisure Time Physical Activity

A 3-item modified version of the Godin Leisure Time Physical Activity Questionnaire

will be used to measure the participants' usual leisure-time exercise habits. It will be administered as part of the survey packet and will be given to the participant during the Screening Visit and the Post-Treatment visit. It is easy to administer and has good test-retest reliability (.81 for total score), and significant correlations with VO^2max. The responses from this survey at baseline will be used to determine if the participant is meeting guidelines for physical activity, as discussed in section 7, and used for randomization.

5.2.3 Accelerometers

At baseline and month 4, participants will wear accelerometers (Actigraph GT3X+) for 7 days to provide an objective measure of physical activity. Before the16 week assessment staff will mail the accelerometers to participants along with the materials needed for the 24 hour recall interviews. The accelerometer, which will be worn while the participant is awake, is small (1.5 x 2 inches) and can either be clipped to the belt or waistband or placed in a pouch attached to a belt that can be worn under clothing. Cutpoints have been established for light, moderate, hard, and very hard activity [63-65]. Participants also will complete a paper-and-pencil exercise diary each day during the week they wear the accelerometer. The diary will ask participants to indicate what type of exercise was performed, duration of the exercise in minutes, and the effort level during the exercise.

5.2.4 Behavioral determinants

Guide to Health Survey

The Guide to Health survey, developed by Anderson-Bill et al, will be used to measure Social Cognitive Theory-based behavioral determinants (social support, self-efficacy, outcome expectations, and self-regulatory behavior) related to healthy eating patterns and physical. The subscales have very good internal consistency (with the exception of one subscale with an alpha of .66, all are between .77 and .95); they were largely responsive to an online intervention targeting physical activity and healthy eating, and were related to behavior change [66, 67]. The diet self-efficacy scales measure healthy eating patterns (e.g., eating more fruits and vegetables, less fat).

Weight Efficacy Life-style (WEL)

The weight efficacy life-style (WEL) questionnaire will be used to assess the participant's confidence that they can regulate their eating. This scale has been shown to have high internal consistency reliability and is responsive to cognitive-behavioral weight loss interventions [68]; it also partially mediated the effect of a group weight loss intervention for older adults [69]. Also, the Guide to Health scales measure self-efficacy for maintaining an exercise program over time and in a range of situations, but in our study of exercise with endometrial cancer survivors we found that self-efficacy for being able to physically do the exercise (task self-efficacy) was also important [70]. Therefore we will use the exercise self-efficacy

scale from that study (internal consistency reliability = .96), which was based on measures by McAuley [71].

Participant-Reported Outcomes Measurement Information System (PROMIS)

The Participant-Reported Outcomes Measurement Information System (PROMIS) global short form will be used to evaluate the effect of the interventions on participant quality of life. This questionnaire assesses physical function, pain, fatigue, emotional distress, and social healthy domains. The PROMIS global short form has been validated and shown to have similar correlations for the global physical health (GPH) and global mental health (GMH) items as similar items from the SF-36 (36-item short- form health survey) and other measures of health related quality of life [72]. The reliabilities for GPH and GMH were 0.81 and 0.86 respectively which compared to SF-36 at 0.88-0.93 for their physical and mental health items [73]. The advantage to using the PROMIS short form versus the SF-36 is that it takes much less time for the participants to complete.

Self-Administered Comorbidity Questionnaire

Because we will not have medical records readily available for our participants, the Self-Administered Comorbidity Questionnaire will be included in the survey packet and be used to assess comorbidity of the participants. The questionnaire has been validated and has shown moderately strong correlation with the Charleston Index, which was used as a standard [74]. It displayed a Spearman correlation coefficient of 0.55 and kappas exceeding
0.46 for almost every item [74]. This questionnaire should serve as a suitable substitute for the medical record.

Demographic Questionnaire

A basic questionnaire will be used to gather demographic information from each participant and will include questions on race, education, employment, and age.

5.3 Screening Visit 2

Once participant eligibility is confirmed based on the blood test results from Visit 1, participant will be scheduled for Visit 2 in the following order:

a) Appointment with MD Anderson's Behavioral Research and Treatment Center (BRTC) for anthropometric assessments. The details of these measurements are described below in section 5.3.2.
b) Laboratory test collection (See Table 5: Study Parameters for the list of laboratory test to be collected for Visit 2).
c) Appointment in Mays Clinic Gynecologic Oncology for history, physical, and

endometrial biopsy (EMB).

5.3.1 Endometrial biopsy

An endometrial pipelle biopsy will be performed at the Screening Visit 2 and at the 4th month on days 115-125 (plus or minus 10 days) into treatment in all participants from (all four arms) both groups.

In addition to histologic examination, each endometrial biopsy will be examined for proliferation markers as well as other biomarkers. The second biopsy in the no treatment group is important in order to determine variability in the biomarkers due to sampling. In preliminary data from the Wyeth-Ayerst Study, we did see variability in certain transcript levels at the 3 and 6 month time points even in the placebo control group. Therefore, we feel strongly in this study that women in both groups undergo a 4 month EMB.

Risks of office endometrial biopsy are small, and while there are no published data on risk of perforation, we believe that the risk is less than 1/1000. Even in those participants in whom uterine perforation occurs, observation alone is usually sufficient. The severe perforations requiring laparotomy and emergent hysterectomy are not seen in office endometrial biopsies, rather in Dilatation and Curettage (D & C) procedures performed in the operating room.

Participants who have no allergies will be instructed to take orally Non- Steroidal Anti-inflammatory Drugs (NSAIDs) before and after the biopsies to reduce pain and cramping. The NSAIDs medications are available over the counter (See Section 5.2 for the recommended dose).

In the second screening visit, if the patient is experiencing discomfort while the provider is attempting to obtain the endometrial biopsy, we may administer topical lidocaine gel to the cervix. This is supported by prior studies which have demonstrated that patients may experience less pain with tenaculum placement and cervical dilation when topical lidocaine is used (Hassan et al, 2005; Davies et al, 1997). Occasionally, some patients may have cervical stenosis, which makes it difficult to dilate the cervix, insert the endometrial suction curette, and obtain an endometrial biopsy. In these instances, prior studies have suggested that the use of vaginal misoprostol may soften the cervix and increase the likelihood that the cervix can be dilated so that the procedure can be performed (Bastu et al, 2013; Ghosh et al; 2013). If cervical stenosis is encountered during the second screening visit, and the study gynecologist is unable to pass the curette or dilator through the cervix, the participant will be given a prescription for misoprostol 200mcg #2 tablets and re-scheduled to return to the clinic for a repeat attempt at performing the endometrial biopsy (within 10 days). The participant will be given instructions to self-administer 1 tablet intravaginally the night before the procedure and one tablet the morning of the procedure. Both lidocaine jelly and misoprostol will be administered as needed. Research Nurse will counsel the participant on the common symptoms of these agents prior to the second screening visit.

5.3.2 Anthropometric assessments

BRTC staff will measure the participants' height, weight, and waist circumference at baseline and 4th month. Height will be measured by having the participant stand evenly without shoes on a flat surface at a right angle to the movable vertical board or cap that is pulled down to lightly touch the most superior point of the head. Weight will be measured using either a standard beam scale or an electronic digital scale. The scale should be on a level floor, and participants should be gowned or wearing light clothing and no shoes. Waist circumference will be measured with the participant standing relaxed with hands hanging at her sides on the exhale. The tension-controlled measurement tape will be placed directly on the skin, horizontally around the narrowest part of the torso. If unable to locate the narrowest part of the waist, the participant will be measured 2-3 inches above the navel. The hip circumference will be measured with the participant standing relaxed with hands hanging at her sides on the exhale. The tension-controlled measurement tape will be placed directly on the skin or over the underwear, horizontally around the widest part of the hip/buttocks area.

The participant will undergo a whole body DEXA to measure body composition. The DEXA, routinely used to determine bone mineral density, has gained support in research application for assessing fat and lean body mass in whole body and in specific body regions [23, 24, 25]. Data from each scan will be as follows: percent body fat and % lean tissue, fat and lean mass for total body region as well as defined body zones (trunk, leg, and arms) expressed in grams.

5.4 Randomization / Visit 3

Participants, who are eligible based on results of the biopsy from Visit 2, will be called to schedule their third visit for randomization.

This study will use a 2 x 2 randomized design with all 100 subjects being randomized: 25 will receive both lifestyle intervention and metformin, 25 will receive the lifestyle intervention plus metformin placebo, 25 will receive metformin only, and 25 will receive metformin placebo but no lifestyle intervention. The participants randomized to the lifestyle change portion of the study will also have their first intervention session schedule for the same day as well. Treatments will last 4 months.

At Randomization / Visit 3, the participant will be:

a) randomized to one of the four intervention groups using the Department of Biostatistics Clinical Trial Conduct Website. See section 7.0 for details.
b) given prescription to fill the study medication at the pharmacy at MD Anderson Cancer Center.
c) participant will be provided education on recording and monitoring daily intake of study medication into the Pill Diary. See Appendix M for Pill Diary.

d) review and report side effects of the study medication.

Once the participant is randomized, the research nurse will notify the pharmacist to inform them of the randomization code in order to keep study staff blinded to assignment to metformin vs. placebo.

In-person toxicity assessments will be performed at the end of the first, second, third, and fourth month (plus or minus 10 days). Phone assessments will be done periodically as needed to assess for adverse effects. At the end of the fourth month of the study, the participant will return for the **Post-Treatment Evaluation** to repeat all measurements (all blood tests, anthropometric assessments, second biopsy, and survey packet) taken at the Screening Visits (1& 2). The participant will also be given a Satisfaction Questionnaire, based on their intervention, in advance to complete and return at this visit.

One year after baseline (+/- 1 month), participants will be contacted to complete a 1 Year Follow-Up Evaluation. This evaluation will include anthropometrics assessments, including a DEXA scan, the survey packet, and blood tests collected from the 4th month visit (see Table 5: Study Parameters for the list of laboratory test to be collected for 1 year Follow-up Evaluation). During this visit, the participant will also be compensated with a parking voucher to cover the cost of parking during the visit.

5.5 Study Compensation

A monetary compensation will be provided in the form of a gift card commensurate with the amount of time, inconvenience, and discomfort experienced by each participant while participating in the obtaining of the endometrial biopsies. The total possible compensation is $125.00 ($50 for baseline and $75 for the 4 month assessment). Participants who are eligible based on the results of Screening Visit 2 tests will receive the first gift card of
$50.00 on the Randomization / Visit 3. Participants found to be ineligible based on baseline EMB results will be contacted by phone and gift card will be mailed to them. Second gift card of $75.00 will be given after completion of all measurements on Post-Treatment Evaluation visit on the 4th month. For all study related assessment visits to MD Anderson each participant will receive parking validation.

5.6 Registration Process

This is a phase II, four-armed, randomized chemoprevention trial. Registration will be a two-step pre-enrollment to enrollment process.

A. New Patient (participant) Intake form will be initiated by study research nurse during phone interview with potential participant. Once form is completed, it will be sent to the MCGOBC to schedule participant for in-person registration.

B. At the first screening visit, participant will register in-person as a new participant at MCGOBC and will be assigned a Medical Record Number (MRN). After they have received an MRN they will meet momentarily with one of the study Gynecologist to obtain consent.

C. Consented participants will be registered in Clinical Oncology Research (CORe) System and will be assigned a unique identifying number at entry into the trial on the first screening visit. This unique subject number will not change during the study.

D. Only participants who meet all eligibility criteria and have successfully completed all laboratory tests and EMB will be randomized to CTC website on the third visit (see section 7.0 for details on randomization).

5.7 Participating Center

The Screening Visits (1 & 2), Randomization / Visit 3, Lifestyle intervention sessions, In-person toxicity assessments, and Post-Treatment Evaluation will occur at the University of Texas M. D. Anderson Cancer Center.

6.0 Treatment Plan

We have chosen to treat for a 4 month period. This period is based on studies demonstrating that metformin will reach full potential in the reduction of hyperinsulinemia and insulin resistance by four months.

6.1 Treatment

Prior to beginning therapy, participants will have a baseline endometrial biopsy performed. If the baseline endometrial biopsy reveals endometrial hyperplasia or cancer or if an office endometrial biopsy cannot be performed the participant will be removed from study. Women with abnormal biopsy results will be referred to contact their Gynecologist from their insurance provider for further treatment for these results.

The study is partially blinded in that investigator and participants will be blinded to the treatment of Metformin versus placebo, but will be un-blinded in regard to the lifestyle intervention. All participants will be followed for at least one month after their last dose of study medication. All treatment-related toxicities will be followed by phone until resolution. All other participants will be randomized to one of four arms:

6.1.1 Metformin and Lifestyle Intervention

- These participants will have a baseline endometrial biopsy.

- See Table 2 in section 3.4 for Metformin Monthly dose and duration.
- Second EMB and post study labs will be done between days 115-125 (plus or minus 10 days).
- For the lifestyle intervention, all participants will receive the intervention described below in section 6.2.

6.1.2 Metformin Placebo and Lifestyle Intervention

- These participants will have a baseline EMB at study entry.
- See Table 2 in section 3.4 for Metformin Placebo Monthly dose and duration.
- The second EMB, as well as post- study labs will be done between 115-125 days (plus or minus 10 days).
- For the lifestyle intervention, all participants will receive the intervention described below in section 6.2.

6.1.3 Metformin and No Lifestyle Intervention

- These participants will have a baseline endometrial biopsy.
- A total of four 30 day cycles (plus or minus 10 days) will be given (120 days).
- See Table 2 in section 3.4 for Metformin Monthly dose and duration.
- The second EMB, as well as post study labs will be done between 115-125 days (plus or minus 10 days).

6.1.4 Metformin Placebo and No Lifestyle Intervention

- These participants will have a baseline EMB.
- A total of four 30 day cycles (plus or minus 10 days) will be given (120 days).
- See Table 2 in section 3.4 for Metformin Placebo Monthly dose and duration.
- The second EMB, as well as post study labs will be done between 115-125 days (plus or minus 10 days).

Participants randomized to receive no lifestyle intervention (control) will be asked to continue their current eating and physical activity patterns for the duration of the study. Participants randomized to the no-lifestyle condition will be given the option of receiving all study materials and up to two meetings with the dietitian after their final assessment and up to one month after.

6.2 Lifestyle Intervention

The lifestyle intervention will use the 4 month intervention developed for the Diabetes Prevention Trial (DPT). This intervention has been found to be feasible, result in weight loss and prevention of diabetes, and produce changes in diet and physical activity. The goals of the intervention are for the participants to lose 7% of their body weight, decrease fat consumption, and increase their energy expenditure by 700

kilocalories per week (equivalent to approximately 2.5 hours per week of moderate intensity walking). Participants can choose whether they want to start with dietary or activity changes first (see Table 3 for schedule of topics), but both goal areas will be addressed.

Strategies to encourage weight loss include self-monitoring of weight and reduction of fat intake (goal: approximately 25% of calories from fat). Individuals who are not able to lose weight with these two strategies will be given a calorie goal during the seventh or eighth week of the intervention sessions in the session titled "Tip the Calorie Balance". Strategies to increase physical activity include self-monitoring, use of a pedometer to increase activity, and provision of opportunities for exercise.

The intervention is delivered as a 16 in-person sessions offered over a 4 month period, once each week. If a participant is travelling when a session is scheduled, or has some other reason why she is not able to attend, the session can be done by telephone. Participants will receive print material, worksheets, measuring utensils, a food scale, and a pedometer.

All print materials have been developed by the DPT and are available for use; they will require only minor modifications for this study. Print material and worksheets to be given to participants are provided in Appendix G. The intervention also involves providing opportunities for supervised exercise (e.g., group exercise class, group walks) at least twice a week for participants. The 16 interventions sessions will be delivered by a master's level Research Coordinator or Research Dietician who will be trained in a manner consistent with the requirements of the DPT. For quality control, 10% of the sessions he/she conducts will be audio-recorded and reviewed by Dr. Basen-Engquist or a Senior Research Coordinator or Research Manager.

Table 3: Schedule of intervention sessions.

	Session
1	Welcome to the Group Lifestyle Balance Program
2	Be a Fat and Calorie Detective
3	Healthy Eating
4	Move Those Muscles
5	Tip the Calorie Balance
6	Take Charge of What's Around You
7	Problem Solving
8	Four Keys to Healthy Eating Out
9	The Slippery Slope of Lifestyle Change
10	Jump Start Your Activity Plan Make Social Cues Work for You
11	Ways to Stay Motivated

12	Preparing for Long-Term Self-Management More Volume, Fewer Calories
13	Balance Your Thoughts for Long-Term Self-Management Strengthen Your Exercise Program
14	Mindful Eating Stress and Time Management
15	Standing Up for Your Health Heart Health
16	Stretching: The Truth about Flexibility Looking Back and Looking Forward

6.3 Supervised Exercise Session

Two opportunities for supervised exercise per week will be offered for participants to increase their level of physical activity. Any other form of exercise will be arranged by the participant herself outside the institution.

6.4 Toxicity assessment

The summary of toxicity assessment for in-person and phone is listed below.

A. In-person assessment include:
- physical exam (only on 1st month)
- weight
- review of study medication side effects (as listed in consent and instruct participant to call if they experience symptoms of abdominal pain, anorexia, nausea, jaundice, and change in urine color)
- ALT and creatinine levels.
- Dispense Pill Diary and prescription.

B. Phone assessment include reported symptoms of:
- gastrointestinal (abdominal pain and severe diarrhea)
- liver (jaundice) and review ALT lab results
- kidney (changes in urine color and amount) and review creatinine lab results
- heart (CHF)
- rare side effects listed in consent

The in-person toxicity assessment will be at the end of each month and will be a month from Randomization / Visit 3. All participants must agree to keep a drug administration diary (Pill Diary). This diary will be signed by both the participant and research nurse and reviewed during each clinic visit to verify compliance. The medication administration record will be filed in the participant's chart. Any discrepancies will be noted along with

the reason, if known. Any remaining pills will also be returned at each clinic visit and documented.

Participants with mild elevations in ALT (3x ULN), will continue study drug at same dose, and have another ALT drawn 30 days later. If ALT levels are >/= 4x ULN, the study drug will be stopped, and participant removed from the study. Participants with an elevation in creatinine of >/= 2.5x ULN will be taken off of the drug and removed from the study. The study drug will be stopped and participant removed from study if the participant reports symptoms of congestive heart failure (CHF) such as: fatigue, tightness or congestion in the chest, edema to ankles or lower extremities, and shortness of breath.

Telephone contacts will be made periodically as needed to assess on the first, second, third, and fourth month (plus or minus 10 days) to encourage compliance and assess for the presence of toxic or adverse effects. Participants experiencing toxic or adverse effects will be referred to MD Anderson health providers for medical care however medical care will be billed to the participant or participant's insurance provider. All participants will be contacted by telephone 4 weeks after completion of the study for follow-up and toxicity assessment.

7.0 Data Monitoring Plan

Participants will be randomized to one of the four arms: metformin, placebo, and metformin / placebo and lifestyle intervention using the Department of Biostatistics Clinical Trial Conduct Website (CTC website). Participants who do not meet these criteria will be considered nonevaluable, removed from study and will not be counted toward the actual accrual goal. We plan to accrue 100 evaluable participants.

We will use a form of adaptive randomization called minimization, which is similar to stratification in that participant characteristics are used to assign them to the treatment conditions [75, 76]. Minimization results in better group balance with respect to participant characteristics. In minimization, before a participant is assigned to a treatment group, the total numbers of participants in each group with similar covariate characteristics are totaled. The totals are based on the marginal sums of the covariates so that each covariate is considered separately. The treatment assignment of a participant is determined based on which group would provide the best overall balance with respect to those covariates. Covariates that will be used in randomization of participants in this study are BMI, age, and whether the participant meets current guidelines for exercise recommended by the Physical Activity Guidelines for Americans. These guidelines recommend that adults engage in moderate physical activity at least 150 minutes per week or in vigorous activity 75 minutes per week [77]. The baseline level of activity for each participant will be measured when they complete the Godin questionnaire, which is part of their survey packet, which will be completed before their Screening Visit 2 appointment. The Research Dietitian/Research Coordinator will determine if the participant is currently meeting these recommendations and provide

this data to the Research Nurse for randomization.

Randomization will be performed by the research nurse using the website created by the Department of Biostatistics. The research nurse will then inform the pharmacist of randomization group (A, B, C, or D) for study drug administration. The Research Dietitian/Research Coordinator who is in charge of administering the lifestyle intervention will be given access to the randomization website and informed of lifestyle intervention randomization code to determine whether the participant has been randomized to the lifestyle intervention.

7.1 Protocol compliance and assessment of toxicity data

The attending physician must see each participant prior to beginning study drug. All required interim and pretreatment data should be available to evaluate toxicities, assign grade, and make a determination regarding continuing treatment.

7.2 Data collection Plans

Protocol data will be entered into the following plans: Clinical Oncology Research System (CORe) / Protocol Data Management System (PDMS), Access Database, ASA server, MD Anderson Cancer Center (MDACC) Behavioral Science server, and excel spreadsheet after each course of therapy. A brief explanation for required but missing data should be recorded as a comment. See below for the list of the specific data stored in PDMS and CORe:

- PDMS
  - Monthly medication administration dates
  - Doses taken
  - Laboratory results
  - Clinic visits
  - Diagnostic test (EMB)
- CORe
  - Pre-registration
  - Adverse events entry

All behavioral and anthropometric data components, including height, weight, waist and hip circumference, exercise diary, and survey packet data will be collected and kept in an Access Database. The ASA24 data is stored on the ASA site's server until we request the data for analysis. The data is sent to us within 48 hours and then the file is stored on our server. The Accelerometer data will also be stored on our server at MDACC (Department of Behavioral Science) until the statistician retrieves the data for analysis. The DEXA scan data is downloaded into a file such as excel (Department of Behavioral Science) and then is stored on the server as well.

8.0 Criteria for Removal from the Study

8.1 Subjects are free to leave the study at any time.

8.2 Participants may be removed from the study at the discretion of the investigator if there is sufficient reason to suspect that the participant is not compliant with the study requirements.

8.3 If at any time during the study, the participant experiences symptoms of congestive heart failure or renal failure, drug will be stopped and the participant taken off study.

8.4 Abnormal baseline endometrial biopsy or the endometrial biopsy is unable to be performed.

8.5 Non-compliance: Non-compliance will be defined as a participant missing more than 4 consecutive doses per month or a total of more than 10 doses during the 4 month period.

8.6 Lost to follow-up: Diligent attempts must be made by telephone and letter to determine the circumstances for loss to follow-up, since loss may be related to study drug.

9.0 Statistical Considerations

This is a partially blinded, randomized study to evaluate the effects of intervention upon Ki-67 and other biomarkers. Participants and researchers will be blinded with regard to assignment of study drug (placebo vs. metformin) but they will not be blinded with regard to assignment of lifestyle intervention. We will use a form of adaptive randomization called minimization, which is similar to stratification in that participant characteristics are used to assign them to the treatment conditions to achieve better group balance. Groups will be balanced with regard to age (50-65 years), BMI (Class I and II: 30.0-39.9 and Class III or greater: ≥40), and whether they meet current exercise guidelines as recommended by the Physical Activity Guidelines forAmericans.

The primary objective of this study is to evaluate the effect of intervention (metformin and/or lifestyle intervention) on Ki-67. Secondary endpoints include examination of intervention on:

(a) genes relevant to estrogen dependent endometrial proliferation, hyperplasia and cancer using Q-PCR (PCNA, sFRP4, RALDH2, IGF-1R, survivin, and EIG121),

(b) biomarkers specific to the effect of metformin treatment (phospho-AMPKa,phospho-ACC, phospho-mTOR and phospho-S6 ribosomal protein.

Additionally, we will examine changes in serum levels of estradiol, estrone, testosterone, DHEA-S, sex hormone binding globulin (SHBG), adiponectin, glucose,

HbA1c, insulin and insulin-like growth factor-1 (IGF-1) and all biobehavioral outcomes, such as changes in body composition, physical activity, quality of life (QoL), diet, etc.

We will analyze differences in Ki-67 and other outcomes using 2x2 ANOVA models. If lifestyle intervention is found to affect change in Ki-67 expression or other outcomes, we will use the methods described by Baron and Kenney [78] to determine whether these changes were mediated by weight loss, body composition changes, physical activity changes, and dietary changes. Differences between arms with regard to adverse events and other toxicities will be examined using Fisher's exact tests. Changes from baseline lab values will be examined 2x2 ANOVA models.
A study of Ki-67 change in participants at high risk for endometrial cancer (Lynch patients) indicated we could expect a mean decrease in Ki-67 of 3.5 (after log-transformation) in the Metformin alone group with a standard deviation of 0.8. For the purposes of estimating power, we assumed that the lifestyle intervention alone group would have the same mean and standard deviation decrease, but we halved mean decrease for our calculations to take into account the non-compliance we might observe in this group. Finally, we estimated power for a moderate interaction effect – half of the metformin intervention effect in addition to the additive effect of metformin and lifestyle intervention. Specifically, we calculated power assuming that the mean decreases in the control arm, metformin alone arm, lifestyle intervention alone arm and metformin plus lifestyle intervention arm were 0, 3.5, 1.75 and 7, respectively. Using a 2-sided test with 5% statistical significance, we will have over 95% power to detect these differences when the common standard deviation is 0.8. Additionally, we will still have adequate power to detect much smaller differences. For example, we will have 80% power to detect a lifestyle alone main effect of 0.3 and still have over 95% power to detect a metformin main effect and an interaction effect of 0.6 and 1.2, respectively, when the common standard deviation is 0.8. Power was calculated using PASS 2005 (copyright © 2005, Jerry Hintze).

All testing will be 2-sided with 5% statistical significance. The primary efficacy analysis will be intent-to-treat. Patients who discontinue early from the study will be included in the analysis as not having a change from baseline values. Additionally, we will use the methods described by Benjamini and Hochberg [51] to control the false discovery rate in the secondary efficacy analyses.

10.0 Reporting Requirements

A data safety monitoring board (DSMB) at MD Anderson will monitor the trial annually for safety.

Criteria for response and toxicity:

10.1 Adverse Event

Any unfavorable or unintended symptom, sign, or disease (including abnormal lab) temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. Such effects can be intervention related, dose related, route related, participant related, and caused by an interaction with another drug.

10.2 Serious Adverse Event

Any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the participant, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity – a substantial disruption of a person's ability to conduct normal life functions.
- A congenital anomaly/birth defect.

10.3 Eliciting Adverse Event Information

Adverse events will be elicited at each clinic visit during participation in the study. Participants will be given frequent toxicity screening (in-person and phone) to review the occurrence of adverse events monthly and throughout the study. All adverse events which are directly observed and all adverse events which are spontaneously reported by the participant are to be documented by the investigator.

10.4 Grading/Rating Scale

All adverse events reported during the study will be evaluated and graded on a scale of 0-5. The CTCAE version 4.0 will be used to determine the grade for all toxicities. For any adverse events which are not listed in the CTCAE version 4.0 the following rating system will be used as listed below in section 10.5.

10.5 Non-NCI Adverse Event Grading Scale Grade/Description

0 No adverse event (absent) or within normal limits
1 Mild adverse event (minor, no specific medical intervention, asymptomatic laboratory

findings only, radiographic findings only, marginal clinical relevance)
2 Moderate adverse event (minimal intervention, local intervention, non-invasive intervention [packing, cautery])
3 Severe and undesirable adverse event (significant symptoms requiring hospitalization or invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation
4 Life threatening or disabling adverse event (complicated by acute, life threatening metabolic or cardiovascular complications such as circulatory failure, hemorrhage, sepsis. Life-threatening physiologic consequences; need for intensive care or emergent invasive procedure; emergent interventional radiological procedure therapeutic endoscopy or operation
5 Death related to adverse event

10.6 Relationship to Study Treatment

The investigator will use the following definitions to assess the relationship of the adverse event to the study treatment:

Definite: Adverse event is clearly related to the study treatment.
Probable: Adverse event is likely related to the study treatment.
Possible: Adverse event may be related to the study treatment.
Unlikely: Adverse event is doubtfully related to the study treatment.
Not Related: Adverse event is clearly not related to the study treatment.

11.0 Laboratory Evaluations and Procedures

11.1 Laboratories

11.1.1 Serum assays will be performed at the MD Anderson Cancer Center Clinical Laboratory (estradiol, estrone, testosterone, DHEA-S, SHBG, adiponectin, glucose, hemoglobin, FSH, A1c, insulin and IGF-1).

11.1.2 The endometrial biopsy slides will be reviewed centrally by the study's co-PI, Russell Broaddus, M.D., Ph.D. at MD Anderson Cancer Center and PI of the Pathology core. A pathology report will be generated and forwarded to the participant's study gynecologist.

Cases with hyperplasia or adenocarcinoma will be confirmed by a second review by Elvio Silva, M.D., a senior gynecological pathologist at MDACC. Dr. Silva is a Professor with more than 25 years of experience and is regarded as an international expert in gynecologic pathology.

11.1.3 Ki-67 immunohistochemical assays, as well as IHC for metformin associated

phosphoproteins, will be performed in the Histology Core Lab at MD Anderson.

11.1.4 Q-PCR for estrogen-regulated genes will be performed in the laboratory of David Loose-Mitchell, Ph.D., U.T. -Houston Medical School (Biomarkers core).

11.2 Collection and Handling Procedures

An endometrial biopsy using 4 mm pipelle will be used to obtain endometrial tissue. Half of the sample will be placed in formalin, and half of the sample will be placed in a cryovial and immediately snap frozen in liquid nitrogen. The sample in formalin will undergo routine histological-processing and paraffin-embedding. One H&E slide will be cut per block. Following central pathologic review, the pathology report will be forwarded to the participant's study gynecologist.

11.3 Shipping Instructions

Snap frozen tissue will be batched and shipped periodically on dry ice to Dr. David Loose-Mitchell at the University of Texas - Houston Medical School, PI of the Biomarkers Core.

Snap frozen tumor will be shipped in liquid nitrogen to M. D. Anderson Gynecologic Oncology Translational Research Tumor Bank.

11.4 Experimental Methodology and Preliminary Results

11.4.1 Handling of Endometrial Biopsies

Once the endometrial biopsy has been performed, half of the biopsy will be flash-frozen in liquid nitrogen, and the remaining half will be fixed in formalin. RNA will be extracted from the frozen portion for the Q-PCR assays. The formalin-fixed portion will undergo routine histological processing and paraffin embedding. One H&E stained slide will be prepared for histological diagnosis. Additional paraffin embedded tissue will be used for immunohistochemical Ki-67 assay and metformin-associated biomarkers (phopho-IHC).

11.4.2 Pathological Examination of Biopsies

A gynecologic pathologist (Russell Broaddus, M.D., Ph.D.,Co-PI and Pathology Core PI) will review all of the endometrial biopsies. Difficult cases will be reviewed with a senior consultant (Elvio Silva, M.D., leader of the Gynecologic Pathology Group, Department of Pathology, M.D. Anderson Cancer Center). The biopsies will be placed into diagnostic categories, such as proliferative phase, secretory phase, atrophic, anovulatory, hyperplasia, hyperplasia with atypia, or carcinoma, according to standard pathological principles.

11.4.3 Frozen Tissue Experiments - Quantitative PCR analysis of Transcript in Endometrial Tissues

The Q-PCR analyses will be performed in the Quantitative Genomics Core Facility at the University of Texas Medical School at Houston. This portion of the project will be under the direction of David Loose-Mitchell, Ph.D. (Biomarkers core PI) and Greg Shipley, Ph.D. This laboratory has been interested in identifying genes in human endometrium whose expression is altered by hormone replacement therapy (HRT). Over the last 3 years we have used several techniques including differential display, microchip array screening, and real-time quantitative PCR to find such genes. The tools we have developed are directly applicable to the investigation of surrogate endpoint biomarkers (SEBs). Identification of appropriate biomarkers is essential for this study. An ideal biomarker is a histological or molecular "red flag" that precedes the onset of cancer. Ideal surrogate endpoint biomarkers are quantitative and are modulated by chemopreventive agents.

In our prior endometrial cancer chemoprevention study of women with HNPCC, we evaluated a panel of 30 biomarkers thought to be relevant in endometrial carcinogenesis. From that study, we have narrowed down the field to a subset of 5 validated, estrogen-regulated genes and a proliferation marker[46].

12.0 Transcripts to be Analyzed by Q-PCR - Endometrial Biopsies

Table 4: Transcripts to be Analyzed and the Rationale.

Transcript	Rationale
PCNA	Marker of proliferation
IGF-IR	The major receptor for IGF-I, an immediate growth regulator of estradiol
RALDH 2	Retinaldehyde dehydrogenase 2 is rate-limiting enzyme for retinoic acid production; induced by E2.
SFRP4	Wnt antagonist; highly induced by E2 in human endometrium
survivin	Inhibitor of apoptosis, modestly induced by E2 in human endometrium
EIG121	Novel estrogen induced gene of unknown function.
Normalizers	
18s ribosomal	essential for quantitating RNA yield
cyclophilin	mRNA not regulated by E2 in the endometrium
b-actin	another mRNA not regulated by E2 in the endometrium

12.1 Preparation of RNA from Endometrial Biopsies for Real- Time Q-PCR

Total RNA will be isolated from endometrial samples using a Qiagen RNeasy Kit (Qiagen, Inc., Chatsworth, CA) and a modification of the manufacturer's protocol.

Endometrial samples containing 5-20ng of tissue will be placed in 1.0 ml freshly prepared lysis buffer containing 10mM b-mercaptoethanol. The tissue is homogenized with a Brinkman PT3000 (Brinkmann Instruments, Westbury NY) set at 30,000 rpm using a small diameter probe (PTDR3007).

The lysate will be centrifuged at 1000xg for 5 minutes to reduce foam and remove debris. The supernatant will be placed in a 1.5ml microfuge tube and the lysate spun at full speed for 3 minutes. An equal volume of 70% ethanol is added to the lysate and mixed thoroughly. The Qiagen columns are loaded (x3) with 700 ml of the lysate/ethanol mixture and centrifuged at 10,000 rpm for 15 s. The RNA is bound to the column matrix and the flow through discarded. After all of the lysate mixture is loaded onto the column, it is washed with 700 ml of wash buffer RW1. The column is then centrifuged at 10,000 rpm for 15 s. and a new receiving tube is attached. The second wash is with 500 ml was buffer RPE at 13,200 rpm for 60 s. The RNA is eluted from the column into a new microfuge tube by applying 55 ml DEPC-treated H_20 treated directly to the column and centrifuging at 10,000 rpm for 60 s. RNA is quantitated by A_{260}. In our previous studies on 290 post-menopausal women, this procedure yielded an average of 16.7 mg per sample (min 0.11, max 117 mg). In that same study, 230 samples were from HRT treated women and yielded an average of 37.3 mg of total RNA.

We typically measure transcripts in 10-20 ng of total RNA. Some of the more abundant RNAs can be measured in as little as 1 ng; in fact, we measure 18S rRNA from a dilution that contains 0.025 ng. If we assume we will need an average of 20 ng per sample per transcript times 4 per quadruplicate determinations, then we will require 80 ng per transcript. To measure the 8 transcripts will require 640ng of RNA. This is less than 1/5 of the average amount of RNA recovered from untreated post- menopausal women. In our recent study, this was obtained in 87% of our post- menopausal, untreated women and in 100% of the samples from treated women. We should be able to recover sufficient RNA from nearly all samples to perform a complete analysis; in that small percentage where RNA becomes limiting we will prioritize our assays as described later in this section.

12.2 Analysis of Q- PCR Transcripts

Transcripts for the 7 genes will be assayed in RNA prepared as described above. For each transcript, compatible PCR primers and a fluorescent hybridization probe (TaqMan probe) will be designed using Primer Express (PE Biosystems). PCR conditions will be optimized for primer and probe concentration and Mg^{2+} concentrations to give assays with a slope of the sRNA standard curve of -3.1 - -3.5 (PCR cycles per log template molecules) and a lower detection limit of $<10^2$ molecules input template. Assays will be assembled in a 96-well plate in which 12 wells are allocated to sRNA standards (10^2 - 10^7 sRNA molecules), 2 wells are assigned to controls (no amplification and no template controls) and 80 wells are allocated to RNA samples. We have found that optimal data is achieved when RT- PCR analyses of unknown RNA's are run in triplicate, and we always include a 4^{th} aliquot of each sample

without added reverse transcriptase (to detect amplification of residual genomic DNA). Using this format of 20 RNA samples are analyzed per 96-well plate. Plates are assembled robotically and the RT and "real-time" Q-PCR runs will be carried out in the ABI 7700. The level of input RNA in unknown samples is calculated by interpolation of the Ct value of the unknown on a sRNA standard curve run in parallel with the unknown samples. The results of the assay are reported as the number of template molecules in the input unknown RNA. Because of occasional inaccuracies in RNA quantitation using spectrophotometric analyses, we will determine the level of three normalizer transcripts (b-actin, cyclophilin, and 18S rRNA) in each sample. Results are then normalized to these three transcripts and are expressed as % normalizer mRNA.

12.3 Real-Time Q-PCR

96- well plates will be assembled robotically using Tecan and Biomek workstations. Aliquots of the RNA (10-20 ng) will be reverse transcribed with SuperScript II reverse transcriptase (1 U/ml; Boeringher Mannheim) in a 10 ml reaction that includes the appropriate transcript-specific reverse primer (300 nM), 1X RT buffer (Gibco-RL), 4 mM $MgCl_2$, 10 mM DTT, and 500 mM dNTPs. The reaction will be incubated at 50°C for 30 minutes and then terminated by incubation at 72°C for 5 minutes. Following reverse transcription of 40 ml of "real-time" PCR reaction mix is added to give a final volume of 50 ml. PCR is performed in a 7700 Prizm Sequence Detector (Perkin Elmer) with preheating at 95°C for 1 minute followed by 40 cycles of annealing and extension (60°C for 60 s.) and melting (95° C for 12 s.).

Dequenching of the fluorescent hybridization probe is continuously monitored and the Ct (PCR cycles to threshold) calculated using Sequencing Detector 1.6.3 software (Perkin Elmer). Unknown RNA samples will be analyzed in triplicate with a control that lacked reverse transcriptase (-RT control). Samples are run in parallel with sRNA standards (0.8 pg - 80 pg) to generate an internal standard curve (Ct versus sRNA template molecules). The number of template molecules in the sample is calculated by interpolation of Ct against the sRNA standard curve.

Amplicon specific sRNAs are generated from appropriate plasmid templates by first synthesizing the target amplicon using the same PCR primers used in the "real-time" PCR reaction. The amplicon is then cloned into the pCRIItopo plasmid (Invitrogen) that includes a T7 site flanking the cloning cassette. sRNA is synthesized from the T7-tailed amplicon template using T7 Mega-script Polymerase (Ambion). sRNA is quantitated by ^{32}P-UTP incorporation and checked for purity by gel electrophoresis and analyzed on a phosphorimager (Molecular Dynamics). Aliquots of sRNA containing known numbers of template molecules are reverse transcribed and subjected to real-time PCR in parallel with unknown RNA samples to quantify transcript abundance.

12.4 Logistics of Real- Time Q-PCR

The studies outlined in this proposal that use frozen specimens total 7 transcripts in 40 women at 2 time points. This will require 30 Q-PCR runs on a 7700. We currently operate 2 ABI 7700 instruments and average throughput is 3 runs per machine per day. Therefore we estimate that the proposed studies can be done in 5 machine days.

12.5 Databases and Data Storage for Real- Time Q-PCR

Data is exported electronically from the 7700 and initial calculations (means of triplicate samples) are performed on a custom spreadsheet. The raw 7700 data and the preliminary calculations are archived on CD-ROM. These data are then electronically transferred to a custom Filemaker Pro database which performs all of the final calculations. Changes to the data contained in the Filemaker database are all logged to a separate, password-protected audit database. The Filemaker database is archived to magnetic tape.

12.6 Immunohistochemistry

Ki-67 immunohistochemistry and phospho-AMPKa, phospho-ACC, phospho-mTOR and phospho-S6 ribosomal protein will be performed in the Histology Core Lab, using paraffin-embedded tissue sections and commercially available antibodies. The percentage cells positive will be recorded, as well as the compartment stained (epithelial cell vs. stromal cell); nucleus vs. cytoplasm vs. cell membrane). Dr. Broaddus will evaluate the immunohistochemistry slides.

13.0 Schedule of Events

Table 5: Study Parameters (see next page)

Study Parameters	TAT[a]	Tube Top	Screening	Screening	Randomization	1st Month	2nd Month	3rd Month	4th Month	1 yr.
		Color	Visit 1	Visit 2	Visit 3	[b]	[b]	[b]	[b]	Follow-Up
Informed Consent			X							
History				X						
Physical				X		X				
Research Red Tube[c]		Red	X						X	X
Research Lavender Tube[c]		Lavender	X						X	X
Insulin (fasting)	4 days	Red	X						X	X
Glucose (fasting)	4 hrs	Mint	X						X	X
FSH (Spec Chem Test)	24 hrs	Gold	X						X	X
Hemoglobin	4 hrs	Lavender	X						X	X
Estradiol	5 days	Red		X					X	X
Estrone	5 days	Red		X					X	X
Testosterone (Chem Test)	24 hrs	Gold		X					X	X
DHEA-S	5 days	Red		X					X	X
Sex Hormone Binding Globulin (SHBG)	5 days	Red		X					X	X
Adiponectin	7 days	Red		X					X	X
A1C (Spec Chem Test)	24 hrs	Lavender	X						X	X
IGF-1	7 days	Red		X					X	X
Serum Creatinine	4 hrs	Mint	X			X			X	X
ALT	4 hrs	Mint	X			X	X[f]	X[f]	X	X
Triglycerides (fasting)	4 hrs	Mint	X						X	X
TSH	24 hrs	Gold	X						X	X
Dispense Medication & Pill					X	X	X	X		
Pill Count						X	X	X	X	
Pre-treatment EMB	72 hrs			X						
Toxicity and Compliance Assessment: Phone[e]						X	X	X	X	
Toxicity Assessment: In-person						X	X	X	X	
Post-treatment EMB	72 hrs								X	
DEXA				X					X	X
waist circumference				X					X	X
hip circumference				X					X	X
BMI				X					X	
ASA-24 (3)[d]			X						X	
Accelerometer			X						X	
Survey Packet				X					X	X

a) *Turn Around Time*
b) *Plus or minus 10 days.*
c) *Research blood for future biomarker analysis.*
d) *1 ASA-24 completed at Screening Assessment. Other 2 ASA-24s complete randomly between screening assessment and 7 days post-biopsy.*
e) *As needed*
f) *Only if ALT levels elevated as listed in CTCAE version 4.0.*

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