Official Title: A Randomized Pilot Study to Evaluate the Effects of a Short Course of Metformin versus No Therapy in the Period Prior to Hysterectomy for Grade 1-2 Adenocarcinoma of the Endometrium in Obese Non-Diabetic Women

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A Randomized Pilot Study to Evaluate the Effects of a Short Course of Metformin versus No Therapy in the Period Prior to Hysterectomy for Grade 1-2 Adenocarcinoma of the Endometrium in Obese Non-Diabetic Women

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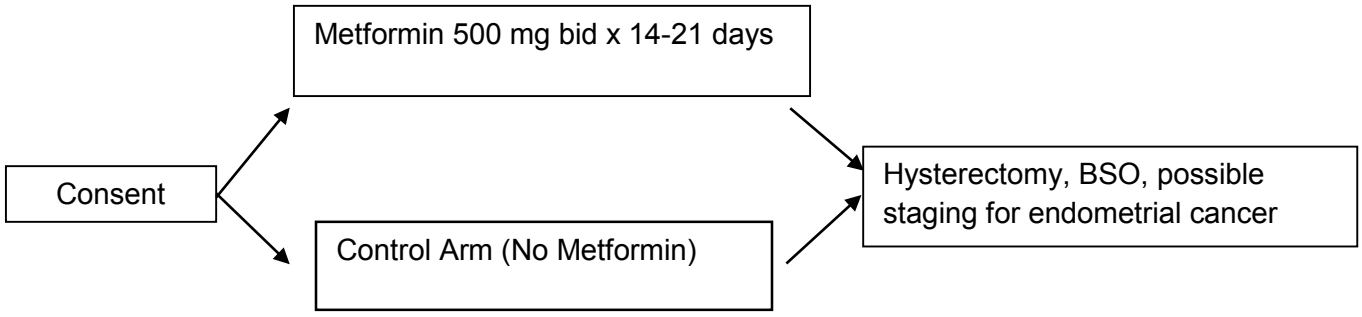
Abbreviations

AE	Adverse event
Akt	serine/threonine protein kinase Akt
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
AMPK	AMP kinase
AST	Aspartate transaminase
ATP	Adenosine triphosphate
BMI	Body mass index
Clin Lab	UAMS Clinical Laboratory
BSO	Bilateral salpingo-oophorectomy
BUN	Blood urea nitrogen
CCTO	Cancer Clinical Trials Office
CO_2	Carbon dioxide
ECC-1	Endometrial carcinoma cell-1
ELISA	Enzyme-linked immunosorbent assay
ER	Estrogen receptor
Erk1/2	Extracellular signal-regulated kinases 1 and 2
FASN	Fatty acid synthase
HbA_1c	Hemoglobin A_1c
HDL-C	High-density lipoprotein cholesterol
HEC-1B	Human endometrial carcinoma-1B
hTERT	Human telomerase
IGF-I	Insulin-like growth factor -1
IGF-II	Insulin-like growth factor -2
IHC	Immunohistochemistry
IRB	Institutional Review Board
IUD	Intrauterine device
Ki67	Antigen KI-67
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
KLF9	Kruppel-like factor 9
LDL-C	Low-density lipoprotein cholesterol
mg	Milligram
miR	Micro RNA
mRNA	Messenger RNA
NIH/NCI	National Institutes of Health/National Cancer Institute
NTU	Non-tumor uterine
PCOS	Polycystic Ovarian Syndrome
PI	Principal Investigator
PR	Progesterone receptor
PTEN	Phosphatase and tensin homolog
RT-PCR	Reverse transcriptase polymerase chain reaction
SD	Standard deviation

TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UTR	Untranslated region
UAMS	University of Arkansas for Medical Sciences

Study Schema



Study Summary

Title	A Randomized Pilot Study to Evaluate the Effects of a Short Course of Metformin versus No Therapy in the Period Prior to Hysterectomy for Grade 1-2 Adenocarcinoma of the Endometrium in Obese Non-Diabetic Women
Short Title	Effect of Metformin on Proliferation Markers in Endometrial Cancer
Protocol Number	138647
Phase	Pilot study
Methodology	A randomized open-label study of metformin vs. no metformin
Study Duration	18 months
Study Center(s)	Single-center
Objectives	To determine metformin tolerability. To determine metformin effect on tissue markers of proliferation, apoptosis, and insulin-mediated signaling. To determine metformin effect on a number of different markers measured in blood and plasma. To determine metformin effect on mRNA levels and miR profiles.
Number of Subjects	40 total: 20 metformin, 20 control
Diagnosis and Main Inclusion Criteria	Obese (BMI≥30) non-diabetic women with newly diagnosed Grade 1-2 adenocarcinoma of the endometrium
Study Product(s), Dose, Route, Regimen	Metformin 500 mg po bid x 14 - 21 days
Duration of administration	14-21 days
Reference therapy	Non-treatment arm
Statistical Methodology	Wilcoxon rank-sum tests will be used to compare treatment arms for differences in each study endpoint.

Protocol Summary

This is a randomized, open-label clinical investigation of the effects of short-term oral metformin therapy on biomarkers for tumor growth in subjects with endometrial cancer. Metformin is a well-tolerated drug widely prescribed for treatment of Type 2 diabetes mellitus and for treatment of Polycystic Ovarian Syndrome (PCOS) in non-diabetic women [1, 2]. Clinical trials are ongoing to explore this possibility in breast cancer (NCT01101438). This investigation is the first study of metformin in endometrial cancer patients, and is designed to understand the mechanism of its anti-cancer actions, if any, and its interactions with biomarkers in endometrial cancer patients.

Forty non-diabetic subjects will be randomized so that 20 will receive oral metformin for 14-21 days and the remaining 20 will receive no metformin for 14-21 days. Subjects will be monitored for adverse events (AEs). All subjects will have blood samples collected on Day 1 (prior to start of metformin for subjects randomized to metformin) and prior to surgery. These samples will undergo metabolic, lipid and tumor-specific biomarker analyses. Tumor and adjacent normal tissue taken from subjects from the diagnosis and at the time of surgery will be analyzed for cellular proliferation, apoptosis and biomarker levels.

The **Primary objectives** of this study are as follows:

1. To determine the effect of metformin on tissue markers of proliferation, apoptosis, and insulin-mediated signaling in endometrial cancer in non-diabetic obese women.
2. To determine the tolerability of a short course of metformin in non-diabetic obese women with newly diagnosed endometrial cancer.

The **Secondary objectives** of this study are as follows:

1. To determine the effect of metformin on HbA_1c and blood lipid profiles measured at baseline and just before surgery.
2. To determine the effect of metformin on the following hormones measured in blood plasma at baseline and just before surgery: Insulin, IGF-I, leptin, and adiponectin.
3. To determine the effect of metformin on the expression levels of mRNAs and miRs measured in blood plasma at baseline and just before surgery.
4. To determine the effect of metformin on the expression levels of mRNAs and miRs measured in tumor and adjacent non-tumoral uterine tissue.

Background and Rationale

Endometrial cancer is the most common gynecologic malignancy in the United States, with approximately 45,000 cases per year [3]. The single biggest risk factor for development of this disease is obesity [4]. There is a direct correlation between the extent of obesity and the development of endometrial cancer. Peripheral adipose tissue contains high amount of the enzyme aromatase, which converts androstenedione into estrone. This source of estrogen then exerts a proliferative effect on the lining of the uterus, leading over time to endometrial hyperplasia and carcinoma. This type of endometrial cancer is referred to as Type I and represents ~80% of all endometrial cancers in this country [5]. More recently it has been shown that obesity is a risk factor for insulin resistance leading to higher levels of circulating insulin. If the insulin resistance is great enough, obesity will lead to diabetes.

Serum insulin levels have now been shown to have a direct correlation with the development of endometrial cancer [6]. Case-control studies have documented higher serum

insulin levels in women who develop endometrial cancer versus women who underwent gynecologic surgery for benign disease [7]. Both insulin and insulin-like growth factor 1 (IGF-1) are highly associated with endometrial cancer. In one study, women with high circulating insulin levels had a hazard ratio of 2.33 for developing endometrial cancer versus women with low levels of insulin, after adjusting for age and estradiol[8].

High insulin levels are associated with the metabolic syndrome, which includes insulin resistance, elevated circulating hormones, obesity, elevated lipids, and can clinically manifest as anovulation and infertility. The metabolic syndrome in women is often referred to as the polycystic ovarian syndrome (PCOS). In these women, medical reduction of circulating insulin leads to improvements in all factors associated with the metabolic syndrome, including an increase in ovulatory cycles and improved pregnancy rates [9]. Metformin, a biguanide compound, is a primary therapy used to treat insulin resistance associated with type II diabetes. Among diabetics on metformin, there is a reduction in the development of cancer and the rate of cancer death [10]. It is not precisely known how metformin reduces the development of cancer. The reduction in cancer is not seen with other diabetic treatments such as sulfonylureas and insulin.

Since the early 1990s, metformin has been used to treat PCOS. A meta-analysis of the use of metformin and polycystic ovary syndrome in 2003 documented from controlled trials a significant improvement, compared with placebo, in ovulation rates, pregnancy rates, fasting insulin levels, blood pressure, and cholesterol[9]. It is important to note that these studies were performed in non-diabetic women. Metformin is now used routinely as therapy for PCOS.

Because high circulating levels of insulin have been associated with the development of endometrial cancer, reduction of insulin levels with metformin may have a direct effect on the development or maintenance of this cancer. Anecdotal reports have occurred of women with early low-grade endometrial cancer being successfully treated with a regimen including metformin [11]. Also, women who had initially responded to progesterone therapy for endometrial cancer and then became resistant, showed a renewal of progesterone response when they were started on metformin [12]. Laboratory data has documented that metformin is a potent inhibitor of endometrial cancer-cell proliferation [13]. Metformin has also been shown to promote progesterone receptor expression in endometrial cancer cells and to reverse progesterone resistance in endometrial cancer cells by several different mechanisms [14, 15]. Progesterone receptor expression and sensitivity in endometrial cancer are associated with improved clinical outcomes.

Arkansas is one of the top states for obesity in the United States. More than 30% of the adult population is characterized as obese, with a Body Mass Index (BMI) of 30 or higher [16]. At UAMS, we see approximately 160 new cases of endometrial cancer in obese women each year. Most of these women are not diabetic, but are at risk for developing diabetes.

The present study proposes to treat obese endometrial-cancer patients with a four-week course of metformin prior to their definitive surgical therapy. This information will be used to apply for further funding to determine the exact role metformin may have in treatment of endometrial cancer. This drug also has the potential to be a chemo-preventive agent in women at high risk for developing endometrial cancer. There is currently no randomized trial to investigate the influence of metformin on endometrial cancer in obese non-diabetic women.

Proliferation Markers

Human endometrial cancer cell lines ECC-1 and Ishikawa, when treated with metformin, showed decreased proliferation and decreased hTERT expression relative to non-treated cells [17]. In women treated with metformin to manage PCOS, sera from PCOS women after 6 months of metformin significantly decreased the invasive properties of ECC-1, in part, by attenuating pro-inflammatory NF-κB expression and activation of Akt and Erk1/2 pathways [18]. Further, the increase in progesterone receptor expression in metformin-treated Ishikawa and HEC-1B cells was associated with pathways targeted by adipokines and insulin.

In this pilot study, we will evaluate the effect of metformin on endometrial tumor-cell proliferation and apoptosis, obesogenic and pro-tumorigenic marker gene expression, and anti-oncogenic micro-RNA (mIR) expression. mIRs are potentially important regulators of expression of tumor-enhancing and tumor-suppressing pathway components. Endometrial tumors and adjacent non-involved tissues will be obtained at surgery. Tissues will be processed for histopathology and immunohistochemistry, and frozen in liquid nitrogen for RNA and protein analyses. Fasting blood sera will be obtained prior to and after the 14-to-21-day treatment period for measurement of insulin, adiponectin, leptin, and IGF-1 levels. Fixed tumor and 'normal' tissues will be analyzed for markers of proliferation (Ki67; phospho-histone H3) and apoptosis (TUNEL), and for expression of estrogen receptor (ER)α, progesterone receptor (PR), and tumor suppressor PTEN by immunohistochemistry. Isolated tissue RNAs will be assessed for PR, PTEN, ERα, KRAS, and hTERT transcripts by quantitative RT-PCR. Protein extracts will be evaluated by Western immunoblotting for PR, PTEN, total and activated Akt and Erk 1/2, and insulin receptor expression. Sera from both groups at both time points will also be subjected to analysis using miR-profiling arrays. Data obtained from analyses of all biological endpoints will be compared using standard statistical procedures.

The efficacy of metformin in potentially reversing the pro-tumorigenic parameters of endometrial cells (to be evaluated in adjacent non-involved endometrium) has not been previously explored. Our studies may provide information on a subset of patients who are more likely to respond to metformin based on their endocrine profiles and clinico-pathologic factors, and hence may lead to metformin as a standard chemopreventive strategy for women with high risk for EC. The analyses of metformin-responsive mIRs that are associated with EC may also offer non-invasive biomarkers for predicting women diagnosed with lower stage of EC and who are likely to suffer from EC recurrence.

Trial Objectives

Primary Objectives

1. To determine the effect of metformin on tissue markers of proliferation, apoptosis, and insulin-mediated signaling in endometrial cancer in non-diabetic obese women. Specifically, to determine the effect of metformin on the following tissue markers measured in tumor and adjacent non-tumor uterine (NTU) tissue:
    a. Proliferation: Ki67, phosphorylated histone H3, estrogen receptor (ER), progesterone receptor (PR), and telomerase (hTERT).

b. Anti-proliferation and apoptosis: PTEN, KLF9, cleaved caspase-3, and TUNEL staining.

c. Insulin-mediated signaling: total and phosphorylated versions of Akt, Erk1/2, AMPK, and receptors for Insulin, IGF-I, and IGF-II.

2. To determine the tolerability of a short course of metformin in non-diabetic obese women with newly diagnosed endometrial cancer.

Secondary Objectives

1. To determine the effect of metformin on HbA_1c and blood lipid profiles measured at baseline and just before surgery.
2. To determine the effect of metformin on the following hormones measured in blood plasma at baseline and just before surgery: Insulin, IGF-I, IGF-II, leptin, and adiponectin.
3. To determine the effect of metformin on the expression levels of mRNAs and miRs measured in blood plasma at baseline and just before surgery.
4. To determine the effect of metformin on the expression levels of mRNAs and miRs measured in tumor and adjacent NTU tissue:

Exploratory Objectives

1. To examine how BMI influences tumor-cell responses to treatment with metformin.
2. To explore the possible interactions of BMI, the plasma miR profiles and tumor and tumor mRNA profiles in final responses of endometrial tissue to metformin.

Trial Design

This is a single-center, two-arm, open-label, randomized proof-of-concept pilot study to ascertain the therapeutic potential of metformin in obese women with Type I endometrial cancer. Forty non-diabetic obese subjects with confirmed stage I or II adenocarcinoma of the endometrium will be recruited from the University of Arkansas for Medical Sciences (UAMS).

A. Method of assigning subjects to treatment groups.

Subjects will be assigned randomly to one of two groups. Group 1 will receive treatment with metformin, while Group 2 will receive no treatment. Subjects assigned to Group 1 will be administered oral metformin at 500 mg twice a day for 14-21 days followed by surgery. These doses are therapeutic, and will permit patients to undergo their surgical therapy within an appropriate time frame.

Subject eligibility will be established before treatment randomization. Once a subject's eligibility is determined, the Research Staff will be notified to implement the randomization process. Randomization will be completed when the Research Staff receives a copy of the signed consent and the physician's orders for study agent to be dispensed.

To minimize imbalance between treatment arms, the randomization procedure will employ a stratified block randomization with randomly chosen blocks of size 2, 4, and 6, and with BMI as the stratification factor. Randomization will be conducted using sealed envelopes located at the Cancer Clinical Trials Office (CCTO). There will be two sets of consecutively numbered envelopes, one set for subjects with BMI from 30 to 39.9 and one set for subject with BMI of 40 or higher. At each randomization, the lowest numbered envelope will be chosen. The Research Staff will open the envelope and assign the subject to the treatment arm based on the contents of the envelope. The appropriate treatment will then be dispensed to the subject.

Methods for ensuring blinding.

Not applicable: this is an open-label study.

Methods for unblinding the study.

Not applicable: this is an open-label study.

Procedure for unblinding in case of medical emergency.

Not applicable: this is an open-label study.

Metformin will be administered to patients (randomized to receive metformin) for a minimum of 14 days and a maximum of 21 days, representing the typical time interval between confirmation of tumor diagnosis and surgical resection. Following surgical resection, any unused study drug will be returned to the physician at the time of the subject's first post-operative visit.

B. Blood Draws and Laboratory Tests

Blood will be drawn from each subject, approximately 18 mL in purple top vacutainers, prior to treatment assignment (if not preformed previously as conventional care) and after completion of the therapy at the time of surgery. The blood sample below will be subjected to the following tests at the UAMS Clinical Laboratory:

a) A comprehensive metabolic panel (glucose, calcium, albumin, total protein, sodium, potassium, CO_2, chloride, BUN, creatinine, ALP, ALT, AST and total bilirubin), to be performed prior to starting treatment only.
b) A lipid panel (cholesterol, HDL-C, LDL-C, triglycerides), to be performed both prior to treatment assignment and at the time of surgery.
c) Determination of HbA1c, to be performed both prior to treatment assignment and at the time of surgery.

The blood samples listed below will be utilized for correlative laboratory studies in the research laboratory of Dr. Rosalia Simmen at the UAMS Biomed Bldg. Aliquots of each blood sample will be centrifuged to separate out the plasma portion. Plasma will be stored at -80°C in the Co-Investigator's lab until analysis is performed.

The following biomarkers will be assayed in the plasma samples collected at baseline and at the time of surgery through enzyme-linked immunosorbent assays (ELISA) or multiplex assays. All of these tests will be performed by laboratory personal in Dr. Rosalia Simmen's lab:

a) Insulin
b) Leptin
c) Adiponectin
d) IGF-I and IGF-II

Remaining plasma samples will be subjected to global profiling of miRs and mRNAs to evaluate/validate the relative expression of specific candidate metformin-regulated miRs and mRNAs (Dr. Frank Simmen's lab). miR and mRNA levels will be determined using quantitative SYBR green-based real time reverse transcriptase polymerase chain reaction (RT-PCR) assays and a microarray platform. These tests will be performed in Dr. Frank Simmen's laboratory by his personnel and/or by commercial providers of microarray profiling services.

C. Tissue Specimens and Laboratory Tests

A sample of tumor with adjacent normal mucosa will be obtained after surgery from the Pathology department from the tissue remaining after normal gross and histological assessments.

Fresh tissue samples will be transported by designated research personnel to Dr. Rosalia Simmen's laboratory at the UAMS Biomed Bldg . Fixed tumor and 'normal' tissues will be subjected to IHC for markers of proliferation (*Ki*-67, phospho-histone H3), and apoptosis (TUNEL, cleaved caspase-3). Tumor (and 'normal') tissues will also be examined by Western blot and/or ELISA for expression levels of FASN, total and phosphorylated insulin receptors, and total and phosphorylated IGF-I receptors. Total and phosphorylated AMPK levels will be assessed by IHC or Western blot. Lastly, tissues will be used for miR and mRNA profiling.

D. Subject Data

Subject data to be obtained and recorded will consist of the following:

a) Patient characteristics: gender; age; BMI; race and ethnicity; smoking status (including approximate packs per day for current and former smokers); alcohol intake.
b) Tumor characteristics: tumor stage; tumor differentiation status (well, moderate, poor); lymph node involvement; depth of invasion; tumor margins.
c) Comprehensive metabolic panel: glucose, calcium, albumin, total protein, sodium, potassium, CO_2, chloride, BUN, creatinine, ALP, ALT, AST and bilirubin.
d) Lipid panel: cholesterol, HDL-C, LDL-C, triglycerides.
e) Plasma markers: plasma HbA1c; plasma insulin; plasma leptin; plasma adiponectin; plasma IGF-I; plasma IGF-II; plasma profiles of miR and mRNA.

f) Tissue markers: endometrial cancer and uterine apoptotic indices; endometrial cancer and uterine proliferative indices; endometrial tumor miR and mRNA profiles; endometrial (adjacent NTU) miR and mRNA profiles.

E. Subject Diary

A subject who is randomized to metformin will receive a diary on Day 1. The subject is to record the date and time of self-administration of the study drug. The diary will be reviewed weekly by members of the study team via telephone for compliance. The diary will be collected at the subject's post-operative visit.

F. Treatment modifications

Patients will be monitored for their ability to tolerate the study medication during weekly follow-up telephone calls. If necessary, anti-diarrheal or anti-emetic therapy may be prescribed to ease any discomfort associated with the medicine. If a patient cannot tolerate the study medication and misses two or more doses in a row, they will be withdrawn from the study.

G. Subject removal

Any patient who develops a grade 3 or higher gastrointestinal toxicity while on study will be discontinued. Subjects that withdraw from the study or that are withdrawn from the study due to unacceptable toxicity will return all unused study drug at their next scheduled clinic appointment.

H. Study Drug

Metformin (Fortamet®; Glucophage®; Glucophage® XR; Glumetza®; Riomet®)

Description:

Meformin is an oral antihyperglycemic drug used in the management of type 2 diabetes.

Molecular formula: C4H11N5 • HCl

Molecular weight: 165.62

Adverse Events:

>10%: Gastrointestinal: Diarrhea. Neuromuscular & skeletal: Weakness.

1% to 10%: Cardiovascular: Chest discomfort, flushing, palpitation. Central nervous system: Headache, chills, dizziness, lightheadedness. Dermatologic: Rash. Endocrine & metabolic: Hypoglycemia. Gastrointestinal: Indigestion, abdominal discomfort, abdominal distention, abnormal stools, constipation, dyspepsia/ heartburn, taste disorder. Neuromuscular & skeletal: Myalgia. Respiratory: Dyspnea, upper

respiratory tract infection. Miscellaneous: Diaphoresis increased, vitamin B_{12} levels decreased, flu-like syndrome, nail disorder

<1%: Megaloblastic anemia

Postmarketing and/or case reports: Lactic acidosis, leukocytoclastic vasculitis, pneumonitis.

Pregnancy and Lactation:

Metformin is classified as FDA pregnancy risk category B. Adverse events have not been observed in animal studies; therefore, metformin is classified as pregnancy category B. Metformin has been found to cross the placenta in concentrations which may be comparable to those found in the maternal plasma. Females of childbearing potential should be instructed to avoid becoming pregnant during the study. It is known that low amounts of metformin are excreted into breast milk.

Administration (Dosing): See Trial Design section above

Storage and Stability:

Tablets: Store between 20 - 25°C; excursion permitted to 15 - 30°C.

Study Population

Subjects are eligible for the study if the following inclusion and exclusion criteria are met:

A. Inclusion Criteria

1. Histological confirmed diagnosis of grade I or II adenocarcinoma of the endometrium
2. Must be obese as defined by a BMI greater than or equal to 30 kg/m^2
3. Candidate for surgical removal of their uterus as part of their endometrial cancer treatment
4. Subjects must have signed informed consent
5. Age 42 - 75 years of age
6. ECOG Performance status of 0 – 2
7. History of adequate renal, liver, and bone marrow function:
    a. Hb: (adequate for surgical intervention, with transfusion if necessary)
    b. WBC: (normal range)
    c. Platelets: (180K/cmm)
    d. LFTs: Normal bilirubin (<2.0mg/dL), AST/ALT (2xULN)
    e. Renal function: creatinine less than 1.4
8. Female subjects must either not be of child-bearing potential or must have a negative urine pregnancy test within 7 days of randomization to metformin. Subjects are considered not of child-bearing potential if they are surgically sterile or they are postmenopausal for greater than 12 months.

B. Exclusion Criteria

1. Poorly differentiated cancer or any of the high-risk subtypes of endometrial cancer including serous, clear cell, or carcinosarcoma
2. History of diabetes mellitus Type 1 or Type 2.
3. Receiving metformin prior to enrollment
4. Known hypersensitivity to metformin.
5. Unable to swallow and retain oral medication.
6. Pregnant or lactating.
7. Previous or concurrent malignancies, except non-melanoma skin cancers, unless curatively treated and with no evidence of recurrence for > 5 years
8. If the physician feels that the candidate is not suitable for the study, he/she will be excluded.
9. Currently taking biguanides, sulfonylurea drugs, thiazolidenediones, insulin, or mTOR or DPP-4 inhibitors or having taken any of these medications during the 12 weeks prior to study participation.
10. Clinical symptoms of gastrointestinal obstruction or bleeding and consideration for immediate surgery or immediate neoadjuvant chemoradiation.
11. History of lactic or other metabolic acidosis.
12. Uncontrolled infectious disease.
13. History of positivity for human immunodeficiency virus (HIV).
14. History of congestive heart failure requiring pharmacologic treatment.
15. History of excessive alcohol abuse, defined by a habitual intake of more than three drinks daily.
16. Mal-absorption syndrome, disease affecting gastrointestinal function, or previous resection of the stomach or small bowel.
17. Current use of medications for weight loss.

Feasibility of Study Population Recruitment:

At the UAMS Gynecologic Oncology clinic, approximately 160 new cases of endometrial cancer in obese women are seen each year (three new cases per week). The majority of these women are not diabetic at the time of the diagnosis of endometrial cancer. This represents the target population for this study. All new patients are seen by Dr. Burnett or Dr. Zorn. Except in rare circumstances, all these women will be scheduled for hysterectomy and bilateral salpingo-oophorectomy to stage their cancer. The typical time period between initially being seen by Dr. Burnett or Dr. Zorn and surgery is approximately 3weeks. Thus, those women who agree to participate in this trial can begin the intervention with metformin and expect no delay to the time of their surgery.

Study Calendar

Procedure	Pre-study	Study Period					Post-Operative clinic visit / Early Termination [f]
		Day 1 (Day of first dose)	**Day 8**			**Days 14 -21** (Surgery)	
Eligibility Assessments							
Informed Consent	X						
Inclusion/Exclusion Criteria	X						
Medical History	X						
Study Assessments							
Physical Examination	X	X					
Physical Measurements [e]	X	X				X	
Demographics [h]	X						
Vital signs [b]		X					
Body Mass Index (BMI)	X					X	
Smoking Status	X						
Alcohol Status	X						
Concomitant Medications [j]	X	X	X [k]			X	
Adverse Events (AEs)		X	X [k]			X	
Telephone contact			X [k]				
Laboratory Test							
CBC Panel [c]	X					X	
Comprehensive Metabolic Panel	X					X	
Lipid Panel (Cholesterol, HDL-C, LDL-C, Triglycerides) *	X					X	
Urine Pregnancy Test [d]		X					
HbA1c *	X					X	
Study labs (send to Simmen lab) [g]	X					X	
Clinical Drug Supplies							
Randomize		X					
Dispense study drug		X					
Dispense patient diary		X					
Review of drug diary			X [k]				
Collect unused study drug							X
Collect subject diary							X
Pathology							
Tumor Stage							X
Tumor Differentiation	X						X
Lymph Node Involvement							X
Tissue Collection (send to Simmen lab)	X[i]					X	

*Above standard of care (investigational-to be paid for by grant).
[a] A comprehensive panel will determine the following serum levels for the following: glucose, calcium, albumin, total protein, sodium, potassium, CO_2, chloride, BUN, creatinine, ALP, ALT, AST, and total bilirubin (standard of care).
[b] Temperature, blood pressure, pulse, respiration rate.
[c] WBC, Platelets, Hb
[d] Within 7 days of randomization
[e] Physical measurements to include ECOG, performance status, weight and height (height only)
[f] Unused drug and diary to be collected during standard of care visit, no other research activities will be performed.
[g] Insulin, Leptin, Adiponectin, IGF-I and IGF-II
[h] Age, sex, race, and ethnicity
[i] If available
[j] Record concomitant medications that are actively being taken at time of consent till completion of study.
[k] Subjects receiving Metformin only

Risks and Benefits

A. Potential toxicities, risks and precautions

Blood Samples: Pain and bruising at the injection site and rare infections can occur. Experienced personnel will perform the phlebotomies using approved techniques. Pressure and dressings will be used to minimize pain, bruising and infection.

Metformin Treatment: Common (50% of the time): Diarrhea; Rare (10 – 25% of the time): Nausea or vomiting, gas; Very rare (1 – 10% of the time): Weakness, indigestion, abdominal discomfort, headache, taste changes, abnormal stools, pain in the muscles, lightheadedness or dizziness, difficulty breathing, nail problems, increased sweating, flu-like symptoms, flushing, feeling of a fast beating heart.

Very rare side effects include Hirsutism (rare), lactic acidosis (3 cases per 100,000 patient years; 1) occurs most often in individuals with concomitant renal and liver disorders, 2) who are 80 years of age or older, 3) who have heart, liver or kidney failure, 4) who have a history of heavy alcohol intake.

B. Potential Benefits

It is unknown if 14-21 days of metformin treatment will provide any therapeutic benefit. For this reason, there is no guaranteed personal health benefit for subjects participating in this study. However, information obtained from this study may improve prevention and treatment of endometrial cancer for individuals in the future.

Adverse Event Reporting

All events listed below must be reported to the UAMS Institutional Review Board (IRB) within 10 days of the event or notification of event.

1). Local AEs that the investigator determines are:

a. Unexpected; and

b. Related to the research; and

c. Involve new or increased risks to subjects or others.

2). Any accidental or unintentional change to the IRB-approved protocol that place one or more subjects at increased risk or affects the rights and welfare of subjects or others.

3). Any new information that indicates an unexpected change to the risks or potential benefits of the research.

4). A breach in confidentiality that may involve risk to subject or others.

5). Any complaint of a participant that indicates an unanticipated risk or any complaint that cannot be resolved by the research team.

6). Incarceration of a subject.

7). Change in FDA labeling

8). Restrictions, terminations, or suspension of the study.

9). Premature completion.

10). Notifications of pending audits, inquiries or investigations by federal agencies.

11). Written reports from study monitor.

Ethical and Regulatory Considerations

A. Recruitment

Subjects will be recruited during clinic visits at the UAMS Gynecologic Oncology clinic.

B. Informed Consent

Subjects will be consented for their participation in the study and the use of their data, tissues and blood samples for research purposes. The principal investigator (PI), a sub-investigator or a designated staff member will discuss the informed consent form with the potential subject. The consent process will take place in a quiet and private room. Subjects may take as much time as needed to make a decision about their trial participation and may take the document home if desired. An interpreter will be utilized if native language is a barrier to understanding the consent form. The individual

obtaining consent will thoroughly explain each element of the document and outline the risks and benefits, alternate treatment(s), and follow-up requirements of the study. Participation privacy will be maintained and questions regarding participation will be answered. No coercion or undue influence will be used in the consent process. No research related procedures will be performed prior to obtaining informed consent. All signatures and dates will be obtained. A copy of the signed informed consent form will be given to the subject. The informed consent process will be documented in each subject's research record.

C. Protocol Management

Study management will be handled by the Cancer Clinical Trials Office (CCTO) in the Winthrop P. Rockefeller Cancer Institute.

D. Drug Accountability

Drug supplies will be kept in a secure, limited access storage area under the recommended storage conditions in the research pharmacy in the Winthrop P. Rockefeller Cancer Institute under the direction of the research pharmacist.

Outcome Measures

A. The **primary endpoints** of this study consist of the following:

a. IHC-based tissue markers of proliferation: Ki67, phosphorylated histone H3, estrogen receptor (ER), progesterone receptor (PR), and telomerase (hTERT).

b. IHC-based tissue markers of anti-proliferation and apoptosis: PTEN, KLF9, cleaved caspase-3, and TUNEL staining.

c. IHC-based tissue markers of insulin-mediated signalling: total and phosphorylated versions of Akt, Erk1/2, AMPK, and receptors for Insulin, IGF-I, and IGF-II.

d. Meformin-related adverse events.

B. The **secondary endpoints** of this study consist of the following:

a. Blood levels of HbA_1c and the lipid-panel analytes (cholesterol, HDL-C, LDL-C, triglycerides).

b. Plasma hormone levels (Insulin, IGF-I, IGF-II, leptin, and adiponectin).

c. Plasma-measured expression levels of mRNAs and miRs, measured using quantitative RT-PCR and miR-profiling microarrays, respectively.

d. Tissue-measured expression levels of mRNAs and miRs, measured using quantitative RT-PCR and miR-profiling microarray, respectively.

Data Handling and Recordkeeping

The PI will carefully monitor study procedures to protect the safety of research subjects, the quality of the data and the integrity of the study. The data will be kept in an electronic database in the office of clinical trials at the Winthrop P. Rockefeller Cancer Institute. Only the PI and Co-I, CRAs, regulatory personnel, biostatistician and lab personnel performing the analyses will have access to the data. In the event that a subject fails screening, only the eligibility criteria case report form will be completed.

Monitoring

Medical Monitor: The Medical Monitor, Principal Investigator and study staff will meet quarterly, once the PI has monitored the data, to review safety data (adverse events, serious adverse events and other scientific observations).

Study Monitor: The study will be monitored to ensure that the rights and well-being of human subjects are protected, that the data are accurate, complete and verifiable from source documents and that the trial is conducted in compliance with currently approved protocol/amendments on a quarterly basis.

Statistical Considerations

General approach: For each tissue endpoint, the tumor-NTU difference will be calculated in each patient as the paired difference in values between tumor and adjacent NTU tissue. For each blood endpoint, the endpoint response to treatment will be calculated in each patient as the paired difference between post-study and pre-study values. All data will be summarized by treatment arm as the mean, standard deviation (SD), median, and range, and will be graphed as box plots. Tumor-NTU differences and endpoint responses will be compared against zero via signed-rank test within each treatment arm. Treatment arms will be compared to each other for differences via Wilcoxon rank-sum tests, with additional details provided below. All statistical comparisons will be two-sided, and will employ a 10% alpha to reduce Type II error in this pilot proof-of-concept study.

Analysis plan for tolerability: Meformin-related adverse events will be summarized by type and treatment arm, both as proportions at each grade and as average grades (i.e., mean scores). For each type, the Wilcoxon rank-sum test will be used to compare treatment arms for differences in average grade.

Analysis plan for the IHC-based primary endpoints: The IHC-based primary endpoints will be measured in tissue, and will include markers of proliferation (Ki67, phospho-histone H3, ERα, PR, and hTERT), anti-proliferation and apoptosis (PTEN, KLF9, cleaved caspase-3 and TUNEL staining), and insulin-mediated signalling (total and phosphorylated versions of Akt, Erk1/2, AMPK, and receptors for Insulin, IGF-I, and IGF-

II). Endpoints measured in tumor tissue, adjacent NTU tissue, and tumor-NTU differences will be summarized and graphed as described above. Wilcoxon rank-sum tests will be used to compare endpoints measured in tumor, and likewise endpoints measured in adjacent NTU tissue, for differences between the metformin and no-metformin treatment arms.

Analysis plan for secondary endpoints measured in blood or plasma: The secondary endpoints measured in blood will be the levels of HbA_1c, cholesterol, HDL-C, LDL-C, and triglycerides. The secondary endpoints measured in plasma will be the plasma hormone levels (Insulin, IGF-I, IGF-II, leptin, and adiponectin) and the plasma-measured expression levels of mRNAs and miRs. Endpoints measured at pre-study and post-study, and endpoint responses to treatment will be summarized and graphed as described above. Wilcoxon rank-sum tests will be used to compare endpoint responses to treatment for differences between the metformin and no-metformin treatment arms.

Analysis plan for secondary endpoints measured in tissue: The secondary endpoints measured in tissue will be the expression levels of mRNAs and miRs. Endpoints measured in tumor tissue, adjacent NTU tissue, and tumor-NTU differences will be summarized and graphed as described above. Wilcoxon rank-sum tests will be used to compare endpoints measured in tumor, and likewise endpoints measured in adjacent NTU tissue, for differences between the metformin and no-metformin treatment arms.

Sample Size and Power: There will be 20 subjects on each treatment arm, yielding a total of 40 subjects. The two-sided Wilcoxon rank-sum test will be used at 10% alpha to compare all endpoints and endpoint responses for differences between the metformin arm and the no-metformin arm. This study will be considered sufficiently powered to meet research objectives if the Wilcoxon rank-sum test has >90% power at 10% alpha to detect a treatment-arm difference of 1.0 SDs or more. Statistical power of the Wilcoxon rank-sum test was calculated using the 2-sample t-test module of PASS 2011 by selecting options corresponding to the worst-case scenario of underlying normal distributions with unknown SDs. Under this worst case scenario, 20 subjects per treatment arm (for a total of 40 subjects) give the two-sided Wilcoxon rank-sum test 91.6% power at 10% alpha to detect a difference of 1.0 SDs between the metformin and no-metformin arms, thereby establishing that this study is sufficiently powered to meet research objectives.

Dissemination of Data

The results will be analyzed after the analysis of all the blood and tissue samples. Then the two groups will be compared and the statistical analysis will be performed. The results will be submitted for presentation at national meetings, manuscripts will be written for publication and the data will be used to apply for additional funding from federal agencies including the National Institutes of Health/National Cancer Institute (NIH/NCI).

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