THOMAS JEFFERSON UNIVERSITY
Sidney Kimmel Cancer Center

A Phase II Study of Metformin, Doxycycline, or a Combination of Both Agents in Head and Neck Squamous Cell Carcinoma

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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:

Title:

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
AKT	Also known as Protein Kinase B
AMPK	AMP-activated Protein Kinase
ATP	Adenosine Triphosphate
BGAL	Beta Galactosidase
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
DLT	Dose Limiting Toxicity
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
ECOG	Eastern Cooperative Oncology Group
ERK	Extracellular Signal-Related Kinases
ESR	Erythrocyte Sedimentation Rate
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GPCR	G Protein Coupled Receptor
HIPAA	Health Insurance Portability and Accountability Act
HOMA	Homeostatic Model Assessment (of Insulin Resistance)
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
mTOR	Mammalian Target of Rapamycin
mTORC1	Mammalian Target of Rapamycin Complex 1
N	Number (typically refers to subjects)
NCI	National Cancer Institute
OXPHOS	Oxidative Phosphorylation
PDE3B	Phosphodiesterase 3B, CGMP-Inhibited
PHI	Protected Health Information
PI	Principal Investigator
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PTEN	Phosphatase and tensin homolog
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
TME	Tumor Microenvironment
TOMM20	Transporter of Outer Mitochondrial Membrane 20
TRAP1	TNF receptor-associated protein 1
TSC2	Tuberous Sclerosis Complex Tumor Suppressor Gene 2
TUNEL	Terminal deoxyribonucleotidyl transferase mediated dUTP-digoxigenin nick end labeling
UP	Unanticipated Problem

Study Summary

Title: A Phase II Study of Metformin, Doxycycline, or a Combination of Both Agents in Head and Neck Squamous Cell Carcinoma

Précis:

Objectives:

Primary:

- To determine if treatment with metformin, doxycycline, or a combination of metformin and doxycycline can increase the percentage of cells that express Caveolin-1 in the cancer associated fibroblasts or stroma of patients with squamous cell carcinoma of the head and neck.

Secondary:

- To determine the effect of metformin, doxycycline, or metformin and doxycycline treatment on the percentage of tumor cells that express MCT4, are TUNEL positive, express BGAL, MCT1 and TOMM20 in HNSCC
- To assess safety and tolerability of metformin, doxycycline, or metformin and doxycycline treatment in subjects with squamous cell carcinoma of the head and neck.

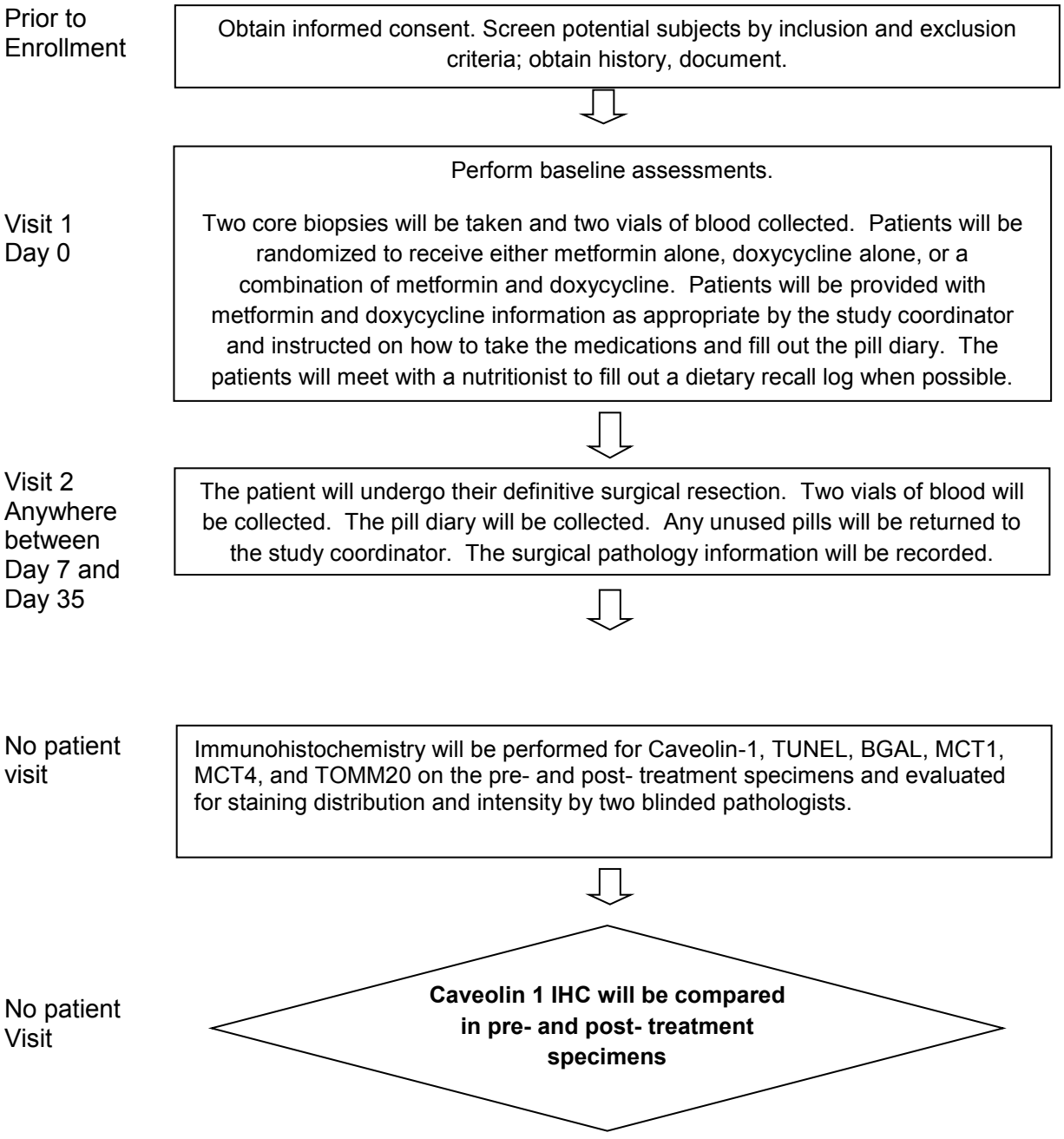
Correlatives:

- To assess the effect of metformin, doxycycline, or 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-treatment and post-treatment tumor samples when sample is available.
- To assess the effect of metformin, doxycycline, or metformin and doxycycline therapy on oncomiR miR-21 after treatment.
- To assess the effect metformin, doxycycline, or metformin and doxycycline therapy on the metabolic state of the patient as characterized serologically by: adipokines and the IGF-1/insulin signaling pathways through assessment of serum triglycerides, IGF-1, IGF-BP3, erythrocyte sedimentation rate, adiponectin, leptin, IGF-1R, exosome evaluation, metabolomics profile, and microRNA expression profiles and physiologically by performing a nutritional assessment via a nutritionist-mediated 3 day dietary recall and comparing a patient's estimated dietary intake against their estimated caloric needs.

Population: Patients aged 18 years and above.

	Known or suspected head and neck squamous cell carcinoma Have a surgical resection planned as a part of their therapy Normal organ function including creatinine less than or equal to 1.5mg/dL
Phase:	II
Number of Sites:	Thomas Jefferson University Hospital
Description of Intervention:	Metformin and doxycycline are the therapeutic agents. The initial starting dose of metformin will be 500 mg orally daily for 2 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 scheduled definitive surgery.
Study Duration:	40 months
Subject Participation Duration:	From the time that patients receive their biopsy and enroll in the trial until their definitive surgery is between 1-5 weeks. The patients will be called 30 days after surgical resection to determine if there were any further adverse effects of the medications given.
Estimated Time to Complete Enrollment:	36 months

Schematic of Study Design:



1 Introduction

1.1 Background Information

Squamous Cell Cancer of the Head and Neck

Head and neck cancers are amongst the most common cancers worldwide accounting for more than 550,000 patients with an estimated 46,000 of those patients in the United States. An estimated 8,650 of those patients in the US will die. About 90% of those cancers are squamous in origin. Head and neck squamous cell carcinomas (HNSCC) arise from the epithelial lining of the oral cavity, oropharynx, larynx, and hypopharynx. Treatment approaches are based upon staging. For early stage tumors and for those with local involvement of lymph nodes, resection is the cornerstone of therapy. Approximately 50% of head and neck cancer patients undergo primary surgery in the treatment of their malignancy. For patients with advanced disease or adverse pathologic features, adjuvant therapy with either radiation therapy or concurrent chemoradiotherapy is used to decrease recurrence rates. Even with adjuvant treatment, 2-year disease free survival can still be as low as 66%, highlighting a need to better understand the biology of these tumors.

The Metabolic Microenvironment as a Potential

Recent studies have contributed significantly to our understanding of the tumor microenvironment and the importance of the interaction between tumor epithelial cells and their surrounding stromal fibroblasts. Epithelial cancer cells are capable of converting the surrounding stroma in to “cancer associated fibroblasts” which generate lactate and pyruvate via aerobic glycolysis and secrete these energy sources. These nutrients can then be incorporated directly into the *tricarboxylic acid cycle* allowing for high efficiency adenosine triphosphate (ATP) generation, and therefore higher proliferative capacity within the cancer cells. This effectively generates a nutrient-rich environment in which the cancer cells can thrive.

Coupled with this evolving understanding of tumor metabolism is an evolving view of carcinogenesis and tumor progression in HNSCC. The high rate of local recurrence in HNSCC has led to the notion of “condemned mucosa” or “field cancerization.” Molecular abnormalities are present in epithelial cells beyond the margin of microscopically detectable carcinoma cells and this mediates, at least in part, field cancerization. We now also recognize that the field at high risk of cancer recurrences is the tumor microenvironment (TME) which contains non-cancerous epithelium, stromal cells such as fibroblasts, immune cells and their extracellular matrix. Our work focuses on the metabolic microenvironment of HNSCC, seeking to better characterize the interaction of tumor cells with the surrounding environmental cells as well as the overall metabolic state of the patient.

Specifically, we are looking at the effect of two agents - metformin and doxycycline - on the metabolic microenvironment of HNSCC.

Metformin and Its Potential as an Antineoplastic Agent

Metformin is a biguanide that is best known for its use in the treatment of type II diabetes. Extensive preclinical data support the effectiveness of metformin as an antineoplastic agent[13]. In head and neck cancer specifically, metformin inhibits proliferation of carcinoma cells and induces apoptosis *in vitro* and *in vivo*, in addition to reducing colony formation with cell cycle arrest *in vitro* [14]. *In vitro* and *in vivo* animal models have also shown that metformin can prevent the conversion of premalignant oral lesions to squamous cell carcinoma. Metformin has been shown to reduce the size and numbers of oral tumors in mice models treated with 4NQO and halt the progression of potential premalignant lesions[15]. This study, by Vitale-Cross et. al. not only showed a decrease in the total number of oral lesions when treated with metformin compared to control, but also showed almost complete absence of transformation to squamous cell carcinoma. Retrospective analyses, while limited due to the number of confounders, have also shown a potential for decreased in cadence of invasive squamous cell carcinoma of the head and neck in patients taking metformin [16, 17].

Mechanism of Action for Metformin:

These observations that metformin may possess antineoplastic properties has stimulated interest in its molecular mechanisms. While the precise mechanisms are still an active area of research, overall metformin works by inducing energetic stress.

One proposal for how this is accomplished is by a direct inhibitory effect on mitochondrial complex I, thus blocking mitochondrial-dependent production of reactive oxygen species (ROS) and adenosine triphosphate (ATP)[18-20]. The decrease in ATP production results in the activation of the liver kinase B1 (LKB1) – adenosine monophosphate-activated protein kinase (AMPK) signaling pathway[18, 21]. 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 regulates metabolism in an attempt to maintain energy homeostasis[22]. 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[23]. Metformin may also have activity that is independent of LKB1. In LKB1-deficient cells metformin is still able to affect the intracellular energy state [24].

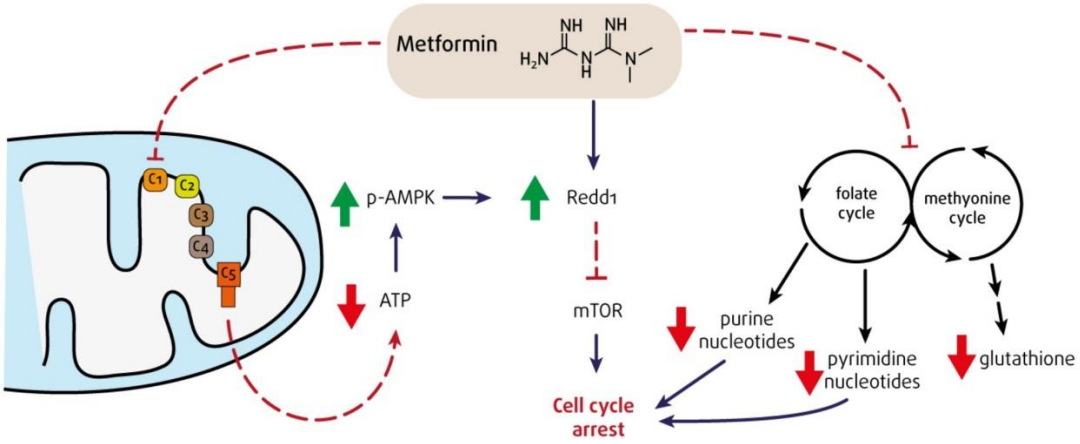


Figure 1. The proposed mechanism of action of metformin in the cell [25].

Another central signaling molecule impacted by metformin is mammalian target of rapamycin (AMPK). In mouse embryonal fibroblasts and several cancer cell lines, AMPK activation by metformin leads to inhibition of mTOR and inhibition of proliferation [24, 26, 27].

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 2 (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 (TSC2) 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[26, 28]. Rozengurt *et al* [29] 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[18] [19, 20].

<u>Epidemiologic Data for Metformin's Efficacy:</u>

Retrospective studies have shown that diabetics treated with metformin have a cancer risk reduction of approximately 40% compared to diabetics not treated with metformin[31, 32].

Other studies have also shown a reduction in the frequency of cancer with metformin use [33]. Evans *et al*. [34] 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).

Also, there is differential expression of the main transporter of metformin into cells between oral premalignant and malignant lesions and head and neck cancer has very high expression of the main transporter of metformin[35].

Current evidence from epidemiologic studies suggests that metformin has clinical activity in breast cancer. Hadad *et al*. [36] 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*.[37] 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.

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[36, 38, 39]. There is one phase II study accruing subjects using paclitaxel plus metformin up to 2500 mg a day or placebo in recurrent or metastatic head and neck cancer [40].

Doxycycline and Its Potential as an Antineoplastic Agent:

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 cancer stem cells or CSCs, across many different tumor subtypes including breast cancer [47]. Clinical trials are ongoing to determine if tetracyclines can be repurposed as anticancer agents.

Mechanism of Action of 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 an additive effect when used in combination to alter the cellular metabolism of cancer cells and their associated stromal fibroblasts.

Preclinical Data in the Martinez Laboratory:

We have shown that head and neck squamous cell carcinomas have high mitochondrial OXPHOS metabolism in highly proliferative cells[30]. Also, there is high Monocarboxylate Transporter 4 (MCT4) expression in HNSCC cancer associated fibroblasts (CAFs) and in carcinoma cells with low proliferation rates[30]. 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[30].

We interrogated HNSCC specimens (N=12) to examine if different metabolic and oxidative stress compartments co-exist in human tumors. A panel of biomarkers (Ki67/TOMM20/COX/MCT1/MCT4) was employed to visualize metabolic compartmentalization. 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-). 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 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.

We have studied a cohort of subjects with head and neck cancer who are either human papilloma virus positive or negative (HPV+ or HPV-) and have determined that high fibroblast MCT4 expression occurs in the majority of cases and TOMM20, which is a biomarker of mitochondrial metabolism, is highly expressed in proliferating carcinoma cells. This type of metabolic compartmentalization is present irrespective of whether the samples are HPV+ or HPV-.

Caveolin-1 or CAV1 is the major structural protein of the caveolae and works as a scaffolding molecule for multiple signaling proteins. Prior work has demonstrated that this protein can induce the invasive phenotype in some solid tumor types. In head and neck cancer, it has also already been shown that high expression levels in the primary tumor are associated with lymph node metastasis (PMID222133373). Loss of CAV1 expression in CAFs is mediated by oxidative stress, autophagy, and glycolysis. In our pilot trial of metformin treatment in HNSCC so far a total of 37 pre- and post-metformin exposure samples have been analyzed showing that exposure to metformin alone can increase the percent of samples showing CAV1 expression in CAFs from an average of 17% to 65% of the samples tested.

Likewise, loss of Beta-galactosidase (BGAL) in tumor stroma is a marker of cancer-associated fibroblasts. In our pilot trial of metformin in HNSCC, stromal BGAL was significantly increased from pre-treatment to post-treatment specimens ($p=0.006$) as scored by our head and neck pathologist.

TUNEL (terminal deoxyribonucleotidyl transferase mediated dUTP-digoxigenin nick end labeling) assay is used as a marker of cellular apoptosis which leads to cell death. In our pilot trial of metformin treatment in HNSCC, 38 pre- and post-metformin exposure samples have shown significantly increased ($p<0.001$) TUNEL staining after exposure to metformin.

1.2 Rationale for the Proposed Study

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 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 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 either the use of metformin or doxycycline alone or a combination of metformin and doxycycline will target mitochondrial pathways and that the combination may possibly be synergistic in impairing mitochondrial function that could lead to rescue of CAV1 expression in CAFs and tumor cell death.

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 day prior to 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 by biopsy to definitive surgical treatment is 3 weeks). We will allow a window of 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 their scheduled definitive surgery.

Rationale for endpoints

Primary*:* We have chosen caveolin-1 (CAV1) expression in tumor stroma as our primary endpoint based on studies revealing that metabolic coupling 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 biopsy 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 section.

Secondary: We have chosen TUNEL, BGAL, MCT1, MCT4 and TOMM20 as secondary end-points of the current clinical trial due to their biologic and prognostic significance as seen in our work in head and neck squamous cell carcinoma. CAV1, BGAL, and MCT4 expression modulate tumor growth in animal models, they are cancer prognostic biomarkers, and preliminary analyses of a pilot study conducted in HNSCC show that metformin modulates CAV1, BGAL and TUNEL expression. MCT4 expression in fibroblasts is associated with advanced stage disease. MCT1 and TOMM20 expression in carcinoma cells is associated with increased functional mitochondrial mass.

Correlative: Mass spectroscopy imaging (MSI) will be performed on paired samples 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 clinical trial. 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 each patient by performing 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 perform a serologic assessment at the time of enrollment and at the time of resection using exosomes, metabolomics, and microRNA expression profiles.

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 performing 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 profile will be built using exosomes and metabolomics, and we will seek to correlate this with the patient's CAV1 expression levels pre- and post-treatment.

We will also further characterize the baseline metabolic state of the patient by performing 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.

1.4 Potential Risks and Benefits

1.4.1 Potential Risks

Metformin- Risks

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]. A common side effect of Metformin is diarrhea. Minor gastrointestinal upset is the most common toxicity, leading to cessation of therapy in less than 5% of individuals. Metformin does not induce hypoglycemia. While metformin alone is rarely associated with low blood sugars (hypoglycemia), it has been reported in cases where people are fasting or undergoing strenuous physical activity. It can also cause low blood sugars in patients with diabetes who are taking other medications to manage their blood sugars such as sulfonylureas

Doxycycline- Risks

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.

1.4.2 **Benefits**

Metformin- Benefits

The possible societal benefits are large since this will allow us to learn about the pharmacodynamic effects of metformin and doxycycline in HNSCC. Information about the expression levels of CAV1, TUNEL, BGAL, 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.

Doxycycline- Benefits

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.

Other Benefits

Patients in this trial will each meet with a certified oncology nutritionist, whenever possible, who will review their dietary choices.

2 Study Objectives

2.1 Objectives

2.1.1 **Primary**

- To determine if treatment with metformin, doxycycline, or a combination of metformin and doxycycline can increase the percentage of stromal cells that express CAV1 in patients with squamous cell carcinoma of the head and neck.

2.1.2 **Secondary**

- To determine the effect of metformin, doxycycline, or metformin and doxycycline treatment on the percentage of tumor cells that are apoptotic as determined by the TUNEL assay, and express MCT4, MCT1, BGAL, and TOMM20 in squamous carcinoma of head and neck region tumor cells.
- To assess safety and tolerability of metformin, doxycycline, or metformin and doxycycline treatment in subjects with squamous cell carcinoma of the head and neck.

2.1.3 Exploratory

- To assess the effect of metformin, doxycycline, or 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-treatment and post-treatment tumor samples.
- To assess the effect of metformin, doxycycline, or metformin and doxycycline therapy on the metabolic state of the patient as characterized serologically by: erythrocyte sedimentation rate, exosome evaluation, metabolomics profile, and microRNA expression profiles and physiologically by performing a nutritional assessment via a nutritionist-mediated 3-day dietary recall and comparing a patient's estimated dietary intake against their estimated caloric needs.

2.2 Endpoints/Outcome Measures

2.2.1 Primary

- Assess the change in the percent of CAFs 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) of the NCI toxicity criteria, i.e. the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. A copy of the CTCAE can be found at http://ctep.cancer.gov. *Any grade 3 or 4 SAE will require immediate notification to the DSMB and IRB.*
- Assess the change in the percent of tumor cells expressing MCT4, that are TUNEL positive and that express MCT1, BGAL and TOMM20 in HNSCC tumors using semiquantitative scoring and digital pathology using Aperio analyses of expression intensity with previously validated algorithms.

2.2.3 Exploratory

- 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-treatment and post-treatment tumor sample.
- To assess 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. Patients will be labeled as below caloric needs, meeting caloric needs, and exceeding caloric needs.
- To assess the effect of combined metformin and doxycycline therapy on exosome evaluation, metabolomics profile, and microRNA expression profile.

3 Study Design

This is a Phase II parallel cohort design trial in which patients with known or suspected head and neck squamous cell carcinoma who are eligible for resection are randomized to receive either metformin (arm A), doxycycline (arm B), or a combination of those two agents (arm C) in the 1-5 week window of time between their diagnostic or confirmatory biopsy and their tumor resection. All medications should cease at least 12 hours prior to time of resection. Immunohistochemical analyses will be performed on the pre-treatment biopsy and the post-treatment resection specimens, allowing us to more fully characterize the metabolic microenvironment of HNSCC and our ability to alter it. No patients will receive a placebo. The treatment takes place in a period of time during which no other standard therapy exists for these patients. All patients will be treated at TJUH and all samples material analyzed here with the exception of MSI performed at Vanderbilt. Patient participation will last only during this window of time between enrollment and surgery, up to 5 weeks, with no additional visits planned. Data collected on the patients will include the IHC and MSI on their specimens, serologic assays on their metabolic state, and a characterization of their general nutritional state via the Harris-Benedict equation.

3.1 Characteristics

Parallel Phase II

3.2 Number of Subjects

33 total, 11 in each arm

3.3 Duration of Therapy

1-5 weeks

3.4 Duration of Follow Up

1 month

3.5 Treatment Assignment Procedures

3.5.1 **Randomization Procedures:** The Division of Biostatistics will generate the randomization schedule prior to the initiation of the study using the method of random permuted blocks. Randomization assignments will be loaded into a REDCap database, and assignments will be accessed by Jennifer Johnson, MD, PhD using the REDCap randomization facility.

3.5.2 **Masking Procedures**: Pathologists will not be given information on the treatment history of the pathologic samples at the time of interpretation. Investigators and clinical coordinators will not be blinded on what treatment patients are receiving.

3.6 Study Timeline

3.6.1 Primary Completion

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.

Visit 1, Day 0: Subjects that meet the inclusion criteria after reviewing plan for tissue diagnosis, laboratory data and clinical chart will be approached the day of their follow up with a subspecialty surgeon. The initial biopsy does not need to take place on Day 0 and previously obtained tissue may be used. The study coordinator will meet with the prospective candidates and go over the protocol, answer questions and obtain informed consent. Once the subject's laboratory results are reviewed and eligibility is confirmed, the patient will be randomized and metformin and/or doxycycline will be dispensed. Medication will be dispensed from Thomas Jefferson University Investigational Drug Service (IDS) Pharmacy after randomization. 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. Patients will also be asked to meet with a nutritionist either in person or by phone within the first 8 days of their enrollment to fill out a 3-day dietary recall log when possible. A nutritionist will either meet with the patients in person or call them within the first 8 days of their enrollment to fill out the 3-day dietary recall log. The nutritionist will do a 2-day guided recall, recording what the patients have eaten in the last 3 days and how much. Two 15 mL vials of blood will be drawn for baseline metabolomics and exosome profiling.

Visit 2, Day of Surgery, Week 1-5 based upon surgery: On the day of surgery, two 15 ml vials of blood will be drawn for post-treatment metabolomic and exosome profiling. A post-treatment tissue sample will be obtained from excess tissue taken from this resection specimen that is not needed in the course of clinical care. On either the day of surgery or up until their first post-op visit, patients will be approached for bottles and pill reconciliation as well as follow up of side effects and adverse events. For patients who, per institutional standard of care, have genetic testing performed on their tumor sample, we will review this information in addition to the remainder of their pathology report.

3.6.2 **Study Completion**

Follow-up: A follow up phone call will be performed 30 days (± 5 days) after the last dose of metformin and/or doxycycline for any other side effects or adverse events. The participant's medical records will be reviewed every 3 months for 12 months to assess final pathologic 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 into the study unless otherwise specified.

4.1 Eligibility Criteria

4.1.1 Inclusion Criteria

Individuals must meet all of the following inclusion criteria in order to be eligible to participate in the study:

1. Diagnosis of head and neck squamous cell carcinoma 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. Patient must be able to swallow pills.
4. Patients with serum creatinine levels less than 1.5 mg/dL
5. Women of childbearing potential must have a negative urine or blood pregnancy test within 14 days of study enrollment.
6. Informed Consent: All subjects must be able to comprehend and sign a written informed consent document.
7. ECOG Performance status ≤1

4.1.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Subjects that do not have a baseline tumor specimen/biopsy prior to starting study medications.
    a. Tumor specimens do not need to be at Jefferson at time of eligibility determination. Tumor specimens held at outside institutions should be requested for analysis of pre-treatment tumor vs post-treatment tumor.
2. Subjects who are pregnant or breastfeeding, or may become pregnant during metformin and doxycycline administration.
3. Received prior cancer therapy for the HNSCC that is being resected.
4. Subjects on metformin or doxycycline for any reason during the preceding 4 weeks.
5. Diabetic subjects that are managed by taking metformin or insulin
6. 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.
7. Patients with serum creatine ≥1.5 mg/dL
8. Patients with history of lactic or any other metabolic acidosis.
9. Patients with history of congestive heart failure stage III or greater.
10. Patients scheduled for definitive cancer surgical resection less than 7 days from beginning of study drug administration or greater than 6 weeks from beginning study

drug administration.

11. 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. Patients who have a history of hepatic dysfunction or hepatic disease and normal liver function tests will be eligible to participate.
12. 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.

13. Patient with prior allergic reaction to metformin, doxycycline, or any other tetracycline antibiotic in the past.
14. 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

4.2 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 HNSCC cohort at Thomas Jefferson University Hospital. By the nature of the diseases covered in this trial there is a male predominance with 45,330 cancer cases in men and 16,430 cases in women projected to be diagnosed in 2016 in the US alone (a 2.8:1 ratio). Our plan will be to monitor accrual in our MDG meetings monthly and to assess whether our accrual ratio matches the expected ratio of the disease. If it does not, we will first query the tumor registry at TJUH to assess our recent male to female ratio for head and neck squamous cell cancer, and if there is still a discrepancy, we will create an action plan.

4.3 Strategies for Recruitment and Retention

33 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 11 patients will be enrolled onto each arm of the trial (see Statistical Analysis Plan).

4.4 Subject Withdrawal

4.4.1 Reasons for Withdrawal

Subjects are free to withdraw from participation in the study at any time upon request.

An investigator may terminate a study subject's participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

4.4.2 Handling of Subject Withdrawals or Subject Discontinuation of Study Intervention

Patients who do not have pre- medication initiation and post-biopsy, or are discontinued from the study will not be considered as evaluable for this study and will be replaced.

Patients will be contacted by phone by a study coordinator 30 days (±5 days) after their planned resection to assess for any further adverse events that were not noted at the time of surgical resection (Visit 2).

4.5 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to investigator, and regulatory authorities. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Insufficient adherence to protocol requirements.
- Data that is not sufficiently complete and/or evaluable.
- Determination of futility.

5 Study Intervention

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 a 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.

The treatments to be used in this trial are outlined in Table 1.

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. Our planned dosing is to use 100 mg every twelve hours until the day prior to their scheduled definitive surgery.

Table 1 Trial Treatments

DRUG	DOSE LEVEL	DOSE	ROUTE	REGIMEN
METFORMIN	1	500 mg daily	Oral	Day 1-3
	2	500 mg every 12 hours (1000mg daily)	Oral	Day4-6
	3	1000 mg every 12 hours (2000 mg daily)	Oral	Day 7-the day prior to surgery
DOXYCYCLINE	-1	100 mg daily	Oral	Only if needed for inability to tolerate level 1
	1	100 mg every 12 hours (200 mg daily)	Oral	Day 1-the day prior to surgery

The same dosing and modifications apply either when given alone or together.

5.2.1 Acquisition

Drug will be obtained through the hospital drug pharmacy and maintained by the Investigational Drug Service Pharmacy.

5.2.2 Formulation, Packaging, and Labeling

Metformin:
Metformin will be provided as 500 mg tablets which are round, white to off-white, film-coated tablets. These are stored at 20°–25° C (68°–77° F); excursions permitted to 15°–30° C (59°–86° F). [See USP Controlled Room Temperature.] It will be dispensed in light-resistant containers labelled as metformin.

Doxycycline:
Doxycycline will be provided as 100 mg white oval tablets containing yellow pellets. These are stored at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F) [see USP Controlled Room Temperature]; dispense in a tight, light-resistant container (USP) labelled as doxycycline.

5.2.3 **Product Storage and Stability**

Drug will be stored in a locked cabinet until given to the participants at room temperature in the Thomas Jefferson University Hospital Investigational Drug Service (IDS) Pharmacy. Access to the locked cabinet will only be granted to the pharmacist. 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.

Each drug is stable at room temperature as above.

5.3 **Dosage, Preparation, and Administration**

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

5.4 **Dose Modifications and Dosing Delays**

Particular attention will be paid in the first three days of treatment. Patients will take 500mg/day for 3 days. From day 4, 500mg twice daily and then in 3 days (day 7) dose escalation to 1000mg twice daily will be achieved. This will be taken until the day before surgery after dinner. A phone call on the day before (or ± 2 days) each dose escalation 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 not be started if CT scan with intravenous contrast is administered to decrease risk of lactic acidosis. After the scan they will be instructed to start the metformin the following day. 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 most recent version (version 4) of the NCI toxicity criteria, i.e. the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

- Grade 1 toxicity: Patient will be maintained on full dose.
- Grade 2 toxicity: Dose will be reduced by 50% 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 by 50%.
- 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 will be provided.

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

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.

5.5 Study Product Accountability

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 in the IDS pharmacy until given to the participants. An approximately 2-week supply of metformin and/or doxycycline will be provided to subjects and the drug will be self-administered by the participants.

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.

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.

5.6 Assessing Subject Compliance with Study Product Administration

Upon enrollment to the trial, depending on which arm the patient is enrolled to, there will be either one or two pill bottles with the appropriate number of metformin and doxycycline tablets distributed to the patients. The pill bottles will be accompanied with detailed instructions on the proper dosage/number of tablets to take daily as noted above. Upon arrival for definitive surgical resection, the bottle will be collected by our trial coordinator and the contents will be evaluated for compliance.

5.7 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

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

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:

Class C:
Carbonic anhydrase inhibitors
Cephalexin
Corticosteroids (orally inhaled)
Corticosteroids (systemic)
Dalfampridine
Dofetilide
Glycopyrrolate
Lamotrigine
Luteinizing hormone-releasing hormone analogs
Pegvisomant
Penicillin
Trospium

Class D:
Bismuth Subsalicylate
Cimetidine
Iodinated contrast agents
Somatropin

5.8 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 Schedule

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	Treatment/Intervention Period [D]							End of [E] Treatment	Follow-up [G]
		Week 1				Week 2[D]		Week 3-5		
		Day 1 -7				Day 8-14		Surgical [F] Resection	Week 6	Week 7
Administrative Procedures										
Informed consent [A] and research authorization/ HIPAA form	X									
Inclusion/Exclusion Criteria	X									
Demographics, Medical history	X									
Clinical Assessment										
Physical examination, vitals, weight, blood pressure	X							X[N]	x	
Height	X									
ECOG Performance status	X								X	
Pathology Report histologic confirmation of disease if available[L]	X							X	-	
Concomitant meds[K]	X	X	X	X	X	X	X	X	X	X
Toxicity and AE Assessment[K]	X	X	X	X	X	X	X	X	x	x

Laboratory tests[B]										
Hematology	X									
Blood Chemistry	X									
pregnancy test[H]	X									
Treatment/ Intervention										
Metformin[C,D]		X	X	X	X	X	X			
Doxycycline[C,D]		X	X	X	X	X	X			
Diagnostic Biopsy or tumor sample[I]	X							X		
Surgical Resection								X		
Correlative Studies										
Serologic metabolic profile (miR21 and exosomes)[F]		X						X		
Nutrition Assessment*		X[M]								

*Nutrition assessment and Harris Benedict Equation will occur on Day 1 (or up to Day 8) of enrollment when possible, and may be done via a phone interview.

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). All are non-fasting labs.
C. Study treatment follow up phone calls will occur on days 3 and 5 ±2 days of dose escalation to evaluate tolerability, document AEs, and concomitant medications. If patient remains on study drugs for >7days, 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/or doxycycline pills should not be taken within 12 hours of scheduled surgery.
D. Patient must receive a minimum of 7 days of metformin and/or doxycycline before having definitive surgery. Patients will stay on metformin and/or doxycycline until the day prior to surgery (up to the maximum of 5 weeks).
E. End of treatment visit may occur within 14 days ± 7 days of stopping study drug.
F. Correlative study blood to be collected the morning of surgical resection. Baseline samples are to be drawn after enrollment but prior to start of study treatment.

G. Follow up phone call will be performed 30 days after last dose of study medication for any other side effects or toxicity and for pathologic staging information.
H. serum or urine pregnancy test must be done within 14 days prior to enrollment on study for all women of childbearing potential
I. If a confirmatory biopsy is performed at TJUH and excess tissue is available tissue will be flash frozen both at the time of biopsy and at the time of resection. A post-treatment tissue sample will be obtained from excess tissue taken from this resection specimen that is not needed in the course of clinical care.
J. Study drug stopped the night prior to surgery.
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. To be performed by a registered dietician, when possible. Must meet with patient within five 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. It will be noted in the patient chart if these assessments could not be completed.
N. Pre-operative nursing or anesthesia exam is acceptable and is to be performed the morning of surgery.

6.1 Pretreatment Period/Screening

Screening Assessment (Day -28 to Day -1)

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
- Concomitant meds
- ECOG performance status
- Discuss concurrent medications
- Vital signs [body temperature, blood pressure, pulse, respiratory rate, weight, height]
- Obtain histologic and/or (if appropriate) radiologic confirmation of disease
- Confirm eligibility according to the inclusion/exclusion criteria
- Laboratory tests:
    - Hematology: CBC with differential
    - Blood Chemistry
        - Creatinine, AST, AT, ALK Phos T Bili > 2.5 x ULN (all are Standard Of Care)
    - Serum or urine pregnancy test (within 14 days prior to enrollment for women of childbearing potential)

6.2 Enrollment/Baseline

- In order to be considered for evaluation, the patient must have an evaluable pre-treatment biopsy specimen.

- 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.

- Patients who are enrolled in the trial will be randomized to receive metformin, doxycycline, or metformin and doxycycline together. Patients will receive a Patient Medication Diary to record the time (AM and PM) and number of pills taken for metformin and/or doxycycline.

- Baseline caloric intake assessment using a dietary log mediated by a nutritionist when possible. This will take place at the patient's baseline assessment and may be done via a phone interview.

- Calculation of the patient's estimated caloric needs via the Harris-Benedict Equation when possible.

*If the nutrition assessment and Harris-Benedict Equation cannot be completed, this will be noted in the patient's chart.

- Complete physical examination including ECOG performance status, vital signs
- The collection of two serum separator tubes to be drawn after enrollment and prior to the start of the study treatment.
- Concurrent medications will be recorded

6.3 Treatment Period

Subjects receiving metformin and/or doxycycline will return to clinic day 1, week 1. Surgical resection will be scheduled a minimum of 7 days after the start of metformin and/or doxycycline, and a maximum of 5 weeks. The participant will receive a Patient Medication Diary to record the time (AM and PM) and number of pills take for both Metformin and Doxycycline as applicable based on their randomization.

The following assessments will be performed at Visit 2, the day of surgery

- Physical exam- Pre-operative nursing or anesthesia exam is acceptable and is to be performed the morning of surgery.
- Concomitant medications
- Adverse event assessment

- Patients will undergo their Surgical Resection at Visit 2.
  - When a pre-treatment sample has been acquired by flash freezing for MSI, and if there is excess tissue is available at the time of resection, this tissue samples will also be frozen in liquid nitrogen for MSI. A post-treatment tissue sample will be obtained from excess tissue taken from this resection specimen that is not needed in the course of clinical care.
- Correlative studies will be drawn at Visit 2: oncomiR miR-21, serum triglycerides, IGF-1, IGF-BP3, ESR, adiponectin, leptin, IGF-1R, exosome evaluation, metabolomics profile, and microRNA expression profile.
- Study drug will be taken up until at least 12 hours before surgery.

6.4 End of Treatment Study Procedures

The following study activities will occur at End of Study Visit, within 14 days ± 7 days of stopping the protocol study treatments:

- Concomitant medications
- Adverse event assessment

6.5 Post-treatment/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 visit if it occurs during timing overlap. The participant's medical records will be reviewed every 3 months for 12 months to assess final pathologic staging.

6.6 Withdrawal Visit/Discontinuation of Therapy

Patients can withdraw at any time during the study if they no longer want to participate in the trial. If withdrawal occurs no further metformin and or doxycycline administration will occur and patients will be required to return remaining tablets which will be logged in to medication administration records and destroyed. If a subject withdraws consent to participate in the study, permission will be sought to use data pertaining to the subject in the analysis as far as they participate and they will be removed from subsequent analyses and replaced.

Description of when a subject's participation in the trial may be discontinued:

Specific reasons for discontinuing a subject from this study are:

1. Voluntary discontinuation by the subject who is at any time free to discontinue their participation in the study, without prejudice to further treatment.
2. Safety reasons as judged by the investigator.
3. Severe non-compliance to the protocol as judged by the investigator.
4. Incorrect enrollment of the subject.
5. Subject lost to follow-up
6. Death

7 Study Procedures and Evaluations

Study procedures for this trial include:

- Review and immunohistochemical staining of a patient's diagnostic biopsy, which was performed as standard of care.
- Randomization to receive metformin alone, doxycycline alone, or metformin and doxycycline together.
- Administration of the oral medication(s) listed above as per randomization until the day before the patient's planned surgery.
- The collection of two serum separator tubes to be drawn after enrollment and prior to the start of the study treatment.
- Meeting with a registered dietician either in person or by phone to do a guided three day dietary recall log within the first 8 days when possible.
- Snap freezing a portion of the patient's biopsy and resection specimen for MSI testing may be performed in a subgroup of specimens.
- Immunohistochemistry on slides from the patient's resection specimen.

7.1 Study Procedures/Evaluations

- Medical history including intercurrent illnesses and specific attention to diabetes, may be obtained from the medical record
- Physical exam: height, weight, obtained from the medical record

- Concomitant meds: prescription medications taken for the 28 days prior to enrollment
- ECOG performance status from the medical record
- Obtain histologic confirmation of disease from the medical record
- Laboratory tests: Hematology- CBC with differential; Blood Chemistry- Creatinine, AST, AT, ALK Phos T Bili to have been performed within 28 days of enrollment and thus may be obtained either from the medical record or as a part of the standard of care

7.2 Laboratory Procedures/Evaluations

7.2.1 Clinical Laboratory Evaluations

- Hematology: CBC with differential
- Blood Chemistry with Creatinine, AST, AT, ALK Phos T Bili
- Serum or urine pregnancy test

7.2.2 Special Assays or Procedures

Research Correlates- exosome evaluation, microRNA and metabolomics profile.
These labs will be obtained from two serum separator tubes which will be provided directly to the Martinez laboratory. After being obtained, the tubes may be kept at room temperature and transported to the laboratory at 233 S. 10th Street suite 909. Contact Diana Whitaker Menezes, MS, Research Associate, 215-955-9774.

7.2.3 Specimen Preparation, Handling, and Storage

Biopsy specimens both pre-treatment and post-treatment are to be processed as formalin-fixed paraffin embedded samples per clinical standards.

Samples for MSI testing will be flash frozen in liquid nitrogen within 10 minutes of removal of the specimen by a member of the Martinez laboratory. Fresh tissue samples will be quick frozen in liquid nitrogen, transferred to appropriate storage vials and stored at -80C until shipping to Vanderbilt MSI Facility. Each sample storage vial will be labeled with a unique ID number without any PHI.

7.2.4 Specimen Shipment

Flash frozen tumor samples will be shipped to the Mass Spectroscopy Imaging facility at Vanderbilt University on dry ice by the Martinez laboratory personnel. For shipping, sample vials are placed into plastic Ziplock bags that are then placed into a styrofoam container of dry ice within a cardboard box. Shipping containers are sent Fed Ex Priority Overnight to the following address:

Mitch Reyzer/Audra Judd, Mass Spectrometry Research Center, 465 21st Avenue South, 9160 MRB III, Nashville, TN 37240

8 Evaluation of Safety

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 subject population being studied;

UAPs are considered to pose risk to subjects or others when they suggest that the research places subjects 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 subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

8.1.3 Serious Adverse Events

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

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- 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 subject and may require medical or surgical 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 informed consent is obtained 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 8.2 Safety Assessment and Follow-Up.

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.

8.4 Safety Reporting

8.4.1 Unanticipated Problem Reporting to IRB

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 subjects 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 subjects or others will be submitted to the IRB at the next continuing review.

8.4.1 Adverse Event Reporting to IRB

Grade 1 AEs are not required to be reported to the IRB.

Grade 2 AEs will be reported to the IRB at the time of continuing review if, in the opinion of the investigator, they represent events that exceed expected frequency or in some other way are judged to be unexpected and possibly associated with increased risk.

8.4.2 Serious Adverse Event Reporting to IRB

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 or definitely 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.

8.4.3 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)

8.4.4 Reporting of Pregnancy

Pregnant women are excluded from this trial. Should a woman of child-bearing potential become pregnant during the course of this trial she will be discontinued from treatment and replaced in the study.

8.5 Halting Rules

This study will be monitored by the SKCC DSMC.
Acute toxicity will be monitored using CTCAE version 4.0 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 DSMC operates in

compliance with a Data and Safety Monitoring Plan (DSMP) that is approved by the Clinical Trials Oversight Committee (CTOC).

10 Clinical Site Monitoring and Auditing

Clinical site monitoring and auditing is conducted to ensure that the rights of human subjects 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 subject 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 DSMP.

11 Statistical Considerations

11.1 Study Hypotheses

Primary: Exposure to metformin and/or doxycycline administered together will alter the metabolic profile of squamous cell carcinoma of the head and neck, specifically as measured by the expression level of CAV1 in tumor-associated stromal cells.

Secondary: Exposure to metformin and/or doxycycline administered together will alter the metabolic profile of squamous cell carcinoma of the head and neck, as measured by the expression levels of BGAL and MCT4 in tumor associated stroma, and TUNEL, MCT1 and TOMM20 in epithelial tumor cells. We hypothesize that these drugs, given either as monotherapy or in combination will be safe and tolerable for patients in the pre-surgical period.

11.2 Analysis Plans

Analysis for primary outcomes: The primary objective of the study is to assess the impact of metformin and or doxycycline on CAV1 expression by immunohistochemistry (IHC) in tumor-associated stromal 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. Comparisons will be made between pre-treatment and post-treatment within each of the cohorts. The study is not powered to make comparisons between the three different cohort groups.

Analysis for secondary outcomes: Analysis of change in TUNEL, BGAL, TOMM20, MCT1, and MCT4 will be performed using the Wilcoxon signed-rank test. Safety will be provided in descriptive tables.

11.2.1 Safety Review

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.

11.3 **Sample Size Considerations**

The sample in each cohort is based on having 85% power to detect an average increase in CAV1 staining of 40% using a two-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 9 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 11 patients per cohort for a total of 33 patients.

11.3.1 **Replacement Policy**

Patients who do not have sufficient pre-treatment biopsy material obtained in the course of their medical care to be evaluated for CAV1 by IHC, or are discontinued from the study will not be considered as evaluable for this study and will be replaced.

11.3.2 **Accrual Estimates**

Our total goal is 33 patients. We estimate this will be possible in a 3-year period with 11 per year.

12 Source Documents and Access to Source Data/Documents

Study staff will maintain appropriate medical and research records for this study, in compliance with ICH E6, and regulatory and institutional requirements for the protection of confidentiality of subject information. Study staff will permit authorized representatives of SKCC and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

13 Quality Control and Quality Assurance

The investigator will allocate adequate time for monitoring activities. The Investigator will also ensure that the medical monitor or other compliance or quality assurance reviewer is given access to all of the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

14 Ethics/Protection of Human Subjects

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6.

14.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to subjects and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the subject. Consent forms will be IRB-approved, and the subject is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the subject and answer any questions that may arise. The subject will sign the informed consent document prior to any study-related assessments or procedures. Subjects will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study. The consent process will be documented in the clinical or research record.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

Pregnant and breastfeeding women are excluded from the trial due to the potential for the use of doxycycline. Otherwise, we will attempt to accrue women and men equally on this study, representative of the epidemiologic distribution of the disease.

14.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to any study information relating to subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study subjects. The clinical study site will permit access to such records.

14.6 Future Use of Stored Specimens and Other Identifiable Data

No identifiable data will be retained.

15 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.

15.1 Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems and adverse events must be reviewed by the investigator or designee.

15.2 Data Capture Methods

Data will be captured on paper case report forms and transferred to an electronic database which will be kept behind a password protected firewall- RedCap.

15.3 Types of Data

Data that will be collected include patient history and physical examination data, safety, laboratory studies, pathology staging studies, and immunohistochemistry as determined in the laboratory.

15.4 Study Records Retention

Study records will be maintained for at least three years from the date that the grant federal financial report (FFR) is submitted to the NIH.

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until 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, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

15.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the subject, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

All deviations from the protocol must be addressed in study subject source documents and promptly reported to the IRB and other regulatory bodies according to their requirements.

16 Study Finances

16.1 Funding Source

The department of medical Oncology at Thomas Jefferson University is funding this trial.

16.2 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).

16.3 Subject Stipends or Payments

Subjects will not receive payment for participation in the study.

17 Publication and Data Sharing Policy

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent

comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

U.S. Public Law 110-85 (Food and Drug Administration Amendments Act of 2007 or FDAAA), Title VIII, Section 801 mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials:"

Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations, of a product subject to FDA regulation;

Trials of Devices: Controlled trials with health outcomes of a product subject to FDA regulation (other than small feasibility studies) and pediatric postmarket surveillance studies.

NIH grantees must take specific steps to ensure compliance with NIH implementation of FDAAA.

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