THOMAS JEFFERSON UNIVERSITY

Sidney Kimmel Cancer Center

A Phase II Study of Metformin in Combination with Doxycycline in Patients with Localized Breast, Uterine and Cervical Cancer

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Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator:

Signed: _____ Date: _____

Name: Jennifer M Johnson, MD PhD

Title: Principal Investigator

Statement of Compliance

This study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and Thomas Jefferson University research policies

List of Abbreviations

AJCC	American Joint Committee on Cancer
AE	Adverse Event/Adverse Experience
AMPK	AMP-activated Protein Kinase
ATP	Adenosine Triphosphate
CAF	Cancer Associated Fibroblast
CAV1	Caveolin-1
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRO	Contract Research Organization
CSC	Cancer Stem Cell
CTCAE	Common Terminology Criteria for Adverse Events
CTO	Clinical Trials Office
DLT	Dose Limiting Toxicity
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
ECOG	Eastern Cooperative Oncology Group
ER	Estrogen Receptor
ESR	Erythrocyte Sedimentation Rate
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HER2	Human Epidermal Growth Factor Receptor 2
HIPAA	Health Insurance Portability and Accountability Act
HNSCC	Head and Neck Squamous Cell Carcinoma
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IHC	Immunohistochemistry
IND	Investigational New Drug Application
IGF-1	Insulin-like Growth Factor 1
IGF-BP3	Insulin-like Growth Factor Binding Protein 3

IGFR	Insulin Growth Factor Receptor
IRB	Institutional Review Board
LKB1	Liver Kinase B1
MCT1	Monocarboxylate Transporter 1
MCT4	Monocarboxylate Transporter 4
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSI	Mass Spectroscopy Imaging
mTORC1	Mammalian Target of Rapamycin Complex 1
N	Number (typically refers to subjects)
NCI	National Cancer Institute
OXPHOS	Oxidative Phosphorylation
PHI	Protected Health Information
PI	Principal Investigator
PR	Progesterone Receptor
PRC	Protocol Review Committee
QA	Quality Assurance
QC	Quality Control
ROS	Reactive Oxygen Species
SAE	Serious Adverse Event/Serious Adverse Experience
SKCC	Sidney Kimmel Cancer Center
SOP	Standard Operating Procedure
TOMM20	Transporter of Outer Mitochondrial Membrane 20
TSC2	Tuberous Sclerosis Complex Tumor Suppressor Gene 2
UP	Unanticipated Problem

Study Summary

Title: A Phase II Study of Metformin in Combination with Doxycycline in Patients with Localized Breast, Uterine and Cervical Cancers

Lead Site: Thomas Jefferson University

Sponsor: Investigator-Initiated Trial

Phase: Phase II

Objectives:

Primary:

- To determine if treatment with a combination of metformin and doxycycline can increase the percentage of cells that express Caveolin-1 in the cancer associated fibroblasts of patients with breast, uterine and cervical cancers.

Secondary:

- To determine the effect of metformin and doxycycline treatment on the percentage of cells that express MCT4 in cancer associated fibroblasts and MCT1 and TOMM20 in the cancer cells of breast, uterine and cervical cancer patients.
- To assess safety and tolerability of metformin and doxycycline treatment in subjects with breast, uterine and cervical cancer.
- To determine the relationship of the percentage of stromal cells expressing CAV1 or MCT4 and tumor cells that express MCT1 and TOMM20 at baseline and after treatment with metformin and doxycycline with the percentage of cells expressing ER and PR for breast, uterine and cervical samples and HER2 in breast cancer samples.

Correlatives:

- To assess the effect of combined metformin and doxycycline therapy on the metabolic profile of cancer cells and stroma using mass spectroscopy imaging (MSI) on paired samples, comparing metabolite profiles in the pre-metformin and post-metformin tumor samples.
- To assess, when possible, the impact of a patient's nutritional status, estimated using 3 day dietary recall versus caloric needs as calculated by the Harris-Benedict equation on the baseline and net change in CAV1.
- To assess the effect of combined metformin and doxycycline therapy on oncomiR miR-21 after intervention.
- To assess the effect of combined metformin and doxycycline therapy on adipokines and the IGF-1/insulin signaling pathways through assessment of serum triglycerides, IGF-1, IGF-BP3, erythrocyte sedimentation rate (ESR), adiponectin, leptin, IGF-1R, exosome evaluation, metabolomics profile, and microRNA expression profiles.

Target Population:

- Patients aged 18 years and above
- Known or suspected breast, uterine (endometrial cancer with histologies including endometrioid, serous, clear cell and carcinosarcoma), or cervical cancer.

- Have surgical intent for resection as a part of their planned therapy
- Performance status ECOG 0-1
- Normal organ function including creatinine <1.5

Required Enrollment

23 patients per cohort (breast and uterine/cervical) for a total of 46 patients

Rationale:

Metformin, a widely used anti-diabetic drug, which functions as a mitochondrial inhibitor, can also be used to selectively target cancer stem cells. Metformin functionally inhibits OXPHOS by targeting complex I of the electron transport chain and can even induce lactic acidosis, as a lethal side effect. As a result, the use of antibiotics, such as doxycycline, may provide a safer and far more effective alternative to anti-cancer therapy with metformin. Thus, future clinical trials for testing the efficacy of mitochondrial-targeted antibiotics in multiple cancer types are now clearly clinically warranted.

Doxycycline is a broad-spectrum antibiotic that is commonly used for the treatment of many bacterial infections, and functions as an inhibitor of protein synthesis in bacteria. Doxycycline also encourages the growth of normal stem cells, has anti-inflammatory properties, and even increases cell lifespan, in certain experimental contexts. Thus, the toxic side effects of anti-cancer therapy would be minimized. Doxycycline has also been used in human tumor xenografts and other animal models to significantly reduce tumor burden and even metastatic cancer cell growth. For example, in pancreatic tumor xenografts (with PANC-1 cells), doxycycline treatment reduced tumor growth by ~80%. In a xenograft model of breast cancer bone metastasis (with MDA-MB-231 cells), doxycycline treatment reduced bone and bone-associated soft-tissue tumor mass by >60% and ~80%, respectively.

Overall, the combination of metformin and doxycycline will co-target mitochondrial pathways and possible be synergistic to impair mitochondrial function that could lead to further cell quiescence.

Study Design:

	Cohort	Treatment	Week	-1	1						2							3	4	5
			Day	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8***	**9**	**10**	**11**	**12**	**13**	**14**		
Eligibility Screening	**1** N=23	Doxycycline[e]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Surgical Resection	
		Metformin		X[a]	X[a]	X[a]	X[b]	X[b]	X[b]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]		
	2 N= 23	Doxycycline[e]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
		Metformin		X[a]	X[a]	X[a]	X[b]	X[b]	X[b]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]		
		Tissue[d]	X																X	

*** If surgery is scheduled past Day 8, patients will stay on metformin and doxycycline until the day prior to surgery.**
a. Metformin will be given initially with a starting dose of 500mg once a day for Days 1-3.
b. Metformin will be increased to 500 mg orally twice a day for Days 4-6
c. If tolerated, beginning Day 7 metformin will be increased to 1000 mg twice a day. Subjects will maintain the maximum tolerated dose (up to 1000 mg twice daily) until the day prior to their scheduled definitive surgery.
d. When possible, baseline biopsy tissue is to be obtained prior to starting study medications. Two serum separator tubes of serum will be taken at the same time as the biopsy specimen (if possible), otherwise done at time of consent. If a pre-treatment sample has already been acquired and flash froze and if there is excess tissue available at resection, the second specimen will also have a piece flash frozen for metabolomic testing.
e. Doxycycline will be taken once every 12 hours, with the last dose being taken at least 12 hours prior to scheduled time of definitive surgery.

Metformin and doxycycline are the therapeutic agents. For metformin, the initial starting dose will be 500 mg orally daily for 3 days which will then be increased to 500 mg orally twice daily, and if tolerated, further increased to 1000 mg twice daily after day 6. Subjects will maintain the maximum tolerated dose (up to 1000 mg twice daily) until the day prior to their scheduled definitive surgery.

For doxycycline the patients will take 100 mg by mouth every twelve hours until the day prior to their surgery.

Criteria for Evaluation:

Primary:

- Assess the impact of metformin and doxycycline on the percentage of cancer associated fibroblasts that stain with 1+ intensity or greater for CAV1 by immunohistochemistry (IHC) pre and post metformin and doxycycline exposure as determined by two blinded pathologists. All patients from whom samples are obtained both pre- and post-treatment will be included in the primary analyses.

Secondary:

- Assess the impact of metformin and doxycycline on the percentage of cancer-associated fibroblasts that express MCT4 and the percentage of cancer cells that express MCT1 and TOMM20. All patients from whom samples are obtained both pre- and post-treatment will be included in the secondary analyses.
- Assess safety and tolerability of metformin and doxycycline treatment in subjects with cancer. Toxicity will be evaluated using the most recent version (version 4) of the NCI toxicity criteria, i.e. the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.
- The staining patterns of CAV1, MCT4, MCT1, and TOMM20 will be compared to the ER, PR, and Her2 expression levels whenever these markers have been performed in the course of clinical care. All patients from whom samples are obtained both pre- and post-treatment will be included in the secondary analyses

Correlative:

- Mass spectroscopy imaging (MSI) will be performed on paired samples, comparing metabolite profiles in the pre-treatment and post-treatment tumor sample. MSI allows assessment of metabolite levels in spatially defined tumor regions and hence will allow the evaluation of metabolite levels in carcinoma and stromal cell compartments. Only patients for whom flash frozen samples can be

obtained both prior to initiation of treatment and in the OR at the time of resection will be considered for this analysis.
- Patients who are able to complete, when possible, a 3 day recall dietary diary with the aid of a nutritionist will also have their caloric needs estimated with a Harris-Benedict Equation.
- Patients from whom serum samples are available will be assessed for oncomiR-21, IGF1, IGF-BP3, ESR, adiponectin, leptin, IGF-1R, and exosome and metabolomics profiles in exploratory analyses.

Statistical Analysis: The primary objective of the study is to assess the impact of metformin and doxycycline on the percent of stromal cell that have CAV1 expression by immunohistochemistry (IHC) in carcinoma cells. All patients from whom samples are obtained both pre- and post-treatment will be included in the primary analyses. Within-patient change in IHC scores will be analyzed using the Wilcoxon signed-rank test.

The sample in each cohort is based on having 80% power to detect an average increase in CAV1 staining of 20% using a one-sided Wilcoxon signed-rank test with alpha=0.05. Based on pilot data in other cancers, we assume the standard deviation of change scores to be 32%. A sample size of 19 per cohort is required under these assumptions. Allowing for approximately 15% of patients to have missing pre- and or post-treatment data, we will recruit 23 women per cohort.

Incidence of Dose Limiting Toxicities (DLTs) will be monitored continuously with potential stopping after 10 patients have been enrolled based on the Bayesian method of Thall and Simon. We are comparing our experimental treatment to a standard of no treatment and assume no DLTs under standard of care. Both agents are extremely safe and DLTs should be exceedingly rare. In 900 patients treated with metformin alone, the discontinuation rate was 0.6%. In 31 patients treated with IV doxycycline, 4 SAEs were observed, but none were related to the study drug. Thus, it is reasonable to assume a prior distribution for the DLT rate in the combination of Beta (0.04,1.96). That is, we assume a 2% event rate for the combination with information equivalent to data from 2 patients. We will stop the study for safety if the posterior probability that the rate of DLTs in the combination treatment is 5% or greater is greater than 90%.

Study Duration: 48 months

Subject Participation Duration: From the time that patients enroll in the trial until their definitive surgery is between 1-5 weeks. The patients' records will then be reviewed every 3 months for the next 12 months.

Estimated Time to Complete Enrollment: 36 months

1 Introduction

This document is a protocol for a human research study. This study will be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background Information

Current treatment Options for Breast, Uterine and Cervical Cancers.

Multiple treatment options exist for “early stage” or “localized” cancers of the breast, uterus, and cervix. Briefly, for breast cancer patients, those patients who are considered to have locoregional disease (AJCC stages I, II, IIB, or T3N1M0) are first offered either lumpectomy or mastectomy for surgical resection. Patients are then stratified based on the surgical resection method chosen, number of positive lymph nodes, and margins to undergo adjuvant therapy with radiation or radiation with chemotherapy. For uterine neoplasms, specifically endometrial cancer, patients typically undergo an initial endometrial biopsy. If patient are medically operable, women will undergo a total hysterectomy, bilateral salpingo-oophorectomy, pelvic and/or paraaortic lymph node dissection, and potential debulking procedure if necessary. Similar to breast cancer, based upon the extent of disease noted these patients may then also go on to have adjuvant radiation with or without chemotherapy. For cervical cancer patients, a majority of these neoplasms are detected by routine pap smears, followed by colposcopy with biopsy. For early stage disease, definitive conization or trachelectomy is the preferred management. For later stage disease, and for patients who refuse or cannot tolerate surgery, combination chemotherapy and radiation is the preferred treatment modality.

Between the time of initial diagnosis (either radiographic or pathologic via biopsy) there is a planning period of on average 2-5 weeks. During this time period, subjects typically receive no active therapy. As described below, there is evidence to suggest that metformin with doxycycline might positively affect tumor cell biology and thereby affect cancer outcomes. We plan to give patients metformin and doxycycline prior to surgery in order to determine the physiological effects on cancer cells. If the results demonstrate that there is a positive effect, this will provide important supportive data for future clinical trials for cancer patients and could alter treatment approaches to patients.

Metformin and Its Potential as an Antineoplastic Agent

Metformin (N, N-dimethylbiguanide) is a biguanide that is best known for its use as first line therapy for type II diabetes patients (1). Metformin specifically inhibits the complex I (NADH:ubiquinone oxidoreductase) of the mitochondrial electron transport chain decreasing cellular respiration and the rate of ATP formation (2) (3) (4). This triggers the activation of the energy sensor AMP-activated protein kinase (AMPK) that regulates cell metabolism and shifting it towards an energy-sparing state (5). This leads to reduced hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity thereby increasing peripheral glucose uptake and utilization without causing hypoglycemia. It is an FDA approved medication for the management of type 2 diabetes mellitus with extensive safety data. In general, clinically significant responses are not seen at doses <1500 mg daily; however, a lower recommended starting dose and gradual increased dosage is recommended to minimize gastrointestinal symptoms.

Extensive preclinical data now also supports the effectiveness of metformin as an antineoplastic agent (6).

Epidemiologic Data for Metformin’s Efficacy:

Retrospective studies have shown that diabetics treated with metformin have a cancer risk reduction of approximately 40% compared to diabetics not treated with metformin (7,8). Other studies have also shown a reduction in the frequency of cancer with metformin use [33]. Evans et al (9) reported that the risk of subsequent cancer diagnosis was reduced in patients with type II diabetes who received metformin (with an odds ratio of 0.85 for any metformin exposure versus no metformin exposure). The protective effect increased with greater metformin exposure (measured as total dose prescribed or total duration of use).

Current evidence from epidemiologic studies suggests that metformin has clinical activity in breast cancer. Hadad et al (11) demonstrated biomarker evidence for anti-proliferative effects of metformin in women with breast cancer by decreasing Ki67 and messenger RNA expression for PDE3B (critical regulator of cAMP levels that affect activation of AMPK). Similarly, Niraula et al (12) showed short-term preoperative metformin with a dosing schedule of 500mg three times daily was well tolerated and resulted in clinical and cellular changes consistent with beneficial anti-cancer effects with increased insulin sensitivity by HOMA in subjects and decreased proliferation and increased apoptosis in carcinoma cells.

Pre-clinical data also supports the potential benefit of metformin in patients diagnosed with cervical cancer. Metformin was shown to induce apoptosis and autophagy in cervical cancer cells that express LKB1. LKB1 is responsible for the phosphorylation and activation of AMPK, which is a potent suppressor of cell proliferation in a variety of cell types, including cervical cancer cells. While LKB1 intact cervical cancer cells showed the greatest response to metformin via LKB1-AMPK-mTOR signaling, it was shown that cervical cancer cells lacking LKB1 were less sensitive to the medication. However, introducing ectopic expression of LKB1 in these cells restored the sensitivity to metformin, which confirms the importance of LKB1 expression in the use of metformin (89).

There are currently multiple completed and on-going clinical trials evaluating the effect of metformin in combination with standard treatment of a variety of malignancies including breast, colorectal, pancreatic, lung, gynecologic, and prostate cancer (13, 14, 15).

<u>Mechanism of Action for Metformin and Doxycycline:</u>
Metformin has been proven to inhibit cell proliferation *in vitro* and/or *in vivo* in several malignancies (17)). Metformin *in vivo* has antineoplastic activity. Experimental work including *in vivo* experiments and clinical trials have demonstrated that metformin has anticancer properties (18)) (19) (20). The mechanism by which metformin has anticancer effects is unknown and the purported mechanisms are numerous and include OXPHOS complex I inhibition, AMPK activation and insulin growth factor signaling (21) (22) (23) (24). While the precise anticancer mechanisms are still an active area of research, metformin works overall by inducing energetic stress. One proposal for how this is accomplished is by its direct inhibitory effect on mitochondrial complex I. Several groups have shown that metformin's ability to limit tumor growth *in vivo* is dependent on mitochondrial complex I (25). Complex I inhibition blocks mitochondrial-dependent production of reactive oxygen species (ROS) and adenosine triphosphase (ATP) (26-28). Catabolite access may determine metformin susceptibility and as supporting examples, cancer cells grown in the absence of glucose and presence of glutamine are more affected by metformin treatment than cells grown in the presence of glucose (29). Metformin sensitivity is determined by glucose availability and oxidative phosphorylation (OXPHOS) capacity(30). The decrease in ATP production results in the activation of the liver kinase B1 (LKB1) – adenosine monophosphate-activated protein kinase (AMPK) signaling pathway (26-28). Activation of this pathway usually occurs during times of hypoxia and nutrient

deprivation, and reciprocally, it can be suppressed in times of “over nutrition” and hyperglycemia. AMPK is a key energy sensor that regulators metabolism in an attempt to maintain energy homeostasis (31). The end result of blocking the LKB1-AMPK signaling pathway is a down-regulation of energy consuming biosynthetic processes including gluconeogenesis, protein and fatty acid synthesis and cholesterol biosynthesis, and promotion of catabolic processes such as fatty acid beta oxidation and glycolysis (32). Metformin may also have activity that is independent of LKB1. In LKB1 deficient cells, metformin is still able to affect the intracellular energy state (33). Metformin also alters the mitochondrial redox state by inhibiting glycerophosphate dehydrogenase (34). Metformin reduces the mitochondrial citric acid cycle and induces aerobic glycolysis as well(35).

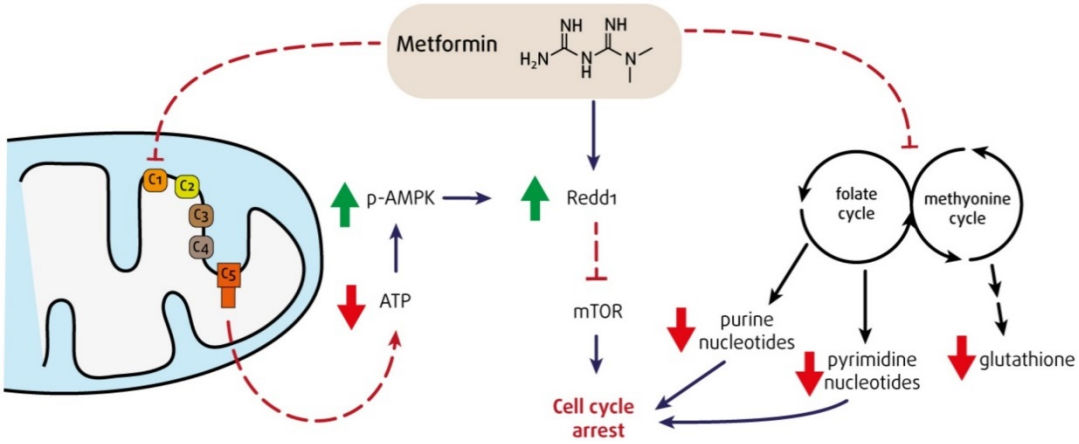

Figure 1. The proposed mechanism of action of metformin in the cell [36].

Another central signaling molecular impacted by metformin is mammalian target of rapamycin (mTOR). In mouse embryonal fibroblasts and several cancer cell lines, AMPK activation by metformin leads to inhibition of mTOR and inhibition of proliferation (37-39).

In addition to the effects of metformin and AMPK on metabolic processes, activation of AMPK results in rapid inhibition of cellular protein synthesis and growth. Mechanistically, AMPK achieves this by phosphorylation and stabilization of the protein product of the tuberous sclerosis complex tumor suppressor gene TSC2, which serves as an integrator of various regulatory inputs implicated in cell growth and transmits them to the master regulator of cellular protein synthesis, mTOR. In addition to mediating the inhibitory effects of AMPK on protein synthesis, TSC2 integrates several regulatory inputs that affect cellular protein translation, notably signals emanating from the availability of oxygen and growth factor– dependent stimulation of the phosphoinositide 3-kinase(PI3K)/phosphatase and tensin homolog(PTEN)/v-akt murine thymoma viral oncogene homolog (AKT) and the Ras/Raf/extracellular signal-regulated kinase (ERK) pathways, two of the most frequently deregulated signaling cascades in human cancer. Metformin has been shown to selectively target stem cells and to have synergistic properties with doxorubicin, paclitaxel and carboplatin allowing for conventional chemotherapy dose reductions in several carcinoma subtypes (40,41). Rozengurt et al identified crosstalk between insulin/insulin-like growth factors (IGF-1) receptors and G protein-coupled receptors (GPCR) signaling systems in pancreatic cancer cells leading to enhanced signaling, DNA synthesis, and proliferation. This crosstalk was dependent on mammalian target of rapamycin complex 1 (mTORC1). They were able to use metformin, which negatively regulates

mTORC1, and disrupt crosstalk between insulin/IGF-1 receptors and GPCR signaling inhibiting growth of pancreatic cancer cell lines in xenograft models. Therefore, metformin decreases oxidative phosphorylation (OXPHOS) metabolism and generation of reactive oxygen species (42).

Other agents are also able to affect mitochondria. In contrast to the mechanism of action of metformin, doxycycline inhibits *mitochondrial* translation and mitochondrial OXPHOS. The translation machinery of mitochondria is distinct from that found in the cytosol and in bacteria although there are also similarities which make it a promising anticancer drug target. Eukaryotic cells originate from a merger of two formerly independent cells—the host cell and the α-proteobacteria, which is a precursor of mitochondria. Each one of these cells contributed a protein synthesis system and hence eukaryotic cells have cytoplasmic and mitochondrial protein translation systems (43). Host cell translation, which occurs in the cytosol, synthesizes almost all cellular proteins, including most mitochondrial proteins. However, 13 key subunits of mitochondrial OXPHOS are mitochondrially translated. Up-regulation of mitochondrial translation occurs with the development of cancer in a subset of human malignancies (44). Inhibition of mitochondrial protein translation also has anticancer activity.

Human mitochondrial ribosomes (mitoribosomes) are composed of a large 39S ribosomal subunit and a small 28S subunit, and by contrast, are highly specialized for the synthesis of 13 membrane proteins in humans that function in energy production (45). Advances in high-resolution cryo–electron microscopy have proven that the molecular structure of mitoribosomes differs dramatically from the “canonical” cytosolic ribosome of bacteria and eukaryotes(44, 46). Also, the structures of mitoribosomes from different species are dramatically different(45). Human mitoribosomes have increased protein mass compared to bacterial ribosomes, which results in a ribosome with a more extensive protein-protein network and an rRNA core better shielded from ROS. This has important implications to develop anticancer drugs that specifically target mitoribosomes (46). The human small mitochondrial 28S ribosomal subunit shares features with the bacterial 30S subunit. As detailed atomic structures of human mitochondrial ribosomes are obtained, this should allow the rational design of compounds that specifically block mitochondrial ribosome activity.

Doxycycline, which inhibits mitochondrial ribosome function, eradicates tumors and specifically CSCs, across many different tumor subtypes including breast cancer (47). Clinical trials are ongoing to determine if tetracyclines can be repurposed as anticancer agents.

<u>Preclinical Data in the Martinez Laboratory:</u>
We and others have shown that breast and uterine cancers have multi-compartment metabolism with high mitochondrial metabolism in carcinoma cells and high glycolytic metabolism in stromal cells. We have also shown that loss of CAV1 in the stroma is a marker of this metabolic heterogeneity. Metformin can rescue CAV1 expression experimentally and abolish metabolic heterogeneity in breast cancer.

Cancer cells have the ability to metabolically reprogram non-cancerous surrounding cells in order to favor nesting behavior, derive nutrients, enhance proliferation, and eventually promote invasion and metastasis (48-51). This parasitic relationship between cancer cells and stromal cells is known as “two-compartment tumor metabolism” (52). In this context, cancer cells rely on oxidative metabolism in order to support their high bioenergetic requirements (53). Simultaneously, the remodeled cancer-associated stroma adopt a catabolic phenotype to provide catabolites to the anabolic cancer cells driving tumor-stroma co-evolution(54). The

presence of CAFs performing aerobic glycolysis is an important feature of multi-compartment metabolism and loss of stromal CAV1 is a marker of this process(48) (55).

Loss of Caveolin-1 (CAV1) in CAFs is sufficient to drive the glycolytic phenotype in the stromal compartment and the increased mitochondrial metabolism in cancer cells (56). CAV1 is the principal structural protein coating caveolae in the plasma membrane, and functions as a scaffolding protein regulating signaling transduction. Downregulation of CAV1 in CAFs induces signaling through transforming growth factor beta (TGF-B), nuclear factor kB (NF-kB), and hypoxia inducible factor (HIF), triggering reactive oxygen reactive species (ROS) generation, inflammation, autophagy and increased catabolism (57). Reduced CAV1 expression overall drives a myofibroblast-like phenotype of CAFs that promotes tumor formation and progression and facilitates invasion and metastasis (56). Loss of CAV1 in CAFs is sufficient to induce further CAV1 downregulation in adjacent fibroblasts without requiring the presence of cancer cells (58). Clinically, loss of stromal CAV1 expression is associated with poor outcomes/prognosis in several cancer types including breast cancer (59, 60) squamous esophageal cancer (61), gastric cancer (62), colorectal cancer (63), pancreatic cancer (64), prostate cancer (66), non-small cell lung cancer (66) and malignant melanoma (67).

In head and neck squamous cell carcinomas (HNSCC) there are very similar features of metabolic heterogeneity to that observed in breast and endometrial cancer. HNSCC have high mitochondrial OXPHOS metabolism in highly proliferative cells (68). Also, there is low expression of CAV1 and high MCT4 expression in HNSCC cancer associated fibroblasts (CAFs) and in carcinoma cells with low proliferation rates (69). MCT4 expression is a marker of pseudohypoxia, oxidative stress and enhanced glycolytic metabolism. We have demonstrated that there is metabolic coupling between highly proliferative carcinoma cells with high OXPHOS metabolism and low proliferative carcinoma cells and fibroblasts (68).

We interrogated HNSCC specimens to examine if different metabolic and oxidative stress compartments co-exist in human tumors. A panel of biomarkers (Ki67/TOMM20/COX/MCT1/CAV1/MCT4) was employed to visualize metabolic compartmentation. Three metabolic compartments were delineated: 1) proliferative and mitochondrial-rich carcinoma cells (Ki67+/TOMM20+/COX+), 2) non-proliferative and mitochondrial-poor carcinoma cells (Ki67-/TOMM20-/COX-), and 3) non-proliferative and mitochondrial-poor tumor stroma (Ki67-/TOMM20-/COX-/CAV1-). High oxidative stress (MCT4+) was specific for cancer tissues.

With this data, we then evaluated its prognostic value in a second cohort (N=40). We found that oxidative stress (MCT4+) in non-proliferating carcinoma cells predicted poor clinical outcome ($p < 0.001$), and was functionally associated with PET-avidity ($p < 0.04$). Similarly, oxidative stress (MCT4+) in tumor stromal cells was associated with higher tumor stage ($p < 0.04$). Leading us to propose that oxidative stress is a hallmark of tumor tissues and fuels mitochondrial metabolism in proliferating mitochondrial-rich cancer cells, via paracrine energy transfer of mitochondrial fuels (such as L-lactate and ketone bodies).

With this, we were able to show for the first time that metabolic and oxidative stress compartmentalization exists in HNSCC mucosa with highly proliferative epithelial cancer cells having high mitochondrial metabolism with lactate and ketone body uptake while as other carcinoma cells and CAFs have low mitochondrial metabolism with high lactate and ketone body generation and high oxidative stress. This metabolic and oxidative stress compartmentalization of HNSCC mucosa shares similarities with that of normal mucosa and

likely drives proliferation via OXPHOS metabolism. MCT4 is an oxidative stress marker and may be a marker of CAFs since it is always absent in normal fibroblasts while it is found in the majority of CAFs. A subgroup of HNSCC has high MCT4 expression in a large carcinoma compartment and this subtype is associated with a poor prognosis.

Furthermore in a pilot trial looking at the effects of metformin on mitochondrial metabolism in HNSCC patients, we have already shown that after an average of 14 days of exposure to metformin as a single agent the average expression of Caveolin-1 was increased from 17% pre-treatment to 60% post- metformin exposure. Analyses from this trial are currently underway.

Doxycycline

Doxycycline is a tetracycline antibiotic commonly used to treat non-gonococcal urethritis, respiratory tract infections, and acne vulgaris among other bacterial infections (69). It is generally bacteriostatic and affects both gram-positive and gram-negative bacteria by transportation into the cell via passive diffusion or through an energy-dependent active transport system. Doxycycline is relatively more lipophilic than other tetracyclines, allowing easy passage through the bacterial lipid bilayer into the cytosol, where reversible binding to the 30S ribosomal subunit occurs; this ultimately inhibits bacterial protein synthesis. High concentrations of antibiotic can also interfere with protein synthesis in mammalian cells, but these cells lack the active transport systems found in bacteria, making eukaryotic cells less vulnerable targets (Clinical Key).

Given that mitochondria have a bacterial ancestry and that the 30S bacterial ribosomal subunit is homologous to the 28S mitochondrial subunit (70), these antibiotics also target mitochondrial translation and impair mitochondrial function. Doxycycline in particular disturbs mitochondrial protein synthesis and metabolic activity while altering gene expression (71). Even at low concentrations, it has been shown to increase glycolytic metabolism and markedly reduce mitochondrial oxygen consumption (72). The crystalline structure of mitochondrial proteins also plays an important role in the efficacy of various drugs on altering mitochondrial metabolism. Lee et al. reported that the target protein in cancer cell mitochondria is TNF receptor-associated protein 1 (TRAP1), and TRAP1 inhibitor efficacy depends on the degree of fit between the crystalline structure of TRAP1 and the structural conformation of the drug in question. This effect of tetracyclines on mitochondrial metabolism is highly relevant for cancer research.

Doxycycline, with its long half-life systemically, has recently become an attractive anti-neoplastic agent. It also encourages the growth of normal stem cells, has anti-inflammatory properties, and even increases lifespan, in certain experimental contexts (73,74). In tumor xenografts and other animal models, doxycycline significantly reduces tumor burden and metastatic cancer cell growth (75). Further studies are needed to elucidate doxycycline's anti-neoplastic properties and support its clinical application in cancer treatment.

Combination of Metformin and Doxycycline for Treatment:
Metformin and doxycycline work through independent mechanisms to block mitochondrial metabolism. We hypothesize that these two FDA-approved medications will be able to provide and additive effect when used in combination to alter the cellular metabolism of cancer cells and their associated stromal fibroblasts.

Our preliminary work in head and neck squamous cell carcinoma has already shown that metformin alone modulates the expression of CAV1 in cancer-associated fibroblasts. We hypothesize that metformin and doxycycline together will be able to increase Caveolin-1 expression in cancer-associated fibroblasts as well as to decrease stromal MCT4 expression and carcinoma TOMM20 expression with altered metabolite profiling. The ability to more fully understand and to modify the metabolic profile of cancer associated fibroblasts may lead to future therapies in breast, uterine (endometrial), and cervical cancers.

1.2 Rationale

Metformin, a widely used anti-diabetic drug, which functions as a mitochondrial inhibitor, can also be used to selectively target cancer stem cells. Metformin functionally inhibits OXPHOS by targeting complex I of the electron transport chain and can even induce lactic acidosis, as a lethal side effect (76). As a result, the use of antibiotics, such as doxycycline, may provide a safer and far more effective alternative to anti-cancer therapy with high metformin doses. Thus, future clinical trials for testing the efficacy of mitochondrial-targeted antibiotics in multiple cancer types are now clearly clinically warranted.

Doxycycline is a broad-spectrum antibiotic that is commonly used for the treatment many bacterial infections, and functions as an inhibitor of protein synthesis in bacteria. Doxycycline also encourages the growth of normal stem cells, has anti-inflammatory properties, and even increases lifespan, in certain experimental contexts (77,78). Thus, the toxic side effects of anti-cancer therapy would be minimized. Doxycycline has also been used in human tumor xenografts and other animal models to significantly reduce tumor burden and even metastatic cancer cell growth (79-82). For example, in pancreatic tumor xenografts (with PANC-1 cells), doxycycline treatment reduced tumor growth by ~80% (83). In a xenograft model of breast cancer bone metastasis (with MDA-MB-231 cells), doxycycline treatment reduced bone and bone-associated soft-tissue tumor mass by >60% and ~80%, respectively (84).

Overall, we hypothesize that the combination of metformin and doxycycline will co-target mitochondrial pathways and possibly be synergistic in impairing mitochondrial function that could lead to further cell quiescence.

Hypothesis: Exposure to metformin and doxycycline administered together will alter the metabolic profile of solid tumor malignancies, specifically with respect to breast, uterine and cervical cancers.

Rationale for Dosage/Route of Administration

Metformin will be administered orally since this is the route of administration currently approved by the FDA. The drug will be initiated at a dose of 500mg daily with dose escalation every 3 days to a goal of 1000mg twice daily to be continued until the time of definitive surgery. There are currently multiple studies on-going using doses from between 500 mg twice daily up to 2500 mg per day in the treatment arms. There are also studies using the extended release form for a dose of 1500 mg daily. We have chosen our starting dose and escalation regimen to minimize side effects. The chosen standing dose is based on metformin's therapeutic range (minimal therapeutic dose in diabetic patients is 1500-2000 mg a day) (85,86). The minimum time of planned exposure to metformin will be 7 days and the maximum planned exposure will be 35 days (average time at TJUH from cancer diagnosis to definitive surgical treatment is 3 weeks). We will allow a window of a 1-5 weeks in the event that there are delays in the surgical scheduling but no patients will receive metformin for more than 35 days.

The usual dose of oral doxycycline is 200 mg on the first day of treatment (administered 100 mg every 12 hours) followed by a maintenance dose of 100 mg/day. The maintenance dose may be administered as a single dose or as 50 mg every 12 hours. In the management of more severe infections (particularly chronic infections of the urinary tract), 100 mg every 12 hours is recommended. The study will be administering 200 mg of doxycycline, given as 100 mg every 12 hours until the day prior to surgery.

Rationale for endpoints

Primary: We have chosen Caveolin-1 expression in tumor stroma as our primary endpoint based on studies revealing that CAV1 stromal expression modulates tumor growth in animal models, is a prognostic biomarker and preliminary analyses of a pilot study conducted in head and neck squamous cell carcinoma. Immunohistochemistry for CAV1 in the tumor-associated stroma will be compared in the pre-treatment specimen and the post-treatment specimens. All stained specimens will be independently viewed and scored by two pathologists who are blinded as to the nature of the samples and specifically as to whether the tissue is pre or post-metformin/doxycycline treatment as previously described by the investigators (68). The stained sections will be scored taking into consideration the intensity of staining and the percentage of stained cells within each tissue core.

Secondary: We have chosen MCT1, MCT4, and TOMM20 as secondary end-points of the current clinical trial due to their biologic and prognostic significance seen in our work in head and neck squamous cell carcinoma. MCT4 expression in fibroblasts is associated with advanced stage disease. MCT1 and TOMM20 expression in carcinoma cells is associated with increased functional mitochondrial mass. Prior work in our laboratory has suggested a correlation between MCT1 expression in breast cancer samples and the Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2 status of the tumors. We will seek similar correlations here.

Correlative: Mass spectroscopy imaging (MSI) will be performed on paired samples from the three subjects, comparing metabolite profiles including ATP, hexose bisphosphates, lactate and pyruvate in the pre-treatment and post-treatment tumor sample. MSI will allow spatial characterization of the metabolic profile of carcinoma and stromal cells within the tumor. MSI will also allow us to determine if distance between carcinoma and stromal cells is associated with different metabolite profiles. MSI testing will be performed at Vanderbilt University as has been performed in the ongoing metformin HNSCC. This testing is performed outside of the KCC because we lack the facilities and expertise for MSI. We will also send tumor samples from our tumor bank, from patients who have not taken metformin or doxycycline, to use as controls. We will also further characterize the baseline metabolic state of the patient by performing, when possible, a nutritional assessment via a nutritionist-mediated 3 day dietary recall and comparing a patient's estimated dietary intake against their estimated caloric needs calculated with the Harris-Benedict equation. This will determine if patients are already calorically restricted, a phenotype similar to that of mitochondrial blockade. Similarly we will do a serologic assessment of their metabolic state will also be performed at the time of enrollment and at the time of resection using the serum markers oncomiR-21, triglycerides, IGF-1, IGF-BP3, ESR, IGFR-1, exosomes, metabolomics, and microRNA expression profiles. These serologic assays have been shown to correlate both with nutritional state and in the case of miR21 with tumor burden.

1.3 **Correlative Studies**

Mass spectroscopy imaging (MSI) will be performed on paired samples, comparing relative proportion of metabolite profiles (specifically ATP, hexose bisphosphates, lactate and pyruvate) in the pre-treatment and post-treatment tumor sample. MSI allows assessment of metabolite levels in spatially defined tumor regions (87) and hence will allow the evaluation of metabolite levels in carcinoma and stromal cell compartments.

To begin to estimate the patient's baseline metabolic state, we will also try to perform a nutritional assessment via a nutritionist-mediated 3 day dietary recall and comparing a patient's estimated dietary intake against their estimated caloric needs calculated with the Harris-Benedict equation. Patients will be characterized as not meeting, meeting, or exceeding their caloric needs.

A serologic metabolic profile will be built using oncomiR-21, triglycerides, IGF-1, IGF-BP3, ESR, IGFR-1, exosomes, and metabolomics and seeking to correlate this with the patient's CAV1 expression levels and baseline and after intervention.

The immunohistochemistry markers described above in the primary and secondary objectives, as well as the MSI and serologic profiling will all also be correlated with the %of cancers cells that stain or estrogen receptor and progesterone receptor for each of the cancer types. Furthermore breast cancer samples will also be assessed for their expression of HER2 via IHC and FISH.

1.4 Potential Risks and Benefits

1.4.1 Potential Benefits

Metformin

The possible societal benefits are large since this will allow us to learn about the pharmacodynamic effects of metformin and doxycycline in breast, uterine, and cervical cancers. Information about the expression levels of CAV1, MCT4, MCT1, TOMM20, and the metabolomics described above will also more broadly allow us to gain new information on tumor metabolism. The study is designed to study as a primary end-point metformin and doxycycline's effects on expression of CAV1 but not to study if this combination of agents improves outcomes as a primary end-point and hence it is unlikely to have significant clinical benefits for subjects with breast, uterine or cervical cancer.

Doxycycline

Doxycycline is relatively attractive as a new anti-cancer agent, as it has a long half-life systemically and has been used successfully for the long-term treatment of patients with urinary tract infections (UTI), prostatitis or acne, for extended periods of time, of up to 4-to-6 months or more (200 mg per day). Doxycycline also encourages the growth of normal stem cells, has anti-inflammatory properties, and even increases lifespan, in certain experimental contexts. Thus, the toxic side effects of anti-cancer therapy would be minimized.

1.4.2 Potential Risks

Metformin

Metformin's most serious toxicity is lactic acidosis, occurring in three of 100,000 patient-years of use. Risk is significantly reduced when metformin use is avoided in those patients with hepatic, cardiac, or renal compromise. However, metformin's risk of lactic acidosis may be overstated since the recent evaluation of metformin associated lactic acidosis cases from 347 trials showed that the risk of lactic acidosis with metformin was not significantly increased compared with

other antiglycemic agents (88). Minor gastrointestinal upset is the most common toxicity, leading to cessation of therapy in less than 5% of individuals. Metformin does not induce hypoglycemia.

Doxycycline
Due to oral doxycycline's virtually complete absorption, side effects to the lower bowel, particularly diarrhea, have been infrequent. The following adverse reactions have been observed in patients receiving tetracyclines:

Gastrointestinal: Anorexia, nausea, vomiting, diarrhea, glossitis, dysphagia, enterocolitis, and inflammatory lesions (with monilial overgrowth) in the anogenital region. Hepatotoxicity has been reported rarely. These reactions have been caused by both the oral and parenteral administration of tetracyclines. Rare instances of esophagitis and esophageal ulcerations have been reported in patients receiving capsule and tablet forms of drugs in the tetracycline class.
Skin: Maculopapular and erythematous rashes. Exfoliative dermatitis has been reported but is uncommon.

Hypersensitivity reactions: Urticaria, angioneurotic edema, anaphylaxis, anaphylactoid purpura, serum sickness, pericarditis, and exacerbation of systemic lupus erythematosus.
Thyroid Gland Changes: When given over prolonged periods, tetracyclines have been reported to produce brown- black microscopic discoloration of thyroid glands. No abnormalities of thyroid function are known to occur.

Combination of these Agents:
No drug-drug interactions are known to occur between these two agents. Metformin is not metabolized and is excreted unchanged in the urine with a half-life of approximately 5 hours. Similarly, doxycycline is not significantly metabolized and no metabolites have been found in man. Some studies have suggested a potential hepatic metabolism pathway. However, no excess toxicity is expected from the combination of these two agents and thus no additive potential harm beyond those associated with the individual agents as described above.

2 Study Objectives

2.1 Objectives

2.1.1 Primary

- To determine if treatment with a combination of metformin and doxycycline can increase the percentage of cells that express Caveolin-1 in the cancer associated fibroblasts of patients with breast, uterine, or cervical cancers.

2.1.2 Secondary

- To determine the effect of metformin and doxycycline treatment on the percentage of cells that express MCT4 in cancer associated fibroblasts and MCT1 and TOMM20 in the cancer cells of breast, uterine and cervical cancer patients.
- To assess safety and tolerability of metformin and doxycycline treatment in subjects with breast, uterine and cervical cancer.
- To determine the relationship of the percentage of stromal cells expressing CAV1 or MCT4 and tumor cells that express MCT1 and TOMM20 at baseline and after treatment

with metformin and doxycycline with the percentage of cells expressing ER and PR for breast, uterine and cervical samples and HER2 in breast cancer samples.

2.1.3 Correlative

- To assess the effect of combined metformin and doxycycline therapy on the metabolic profile of cancer cells and stroma using mass spectroscopy imaging (MSI) on paired samples, comparing metabolite profiles in the pre-metformin and post-metformin tumor sample.
- To assess, when possible, the impact of a patient's nutritional status, estimated using 3-day dietary recall versus caloric needs as calculated by the Harris-Benedict equation on the baseline and net change in CAV1.
- To assess the effect of combined metformin and doxycycline therapy on oncomiR miR-21 after intervention.
- To assess the effect of combined metformin and doxycycline therapy on adipokines and the IGF-1/insulin signaling pathways through assessment of serum triglycerides, IGF-1, IGF-BP3, erythrocyte sedimentation rate (ESR), adiponectin, leptin, IGF-1R, exosome evaluation, metabolomics profile, and microRNA expression profile.

2.2 Endpoints/Outcome Measures

2.2.1 Primary

- Assess the change in the percent of stromal cells expressing CAV1 at an intensity of 1+ or greater after treatment with metformin and doxycycline as determined by two blinded pathologists.

2.2.2 Secondary

- Assess safety and tolerability of metformin and doxycycline treatment in subjects with cancer. Toxicity will be evaluated using the most recent version (version 4.03) of the NCI toxicity criteria, i.e. the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.
- Assess the change in the percent of stromal cells expressing express MCT4 and the percent of tumor cells that express MCT1 and TOMM20 in the cancer cells of breast, uterine and cervical cancer patients using Aperio analyses of expression intensity with previously validated algorithms.
- Assess the relationship of the percentage of stromal cells expressing CAV1 or MCT4 and tumor cells that express MCT1 and TOMM20 at baseline and after treatment with metformin and doxycycline with the percentage of cells expressing ER and PR for breast, uterine and cervical samples and HER2 in breast cancer samples.

3 Study Design

This is a 2 parallel cohort Phase II study. Metformin and doxycycline are the therapeutic agents. For metformin, the initial starting dose will be 500 mg orally daily for 3 days which will then be increased to 500 mg orally twice daily for 3 days, and if tolerated, further increased to 1000 mg twice daily after day 6. Subjects will maintain the maximum tolerated dose (up to 1000 mg twice daily) until the day prior to their scheduled definitive surgery. All medications should cease at least 12 hours prior to time of surgery.

For doxycycline the patients will take 100 mg by mouth every twelve hours until the day prior to their surgery. The last dose should be taken no closer than 12 hours prior to time of surgery.

Table 1. Study Design

	Cohort	Treatment	Week	-1	1							2							3	4	5
			Day	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8***	**9**	**10**	**11**	**12**	**13**	**14**			
Eligibility Screening	**1** N=23	Doxycycline[e]			X	X	X	X	X	X	X	X	X	X	X	X	X	X			
		Metformin			X[a]	X[a]	X[a]	X[b]	X[b]	X[b]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	Surgical Resection		
	2 N= 23	Doxycycline[e]			X	X	X	X	X	X	X	X	X	X	X	X	X	X			
		Metformin			X[a]	X[a]	X[a]	X[b]	X[b]	X[b]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]			
		Tissue[d]		X															X		

*** If surgery is scheduled past Day 8, patients will stay on metformin and doxycycline until the day prior to surgery.**

a. Metformin will be given initially with a starting dose of 500mg once a day for Days 1-3.
b. Metformin will be increased to 500 mg orally twice a day for Days 4-6
c. If tolerated, beginning Day 7 metformin will be increased to 1000 mg twice a day. Subjects will maintain the maximum tolerated dose (up to 1000 mg twice daily) until the day prior to their scheduled definitive surgery.
d. When possible, baseline biopsy tissue is to be obtained prior to starting study medications. Two serum separator tubes of serum will be taken at the same time as the biopsy specimen (if possible), otherwise done at time of consent. If a pre-treatment sample has already been acquired and flash froze and if there is excess tissue available at resection, the second specimen will also have a piece flash frozen for metabolomic testing.
e. Doxycycline will be taken once every 12 hours, with the last dose being taken at least 12 hours prior to scheduled time of definitive surgery.

Eligibility Screening: Trial Coordinator will screen the office charts for possible inclusion in the trial. Labs for the screening part will be available from pre-admission testing that is standard of care for initial biopsy. Participants of child bearing potential will have a pregnancy test within 14 days prior to enrollment.

Visit 1, Day 0: Subjects that meet the inclusion criteria after reviewing diagnosis, laboratory data and clinical chart will be approached the day of their consult or follow up with a subspecialty surgeon. The study coordinator will meet with the prospective candidates and go over the protocol, answer questions and obtain informed consent. If the subject is agreeable to the study, two 8.5 mL vials of blood will be drawn for baseline metabolomics and exosome profiling. After eligibility is confirmed, metformin and doxycycline will be dispensed. Instructions on how to take study drug will be given and times for follow up phone calls for tolerability and safety will be arranged with participants. When possible, patients will be given the option to meet with a nutritionist either in person or by phone within the first 7 days of their enrollment to fill out a 3 day dietary recall.

Visit 2,Day of Surgery: Patients will be approached for bottles and pill reconciliation as well as follow up of side effects and adverse events the day of final surgery. Two 8.5 mL vials of blood will be drawn for post-treatment metabolomic and exosome profiling. A specimen of the removed tumor will be taken for genomic analysis.

Follow-up: A follow up phone call will be performed 30 days (±5 days) after the last dose of metformin and doxycycline for any other side effects or adverse events. The participant's medical records will be reviewed every 3 months (±1 month) for 12 months to assess: overall survival, frequency of metastasis, and staging.

4 Study Enrollment and Withdrawal

Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion and exclusion criteria. Results of all baseline evaluations will be reviewed by the Principal Investigator or treating physician prior to enrollment, to verify that all inclusion and exclusion criteria have been satisfied. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to screening procedures being performed. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

4.1 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Diagnosis of localized breast, uterine or cervical cancer that is either biopsy proven or suspected based on history, physical, and/or radiographic findings, and who are planned for definitive resection of the tumor without the use of neoadjuvant chemotherapy or radiation therapy at TJUH are eligible to participate.
2. Subjects must be ≥ 18 years of age at time of consent.
3. Subjects must be newly diagnosed or suspected to have breast, uterine (endometrial cancer with histologies including endometrioid, serous, clear cell, and carcinosarcoma) or cervical cancer.
4. Patient must be able to swallow pills.
5. Patients with serum creatinine levels less than 1.5 mg/dL.
6. Women of child bearing potential must have a negative urine or blood pregnancy test within 14 days of study enrollment.
7. Informed Consent: All subjects must be able to comprehend and sign a written informed consent document.
8. ECOG Performance status ≤1

4.2 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. Received any prior cancer therapy for the breast, uterine, or cervical cancer that is being resected, including progesterone therapy for endometrial cancer patients.
    a. Patients may have had prior therapy for other contra-lateral breast cancer.
2. Subjects who are pregnant or breastfeeding or may become pregnant during metformin and doxycycline administration.
3. Subjects on metformin or doxycycline for any reason during the preceding 4 weeks.
4. Diabetic subjects that are managed by taking metformin or insulin.
5. Subjects who have received iodinated contrast dye must wait 12 hours prior to starting Metformin. If a CT scan with contrast is scheduled after screening and consent, the

metformin cannot be taken until after the CT with contrast has been completed and they have waited 12 hours.

6. Patients with serum creatinine level greater than 1.5 mg/dL.
7. Patients with history of lactic or any other metabolic acidosis.
8. Patients with history of congestive heart failure stage III or greater.
9. Patients scheduled for definitive cancer surgical resection less than 7 days from beginning of study drug administration or greater than 5 weeks from beginning study drug administration.
10. Patients with history of hepatic dysfunction or hepatic disease and abnormal liver function tests defined as AST, ALT, Alk Phos, and or total bilirubin greater than 2.5 times the upper limit of normal.
    a. Patients who have a history of hepatic dysfunction or hepatic disease and normal liver function tests will be eligible to participate.
11. Patients with a current history (in the past 30 days) of heavy drinking which is defined in accordance with CDC definition as more than 8 drinks per week for women and more than 15 drinks per week for men. A standard drink contains .6 ounces of pure alcohol. Generally, this amount of pure alcohol is found in 12-ounces of beer, 8-ounces of malt liquor, 5-ounces of wine, 1.5-ounces or a “shot” of 80-proof distilled spirits or liquor (e.g., gin, rum, vodka, or whiskey). While on study, patients should limit their alcohol consumption to no more than 8 drinks per week for women and no more than 15 drinks per week for men. Patients who feel they cannot comply with this recommendation are not eligible. Prior allergic reaction to metformin, doxycycline, or any other tetracycline antibiotic in the past.
12. Patient is on medications that are contraindicated with metformin or doxycycline under current FDA recommendations. The following is a list of medications identified as class D (consider therapy modification) when treatment with metformin or doxycycline is considered:
    - Class D:
        - Bismuth Subsalicylate
        - Cimetidine
        - Iodinated contrast agents
        - Somatropin

4.3 Gender/Minority/Pediatric Inclusion for Research

We will not exclude potential subjects from participating in this study based on ethnic origin or socioeconomic status. Every attempt will be made to enter all eligible patients in this protocol and therefore address the study objectives in a patient population representative of the entire breast, uterine and cervical cancer population treated at Thomas Jefferson University Hospital. By the nature of the diseases covered in this trial the majority enrolled will be female. We will not exclude male patients with breast cancer.

4.4 Recruitment and Screening

46 subjects will be recruited. No advertisement will be conducted. Screening requirements include serum measurement of creatinine, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase taken on a non-fasting blood sample. Potential research subjects will be identified by a member of the patient’s treatment team, the protocol investigator, or research team at participating centers from Medical Oncology, Radiation Oncology and Surgical offices. Investigators will screen the patient’s medical records for suitable research study subjects and discuss the study and their potential for enrolling in the

research study. Patients will be screened based on pathology, image studies etc. A maximum of 23 patients will be enrolled onto each arm of the trial (see Statistical Analysis Plan Section 9).

4.5 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.
Subjects may be discontinued from the trial for any of the following reasons:

- The subject withdraws consent.
- Documented Disease progression
- Unacceptable adverse experiences as described in Section 8
- Investigator's decision to withdraw the subject
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 Trial Flow Chart. After the end of treatment, each subject will be followed for 30 days for adverse event and 90 days for serious adverse events monitoring as (described in Section 8.4).

4.5.1 Subject Replacement Strategy

Patients who are discontinued from the study for any reason outlined in Section 4.5 will not be considered as evaluable for this study and will be replaced.

5 Study Treatments

5.1 Study Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

5.2 Study Product Description

Metformin:
Metformin is a biguanide drug currently approved for the treatment of type 2 diabetes mellitus by the FDA. It is currently being investigated in multiple cancer treatment trials.

Metformin is the therapeutic agent in the protocol. The initial starting dose will be 500mg orally daily for 3 days which then will be increased to 500 mg twice daily and, if tolerated, further increased to 1000mg twice daily after day 6. Patients will maintain 1000 mg twice a day dosing until the day prior to their scheduled definitive surgery. This dose schedule has been shown to be well tolerated and was able to promote cellular changes consistent with beneficial anti-cancer effects in breast cancer patients (32).

The treatments to be used in this trial are outlined below in Table 2. Trial treatment should begin within 5 days of eligibility confirmation.

Table 2 Trial Treatments

Drug	Dose Level	Dose	Route of Administration	Regimen
Metformin	-1 1 2	500 mg QDay 500 mg BID 1000 mg BID	Orally	Day 1-3 Day 4-6 Day7- Day Before Surgery
Doxycycline	-1	100 mg Q12 hours	Orally	Day 1- Day Before Surgery

Doxycycline:
Doxycycline is an antibiotic provided in the formulation doxycycline hyclate. Doxycycline is virtually completely absorbed after oral administration. Following administration of a single 200 mg dose to adult volunteers, average peak serum doxycycline levels were 2.6 mcg/mL at 2 hours, decreasing to 1.45 mcg/mL at 24 hours. The mean Cmax and AUC 0-∞ of doxycycline are 24% and 13% lower, respectively, following single dose administration of DORYX tablets, 100 mg with a high fat meal (including milk) compared to fasted conditions. The mean Cmax of doxycycline is 19% lower and the AUC 0-∞ is unchanged following single dose administration of DORYX Tablets, 150 mg with a high fat meal (including milk) compared to fasted conditions. The clinical significance of these decreases is unknown.

Tetracyclines are concentrated in bile by the liver and excreted in the urine and feces at high concentrations and in a biologically active form. Excretion of doxycycline by the kidney is about 40%/72 hours in individuals with a creatinine clearance of about 75 mL/min. This percentage may fall as low as 1-5%/72 hours in individuals with a creatinine clearance below 10 mL/min.

Studies have shown no significant difference in the serum half-life of doxycycline (range 18-22 hours) in individuals with normal and severely impaired renal function. Hemodialysis does not alter the serum half-life.

Doxycycline is an FDA approved product that is indicated for the prophylaxis against and treatment of both bacterial and protozoal infections. For adults the dose is 200 mg on the first day of treatment administered as 100 mg every 12 hours followed by a maintenance dose of 100 mg daily. The maintenance dose may be administered as a single 100 mg dose of 50 mg dose every 12 hours. Serious infections are treated with a sustained dose of 200 mg per day in divided doses. Treatment for uncomplicated bacterial infections typically lasts for 7 days total. Prophylaxis for malaria can be continued daily for the period of time the patient is in a malarious area and continued for 4 weeks thereafter, up to 4 months.

5.2.1 Dose Calculation

Rules for dose modification: Toxicity monitoring, dose modification and treatment of complications:
Particular attention will be paid in the first three days of treatment. Patients will take 500mg/day for 3 days. If tolerated, starting day 4, patients will increase to 500mg twice daily. If this dose increase is tolerated, then on day 7 the patient will be dose escalated to 1000mg twice daily. The maximum tolerated dose will be taken until the day before surgery with food. A phone call on or around the day of each dose escalation (± 2 days) will be made in order to evaluate the tolerability of the drug and also weekly thereafter. Patients will be instructed to contact the clinical investigators should any toxicity occur during the study (66).

Metformin will be held for 12 hours if CAT scan intravenous contrast is administered to decrease risk of lactic acidosis. This approach is more stringent than the most recent recommendation of the American College of Radiology which does not recommend holding metformin in the absence of comorbidities (renal insufficiency, liver dysfunction, alcohol abuse, cardiac failure, myocardial or peripheral muscle ischemia, sepsis or severe infection) which are exclusion criteria for this clinical trial (29).

Toxicity will be evaluated using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

- Grade 1 toxicity: Patient will be maintained on full dose.
- Grade 2 toxicity (probably or definitely drug related): Dose will be reduced back to the maximum tolerated dose until grade 1 or lower. If symptoms are not resolved within 3 days the treatment will be discontinued definitively.
- Grade 3 toxicity (probably or definitely drug related): Treatment will be interrupted and toxicity reassessed daily. If toxicity improves to grade 2, dose will be reduced back to the maximum tolerated dose.
- Grade 4 toxicity (probably or definitely drug related): Treatment will be discontinued definitively.

In case of grade 1 or 2 diarrhea (the most frequent side effect) a concomitant administration of loperamide may be provided, and dose may be reduced back to the maximum tolerated dose or temporarily halted at the treating physician's discretion. Clear documentation of this will be recorded in the patient's research chart.

If patient experiences continued intolerable side effects, dose management may be discussed with a study investigator and dose modification can be recommended as needed. Clear documentation of this will be recorded in the patient's research chart.

If doses of either medication are missed due to toxicity they should not be replaced. If a dose is not taken due to an error, it may be taken up to 6 hours later. If vomiting occurs within 30 minutes of intake, that dose may be repeated. These events should be recorded by the patient on their pill diary.

5.2.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

5.2.3 Clinical Supplies Disclosure

The subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

5.2.4 Receiving, Compliance, Storage and Return

Receipt of Drug Supplies

Metformin and doxycycline tablets will be provided by the Department of Medical Oncology and will be coordinated through the Thomas Jefferson University Hospital Investigational Drug Service (IDS) Pharmacy. Drug will be stored in locked cabinet until given to the participants. A study investigator will prescribe the approximate amount of medication needed for the treatment period, and the metformin and doxycycline will be self-administered by the participants based on dose escalation timeline and study treatment follow-up phone calls.

Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.
At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

Subject Compliance Monitoring

There will be two pill bottles with the appropriate number of metformin and doxycycline tablets distributed to the patients upon enrollment into the trial. The pill bottles will be accompanied with a pill diary for each medication and detailed instructions on the proper dosage/number of tablets to take daily as noted above. Upon arrival for definitive surgical resection, the diaries and bottles will be collected by our trial coordinator and the contents will be evaluated for compliance.

Storage Requirements

Drug will be stored in a locked cabinet until given to the participants at room temperature in the coordinated through the Thomas Jefferson University Hospital Investigational Drug Service (IDS) Pharmacy. Access to the locked cabinet will only be granted to the study coordinator. The investigator must ensure that it is stored in accordance with the environmental conditions as defined in the Investigator Brochure. Clinical supplies may not be used for any purpose other than that stated in the protocol.

Returns

The investigator is responsible for keeping accurate records of the clinical supplies received from the Sponsor or designee, the amount dispensed to the subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

5.2.5 Dose Limiting Toxicity Criteria

Dose-limiting toxicities (DLTs) will be graded in severity according to the guidelines outlined in the NCI- CTCAE version 4.03. Dose-limiting hematologic and non-hematologic toxicities will be defined differently, and will be based on events that occur during study drug administration and for 30 days after the completion of the therapy. In order to be declared a dose-limiting toxicity, an adverse experience must be related (definitely, probably, or possibly) to study therapy.

5.3 Surgical Resection

Surgical Resection will occur immediately following the last scheduled dose of metformin and doxycycline. Surgical technique to be used will be at the surgeon's discretion. Every effort should be made to have tumor tissue flash frozen as quickly as possible after resection. AEs

and concomitant medications will be evaluated and recorded prior to surgery. Adverse events and concomitant medications considered by the Investigator to be related to surgical resection will be recorded in appropriate source documentation; however, such AEs and concomitant medications will not be recorded on CRFs.

5.4 Concomitant Medications/Treatments

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If the patient is on any of the Class C medications as outlined in Section 5.4.2, they should be monitored closely for any potential interactions or side effects. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Sponsor. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the Investigator, the Sponsor, and the subject.

5.4.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication and therapies will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date should also be included on the CRF. These will be cross referenced against the list of drugs in section 4.2 (Exclusion Criteria) to ensure that no agents that are contraindicated with metformin or doxycycline are used.

All concomitant medications received within 28 days before informed consent is signed and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered to treat SAEs and ECIs should be recorded as defined in Section 8.2

5.4.2 Prior and Concomitant Medication

All medications are permitted except those that are contraindicated with metformin and doxycycline under current FDA recommendations. It is important to note that the medications that are contraindicated with metformin are contraindicated due to concern for theoretical interactions. Class C medications are allowed if they are established at baseline. The treating physician should be made aware of any new Class C medications taken during therapy for proper monitoring of potential interactions. The following is a list of medications identified as class C (monitor therapy) and class D (consider therapy modification) when treatment with metformin or doxycycline is considered:

<u>Class C:</u>
Carbonic anhydrase inhibitors
Cephalexin
Corticosteroids (orally inhaled)
Corticosteroids (systemic)
Dalfampridine
Dofetilide
Glycopyrrolate
Lamotrigine
Luteinizing hormone-releasing hormone analogs

<u>Class D:</u>
Bismuth Subsalicylate
Cimetidine
Iodinated contrast agents
Somatropin

Pegvisomant
Penicillin
Trospium

5.5 Dietary Restrictions

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting. Patients will be asked to fill a dietary log at the beginning the study when they speak with a registered dietician.

6 Study Procedure

All patients will be evaluated by a treating surgeon. Initial visit will include overall assessment of health as well as determination of eligibility. A study calendar details the requisite pretreatment evaluations.

Study Procedure	Screening[A]	Treatment/Intervention Period[D]							End of Treatment Visit[E]	Long term Follow-up[G]
		Week 1				Week 2-5[D]		Surgical Resection[F]		
		Day 1 -7				Day 8+				
Administrative Procedures										
Inclusion/Exclusion Criteria	X									
Pregnancy Test	X[H]									
Demographics, Medical history	X									
Clinical Assessment										
Physical examination[I]	X[J]								X	
ECOG Performance status	X								X	
Pathology Report histologic suspicion or confirmation of disease[L]	X							X		
Concomitant meds[K]	X		X[¥]	X[¥]			X[¥]	X	X	X
Toxicity and AE Assessment[K]	X		X[¥]	X[¥]			X[¥]	X	X	X

Laboratory tests[B]										
Hematology and Blood Chemistry	X									
Treatment/ Intervention										
Metformin[c]		X	X	X	X	X	X			
Doxycycline[c]		X	X	X	X	X	X			
Diagnostic Biopsy or tumor sample[O]	X									
Surgical Resection								X^{N}		
Correlative Studies										
Serologic metabolic profile (miR21 and exosomes)		X						X		
Nutrition Assessment*		X^{M}								

* Nutrition assessment will occur within seven days of enrollment when possible, and may be done via a phone interview.
¥ Con Meds and AE/Toxicity Assessments should be performed only during day 3, day 5, and (if applicable) subsequent weekly follow-up phone calls.

A. Written informed consent must be obtained before any study-specific screening assessments are performed. Lab results performed as standard-of-care prior to obtaining informed consent and within 28 days prior to enrollment may be used; such tests do not need to be repeated for screening. Trial treatment should begin within 5 days of eligibility confirmation.
B. Blood Chemistry includes: Creatinine, AST, ALT, ALK Phos,T Bili > 2.5 x ULN. Hematology includes: CBC (with differential). Non-fasting labs. If labs were completed within 28 days prior to enrollment, these values can be used for eligibility/screening and labs need not be repeated.
C. Study treatment follow-up phone calls will occur on days 3 ±2 days and 5 (± 2 days) of dose escalation to evaluate tolerability, document AEs, and concomitant medications. If patient remains on study drugs for >7 days, study treatment follow-up phone calls will occur a minimum of once a week until surgery to evaluate tolerability, AEs, and con meds. Last doses of Metformin and Doxycycline pills should not be taken up to 12 hours before scheduled surgery time.
D. Patient must receive a minimum of 7 days of metformin and doxycycline before having definitive cancer surgery. Patients will stay on metformin and doxycycline until the day prior to surgery (up to the maximum of 5 weeks).
E. End of treatment visit may occur within 21 days ±7 days of stopping study drug.
F. Correlative study blood to be collected the morning of surgical resection.

G. Follow up phone call will be performed 30 days (± 5 days) after last dose of study medication for any other side effects or toxicity. Subject medical records will be reviewed every 3 months (± 1 month) for 12 months.
H. Urine or blood pregnancy test must be done within 14 days prior to enrollment on study for all women of childbearing potential.
I. Physical examinations should include vital signs, weight and blood pressure
J. Screening physical examination should include height.
K. Concomitant medication reconciliation, and Toxicity and Adverse Event assessments should be done at every patient contact to fully capture any potential dose limiting toxicities or AEs that affect the patient's ability, willingness, and eligibility to continue taking study medications.
L. Operative reports should be included with pathology reports in source documents when applicable.
M. This optional assessment should be performed by a registered dietician. When applicable, the RD will meet with the patient within seven days of enrollment and record the patient's last 3 days of caloric intake and estimate their caloric intake versus their caloric needs per the Harris Benedict Equation.
N. Tumor tissue should be flash frozen as quickly as possible after resection.
O. Baseline tissue specimen will be obtained for comparative analysis when available.

6.1 Screening Assessment

Before initiating any screening activities, the scope of the study will be explained to each patient during informed consent. Patients should be advised of any known risks inherent in the planned procedures, any alternative treatment options, their right to withdraw from the study at any time for any reason, and their right to privacy. After this explanation, patients should be asked to sign and date a Notice of Privacy Practice research authorization/HIPAA form and an IRB-approved statement of informed consent that meets the requirements of the Code of Federal Regulations (Federal Register Vol. 46, No. 17, January 27, 1981, part 50). During the screening period, subject eligibility will be determined according to the inclusion and exclusion criteria (Section 4.1,4.2). The following assessments will be performed during this time:

- Obtain informed consent and research authorization
- Inclusion/Exclusion Criteria
- Record demographics (including age) and medical history

- Physical exam
- Urine or Blood Pregnancy test (within 14 days for women of child bearing potential)
- Discuss and record concomitant medications
- ECOG performance status, vital signs [body temperature, blood pressure, pulse, respiratory rate, weight, height]
- Obtain histologic or (if appropriate) radiologic confirmation of disease
- Confirm eligibility according to the inclusion/exclusion criteria
- Laboratory tests (within 28 days of enrollment):
    - Hematology (CBC with diff)
    - Blood Chemistry
        - Creatinine, AST, AT, ALK Phos T Bili > 2.5 x ULN (all are SOC)
- If possible, baseline caloric intake assessment using a dietary log mediated by a nutritionist. This will take place at the patient's baseline assessment if possible and may be done via a phone interview.
    - If caloric intake is completed, a calculation of the patient's estimated caloric needs via the Harris-Benedict Equation will be performed.

6.2 Treatment Period (Week 1 Day 1 – day prior to Surgical Resection)

After screening is completed and eligibility is confirmed, Metformin and Doxycycline will be ordered by a study investigator and the medication will be dispensed by the Investigation Drug Service pharmacy. The date subjects begin taking metformin and doxycycline will be considered Week 1 Day 1, and a return to clinic is not necessary. Surgical resection will be scheduled a minimum of 7 days after the start of metformin and doxycycline and maximum of 5 weeks. The participant will receive a Patient Medication Diary to record the time (AM and PM) and number of pills to take for both Metformin and Doxycycline.

See Study Calendar (Section 6 above) for when the following assessments will be performed during the treatment period:

- Study Treatment:
    - Metformin
    - Doxycycline
- Study treatment follow-up phone calls (Day 3 $\pm$2 days and Day 5 $\pm$2 days from start of medication, and weekly thereafter until surgical resection)
- Concomitant medications

- Adverse event assessment

6.3 Surgical Resection

The following study activities will occur on the day of surgical resection:

- Two SST red-top tubes of blood for correlative studies: oncomiR miR-21, serum triglycerides, IGF-1, IGF-BP3, ESR, adiponectin, leptin, IGF-1R, exosome evaluation, metabolomics profile, and microRNA expression profile.
- Definitive cancer surgery
    - Pathology report needed
    - Tumor tissue
        - Exosome evaluation, metabolomics profile, and microRNA expression profile.
- Return all Metformin and Doxycycline medication bottles (including any unused tablets/pills)
- Collection of pill diaries

6.4 End of Treatment Study Visit (Within 21 Days ±7 of stopping protocol study medications)

The following study activities will occur at End of Treatment Visit after patients have completed treatment with metformin and doxycycline:

- Physical exam
- Vital signs
- ECOG performance status
- Concomitant medications
- Adverse event assessment

6.5 Long term Follow-up

A follow up phone call will be performed 30 days (±5 days) after the last dose of metformin and doxycycline for any other side effects or adverse events. This is allowed to coincide with the End of Treatment study visit if it occurs during timing overlap. Subjects will be followed for 12 months following the End of Treatment visit or until death. Subjects withdrawn from the study because of AEs will be followed until the adverse event has either resolved or stabilized. Reasons for premature withdrawal should be determined and noted.

The participant's medical records will be reviewed every 3 months (±1 month) for 12 months to assess overall survival and final pathologic staging.

7 Study Procedures and Evaluations

The Trial Flow Chart- Section 6 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator. Tumor biopsies will be formalin fixed and paraffin embedded. If a confirmatory diagnostic core biopsy is required and there is excess tissue available not needed in the course of clinical care, this tissue will be flash frozen in liquid nitrogen. When a pre-treatment sample has been acquired in this manner, and if there is excess tissue is available at the time of resection, these tissue samples will also be frozen in liquid nitrogen as well.

When possible, a registered dietician will meet with the patients within the first seven days of enrollment and through an interview record their last 3 days of caloric intake per their recollection and record this on a specific case report form. The dietician will also then use their height, weight, age, gender, and self-reported activity level per the Harris Benedict Equation to estimate their caloric intake versus their caloric needs.

Serologic Analysis of the metabolic profile of the patients will be performed at the time of enrollment and the time of resection. Two serum separator tubes of blood will be collected and transported at room temperature before being stored at -4 degrees C in a locked refrigerator.

7.1 Administrative Procedures

7.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial. Consent must be documented by the subject's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the subject before participation in the trial. The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature.

7.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial. Eligibility will be confirmed prior to the patient starting study medication.

7.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.4 Prior and Concomitant Review

Prior Medication

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

Concomitant Medications/Therapies

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.5 Disease Details and Treatments

Prior Cancer History

The investigator or qualified designee will obtain prior and current details regarding disease status.

Prior Cancer Treatment
The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

Subsequent Anti-Cancer Therapy Status
The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into follow-up.

7.2 Clinical Assessment

7.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart (Section 6) and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow- up period according to NCI CTCAE Version 4.03 (see Section 8.3). Toxicities will be characterized in terms of seriousness, causality, toxicity grading, and action taken with regard to trial treatment. Please refer to Section 8.2 for detailed information regarding the assessment and recording of AEs.

7.2.2 Physical Examination

The investigator or qualified designee will perform a physical examination during the screening period. Clinically significant abnormal findings should be recorded as medical history. A physical examination will be performed at the end of therapy study visit as specified in the Trial Flow Chart (Section 6.0).

7.2.3 Vital Signs

The investigator or qualified designee will take vital signs at screening, and at the end of therapy study visit as specified in the Trial Flow Chart (Section 6.0).Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.2.4 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status at screening and at the end of therapy study visit as specified in the Trial Flow Chart (Section 6.0).

7.3 Laboratory Safety Evaluations

Laboratory tests for hematology, chemistry, and others are specified in Table 3. The total amount of blood to be drawn over the course of the trial (from pre-trial to post-trial visits), including approximate blood by visit and by sample type per subject can be found in the Procedures Manual.

Table 3 Laboratory Tests

Hematology	Chemistry	Correlatives
Hematocrit	Creatinine	miR21
Hemoglobin	Alkaline phosphatase	IGF-1

Platelet count	Alanine aminotransferase (ALT)	IGF-BP3
WBC (total and differential)	Aspartate aminotransferase (AST)	ESR
Red Blood Cell Count	Total Bilirubin	Adiponectin
Absolute Neutrophil Count		Leptin
		IGF-1R
		Exosome profile
		Metabolomics profile

Screening laboratory tests should be performed within 28 days prior to enrollment. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to enrollment on this treatment trial.

7.3.1 **Correlative Studies**

Serum studies as described above will be drawn anytime during the screening period and/up to Week 1 Day 1 and at the time of surgical resection.

8 Safety Evaluations

8.1 Specification of Safety Parameters

8.1.1 Unanticipated Problems

Unanticipated problems (UAPs) include, in general, any incident, experience, or outcome that meets the following criteria:

- unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

UAPs are considered to pose risk to participants or others when they suggest that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.1.2 Adverse Events

An adverse event is any untoward or unfavorable medical occurrence in a human participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's participation in the research, whether or not considered related to the participant's participation in the research.

8.1.5 Serious Adverse Event (SAE)

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the participant at immediate risk of death from the event as it occurred)

- Is disabling or incapacitating
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant or may require intervention to prevent one of the outcomes listed in this definition.

8.2 Safety Assessment and Follow-Up

The PI will follow adverse events with start dates occurring any time after study drug administration until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator (or designee) will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3 Recording Adverse Events

The following subsections detail what information must be documented for each adverse event occurring during the time period specified in Section **Error! Reference source not found. Error! Reference source not found.**.

8.3.1 Relationship to Study Intervention

The relationship to study intervention or study participation must be assessed and documented for all adverse events. Evaluation of relatedness must consider etiologies such as natural history of the underlying disease, concurrent illness, concomitant therapy, study-related procedures, accidents, and other external factors.

The following guidelines are used to assess relationship of an event to study intervention:

1. Related (Possible, Probable, Definite)
    a. The event is known to occur with the study intervention.
    b. There is a temporal relationship between the intervention and event onset.
    c. The event abates when the intervention is discontinued.
    d. The event reappears upon a re-challenge with the intervention.
2. Not Related (Unlikely, Not Related)
    a. There is no temporal relationship between the intervention and event onset.
    b. An alternate etiology has been established.

8.3.2 Expectedness

The PI is responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention. Risk information to assess expectedness can be obtained from preclinical studies, the investigator's brochure, published medical literature, the protocol, or the informed consent document.

8.3.3 Severity of Event

Adverse events will be graded for severity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Any adverse event that changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets (Table 4).

Table 4 Evaluating Adverse Events

V4.03 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of metformin or doxycycline product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the metformin or doxycycline product to be discontinued?	
	Exposure	Is there evidence that the subject was exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the metformin or doxycycline? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

8.3.4 Intervention

Any intervention implemented to treat the adverse event must be documented for all adverse events.

8.4 Safety Reporting

8.4.1 Reporting to IRB

8.4.1.1 Unanticipated Problems

All incidents or events that meet criteria for unanticipated problems (UAPs) as defined in Section 8.1.1 Unanticipated Problems require the creation and completion of an unanticipated problem report form (OHR-20).

UAPs that pose risk to participants or others, and that are not AEs, will be submitted to the IRB on an OHR-20 form via the eazUP system within 5 working days of the investigator becoming aware of the event.

UAPs that do not pose risk to participants or others will be submitted to the IRB at the next continuing review.

8.4.1.2 Adverse Events

Grade 1 AEs will be reported to the IRB at continuing review.

Grade 2 AEs will be reported to the IRB at the time of continuing review.

8.4.1.3 Serious Adverse Events

SAEs will be reported to the IRB on OHR-10 forms via the electronic reporting system (eSAEy) according to the required time frames described below.

Grade 3-4 AEs that are unexpected and deemed to be at least possibly related to the study will be reported to the IRB within 2 working days of knowledge of the event.

Grade 3-4 AEs that are deemed unrelated to the study will be reported to the IRB within 5 working days.

Grade 5 AEs will be reported to the IRB within one working day of knowledge of the event.

All SAEs will be submitted to the IRB at continuing review, including those that were reported previously.

8.4.2 Reporting to SKCC DSMC

All AEs and SAEs, safety and toxicity data, and any corrective actions will be submitted to the DSMC per the frequency described in the SKCC DSMP. The report to the SKCC DSMC will also include any unanticipated problems that in the opinion of the PI should be reported to the DSMC.

For expedited reporting requirements, see table below: DSMC
AE/SAE Reporting Requirements

	Grade 1	Grade 2		Grade 3				Grades 4 and 5
				Unexpected		Expected		
	Unexpected and Expected	Unexpected	Expected	With Hospitalization	Without Hospitalization	With Hospitalization	Without Hospitalization	Unexpected and Expected
Unrelated Unlikely	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	5 Working Days	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	5 Working Days	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase I - 48 Hours (Death: 24 Hours) Phase II - 5 working days
Possible Probably Definite	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	48 Hours (Death: 24 Hours)	Phase I - 48 Hours Phase II - 5 working days	48 Hours (Death: 24 Hours)	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase I and Phase II - 48 Hours (Death: 24 Hours)

8.4.3 Reporting of Pregnancy

If the female partner of a male patient becomes pregnant during the course of the study, the treating physician must be notified immediately. All confirmed pregnancies must be immediately reported to the medical monitor. All pregnancies will be followed until resolution (i.e., voluntary or spontaneous termination or birth) and assessed for congenital anomalies and birth defects.

8.5 Halting Rules

This study will be monitored by the DSMC committee.

Acute toxicity will be monitored using CTCAE version 4.03 criteria during therapy and up to 30-days post completion of surgical resection. If the lowest dose of metformin and doxycycline develop toxicity ≥4, the study may be terminated after discussion with PI and sponsor.

9 Study Oversight

In addition to the PI's responsibility for oversight, study oversight will be under the direction of the SKCC's Data and Safety Monitoring Committee (DSMC). The SKCC DSMC operates in compliance with a Data and Safety Monitoring Plan (DSMP) that is approved by the NCI.

10 Statistical Analysis Plan

10.1 General considerations:

All analyses will be performed separately by cohort.

10.2 Analysis for primary outcomes.

The primary objective of the study is to assess the impact of metformin and doxycycline on CAV1 expression by immunohistochemistry (IHC) in carcinoma cells. All patients from whom samples are obtained both pre- and post-treatment will be included in the primary analyses. Within-patient change in IHC scores will be analyzed using the Wilcoxon signed-rank test.

10.3 Analysis for secondary outcomes.

Analysis of change TOMM20, MCT1, and MCT4 will be performed using the Wilcoxon signed-rank test. Analysis of secondary clinical outcomes will be primarily descriptive. Distribution of progress-free survival and overall survival will be estimated using the Kaplan-Meier method. The objective response rate will be estimated along with an exact 95% binomial confidence interval.

10.4 Sample size justification

The sample in each cohort is based on having 80% power to detect an average increase in CAV1 staining of 20% using a one-sided Wilcoxon signed-rank test with alpha=0.05. Based on pilot data in other cancers, we assume the standard deviation of change scores to be 32%. A sample size of 19 per cohort is required under these assumptions. Allowing for approximately 15% of patients to have missing pre- and or post-treatment data, we will recruit 23 women per cohort. Cohort 1 will consist of breast cancer patients and cohort 2 will include both uterine and cervical (gynecologic) cancers.

10.5 Interim analysis and early stopping.

There will be no interim analysis for efficacy or futility.

10.6 Stopping rules for safety

Incidence of DLTs will be monitored continuously with potential stopping after 10 patients have been enrolled based on the Bayesian method of Thall and Simon. We are comparing our experimental treatment to a standard of no treatment and assume no DLTs under standard of care. Both agents are extremely safe and DLTs should be exceedingly rare. In 900 patients treated with metformin alone, the discontinuation rate was 0.6%. In 31 patients treated with IV doxycycline, 4 SAEs were observed, but none were related to the study drug. Thus, it is reasonable to assume a prior distribution for the DLT rate in the combination of Beta(0.04,1.96).

That is, we assume a 2% event rate for the combination with information equivalent to data from 2 patients. We will stop the study for safety if the posterior probability that the rate of DLTs in the combination treatment is 5% or greater is greater than 90%. Stopping rules are outlined in the table below.

Number of patients enrolled	Stop if number of patients with Grade 3 or higher toxicities is ≥
10	2
11-22	3
23-35	4
36	5

Thall C.P., Simon R. Practical Bayesian guidelines for phase IIB clinical trials. Biometrics. 1994;50:337–349.

11 Data Handling and Record Keeping

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate case histories of study subjects, including accurate case report forms (CRFs), and source documentation.

11.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

11.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diary or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media x-rays, subject files and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

11.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

11.4 Study Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained**.**

12 Clinical Site Monitoring and Auditing

Clinical site monitoring and auditing is conducted to ensure that the rights of human participants are protected, that the study is implemented in accordance with the protocol and/or other operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring and auditing for this study will be performed in accordance with the SKCC's Data and Safety Monitoring Plan (DSMP) developed by the SKCC Data and Safety Monitoring Committee (DSMC). The DSMP specifies the frequency of monitoring, monitoring procedures, the level of clinical site monitoring activities (e.g., the percentage of participant data to be reviewed), and the distribution of monitoring reports. Some monitoring activities may be performed remotely, while others will take place at the study site(s). Appropriate staff will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the details described in the SKCC DSMP.

13 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.
This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator before commencement of this study.

All subjects for this study will be provided a consent form that is compliant with local and federal regulations, describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB- approved

consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

13.1 Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The investigator will be thoroughly familiar with the appropriate use of the drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

13.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB/IEC will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB/IEC approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients (including diary cards), safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC by the investigator.

13.3 Patient Information and Informed Consent

Before obtaining consent, members of the study team will review the rationale for the treatment program with the patient. The discussion will review the alternatives available (including hormonal therapy, chemotherapy, or supportive care as appropriate), the potential benefits of this program, the risks and the probability of their occurrence, and the procedures to minimize these risks. Should an AE occur, the provisions available to ensure medical intervention will also be reviewed. Why the risks are reasonable in relation to the anticipated benefits, incentives, or costs that will or may be incurred as a result of participating in the study, as well as the efforts to maintain confidentiality, will also be discussed with the patient.

Patients will be required to sign and date a statement of informed consent that meets the requirements of the Code of Federal Regulations (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the IRB. The medical record will include a statement that written informed consent was obtained (and document the date that it was obtained) before the patient is enrolled in the study. The original signed document will become part of the patient's medical record.

The consent form will include the following:

- the nature and objectives, potential toxicities, and benefits of the intended study
- the length of therapy and likely follow-up required
- alternatives to the proposed therapy (including available standard and investigational therapies)
- the name of the investigator(s) responsible for the protocol
- the right of the patient to accept or refuse treatment and to withdraw from participation in this study

- informed consent documents and sections in the research authorization/HIPAA forms should include who will have access to the subjects data and medical records

13.4 Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. After this discussion, they will be asked to sign a Notice of Privacy Practice research authorization/HIPAA form. The original signed documents will become part of the patient's medical records, and each patient will receive a copy of the signed documents. The use and disclosure of protected health information will be limited to the individuals described in the research authorization form. The research authorization form must be completed by the principal investigator and approved by the IRB.

13.5 Protocol Compliance

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB/IEC and the appropriate regulatory authority(ies). Changes to the protocol will require approval from TJUH at SKCC and written IRB/IEC approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB/IEC may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB/IEC. The investigator will submit all protocol modifications to TJUH at SKCC and the regulatory authority(ies) in accordance with the governing regulations.

Any departures from the protocol must be fully documented in the source documents. On- site Audits

Regulatory authorities, the IEC/IRB and/or TJUH at SKCC clinical quality assurance group may request access to all source documents, data capture records, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

13.6 Drug Accountability

Accountability for the drug at all study sites is the responsibility of the principal investigator. The investigator will ensure that the drug is used only in accordance with this protocol. Drug accountability records indicating the drug's delivery date to the site (if applicable), inventory at the site (if applicable), use by each patient, and disposal of the drug will be maintained by the clinical site. Accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), and patient numbers.

13.7 Terminating or Modifying the Study

Adverse event and laboratory data from this trial will be assessed by the lead site or the sponsor's medical monitor on an ongoing basis. SAEs will be reviewed as they are reported to the lead site/sponsor, and the medical monitor will make an assessment regarding the safety of continuing or modifying the study. This assessment will be shared with the investigators either in writing or as part of a teleconference. Should the assessment of either the lead site/sponsor or the principal investigator be that the study should be terminated, the study will be closed to further accrual. Patients who are receiving metformin and doxycycline will be assessed individually by the investigator to see if it is in the patients' best interest to continue, which might

be the case for a patient that is responding to the intervention. Follow-up safety assessments will be performed for all patients who are terminated from the study prematurely.

Written notification documenting the reason for study termination will be provided to the investigator or by the terminating party. Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients
- Failure to enter patients at an acceptable rate
- Insufficient adherence to protocol requirements
- Insufficient complete and/or evaluable data
- Plans to modify, suspend or discontinue the development of the drug

Should the study be closed prematurely, all study materials must be returned

13.8 Record Retention

The investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s).

14 Study Finances

14.1 Funding Source

This study is financed through a grant from the Prostate Cancer Foundation.

14.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All Jefferson University Investigators will follow the TJU Conflicts of Interest Policy for Employees (107.03).

14.3 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

15 Publication Plan

The PI holds the primary responsibility for publication of the any results of the study. Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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