Thomas Jefferson University

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Kimmel Cancer Center

Pilot study of metformin in head and neck cancer and its effect on proinflammatory cytokines and exosomes implicated in acute and chronic toxicity

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CONFIDENTIAL

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List of Abbreviations

AKT: also named protein kinase B
AMPK: adenosine monophosphate protein kinase
ATP: adenosine triphosphate
CAFs: cancer associated fibroblasts
COX: cytochrome c oxidase
ERK: extracellular regulated kinase
FDG: fluoro deoxy-glucose
GPCR: G protein-coupled protein receptor
HOMA: homeostatic model assessment
HNSCC: head and neck squamous cell carcinoma
IGF-1: insulin growth factor 1
IHC: immunohistochemistry
IL-6: interleukin 6
IL-1 Beta: interleukin 1 beta
Ki67: Kiehl-67 antibody
LKB1: liver kinase B1
mTOR: mammalian target of rapamycin
mTORC1: mTOR complex 1
OXPHOS: oxidative phosphorylation
PDE3B: phosphodiesterase 3B
PET: positron emission tomography
PI3K: phoshoinositide 3 =kinase
PTEN: phosphatase and tensin homolog
RAS: rat sarcoma oncogene
RAF: rapidly accelerated fibrosarcoma
ROS: reactive oxygen species
TNF-a: tumor necrosis factor alpha
TSC2: tuberous sclerosis 2

Study Summary

Title	**Pilot study of metformin in head and neck cancer and its effect on proinflammatory cytokines and exosomes implicated in acute and chronic toxicity**
Short Title	*Metformin in radiation treatment of head and neck cancer*
Protocol Number	*IRB# 16D.639/JT#9410*
Phase	Pilot
Methodology/Study Design	Pilot Feasibility trial
Study Duration	24 months
Study Center(s)	Single Center- Jefferson
Objectives	To assess the effect of metformin on radioprotection in normal tissue for subjects undergoing head and neck radiation. We will evaluate metformin's ability to decrease proinflammatory cytokines and decrease mucositis, xerostomia, fatigue and dysphagia.
Number of Subjects	50
Diagnosis and Main Inclusion Criteria	Subjects with a biopsy proven diagnosis of head and neck cancer, and who have a scheduled appointment for either postoperative adjuvant radiation or definitive radiation.
Study Therapy, Dose, Route, Regimen	Metformin is the therapeutic agent in the protocol. The initial starting dose will be 500mg orally daily for 3 (+2 days) days which then will be increased to 500 mg twice daily and, if tolerated, further increased to 1000mg twice daily after day 6 (+2 days). Subjects will maintain 1000 mg twice a day dosing until two weeks post radiation. Subjects will be randomized to 1:1 to take metformin or placebo.
Duration of administration and follow-up	The initial starting dose will be 500mg orally daily for 3 (+2 days) days which then will be increased to 500 mg twice daily and, if tolerated, further increased to 1000mg twice daily after day 6 (+2 days). Subjects will maintain 1000 mg twice a day dosing until two weeks post radiation. The participant's medical records will be reviewed every 3 months for 12 months. 2 vials of Blood and 5cc of saliva samples will be obtained before, during and immediately after treatment and every three months thereafter for one year
Reference therapy	*None*

Statistical Methodology	The primary objective of the study is to assess the impact of metformin on cytokine and exosome profiles. All subjects from whom blood and saliva samples are obtained both pre-, during and post-treatment will be included in the primary analyses. Change in cytokine profiles will be evaluated using mixed effects linear regression. Analysis of secondary outcomes will be primarily descriptive. The distribution of swallowing function and mucositis scores will be summarized. The objective response rate will be estimated along with an exact 95% binomial confidence interval.
Schema	50 HNSCC patient candidates for adjuvant or neoadjuvant radiation → Pre-metformin blood and saliva samples → Randomized Metformin Treatment* (n=25) vs Placebo** Treatment (n=25) → external beam radiation (sample collected halfway through) → Post-metformin blood and saliva sample *Metformin (500 mg/day for 3 (+ 2 days) days followed by 500 mg twice daily for 3 days followed by 1000mg twice daily from day 6 (+2 days). Subjects will maintain 1000 mg twice a day dosing until two weeks post radiation.) ** Placebo (1 tab taken BID) No ramp up phase

1.0 INTRODUCTION

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

1.1 Specific Aims and Hypothesis

Purpose of the study: To assess the effect of metformin on inflammatory pathways in subjects with cancer of the head and neck undergoing external beam radiation. We will use analysis of cytokines/chemokine profiles expression and exosome profiles in subjects that receive metformin during their radiation treatment.

- **Primary objective:** Assess the impact of metformin on cytokine/chemokine and exosomes on blood and saliva during and after radiation.

- **Secondary objectives**: Assess safety and tolerability of metformin treatment in subjects with head and neck cancer. Also, to assess the potential impact of metformin on the following: xerostomia, dysphagia, mucositis, fatigue as measured by EORTC QLQ – H&N35, Xerostomia Score, mucositis score and Multidimensional Fatigue Inventory (MFI) (See appendix).

1.2 Background and Rationale:

Effects of Radiation on Normal Tissue and Systemic Effects

Treatment for Head and Neck Cancer has used radiation as one of the foundational components to achieve expected cure rates. Protocols have been well established to maximize tumor kill rate and minimize collateral damage to surrounding tissue, yet the effects of radiation on normal tissue remains profound in the head and neck region. Oral mucositis, swallowing, xerostomia and fatigue are the main drivers that affect quality of life status post treatment.

Oral mucositis is a common, problematic, and painful complication of cancer therapy, in the regimens that include radiation therapy for the head and neck cancer[1]. Oral mucositis is a treatment-induced morbidity that has substantial impact on day-to-day functioning. In addition to the common need for, and inadequate pain control with, narcotics, the profound clinical impact of oral mucositis also includes weight loss, difficulty eating and swallowing, dehydration, need for nutritional support, and reduced performance status[2], as well as secondary infections and diminished quality of life (QOL)[3].

A majority of patients receiving combined chemoradiotherapy for head and neck cancer (HNC) can be expected to develop severe (WHO Grade 3-4) oral mucositis[4] and nearly all HNC patients receiving RT with concurrent cisplatin are expected to develop oral mucositis (WHO Grade 2 or higher) [5].
Standard chemoradiotherapy for locally advanced head and neck (HNC), whether in the post-operative or definitive setting, currently consists of intensity-modulated radiation therapy (IMRT) plus/minus systemic therapy. The monoclonal antibody cetuximab has also been shown to increase the efficacy of radiotherapy for locally advanced head and neck cancer [6], and the combination of IMRT plus either single-agent cisplatin or single-agent cetuximab is currently being tested head-to-head for patients with human papilloma virus (HPV)-related oropharyngeal cancer in the ongoing RTOG 1016 trial. In addition, two recently published Phase 3 studies of palifermin in patients receiving RT plus single-agent cisplatin for HNC reported an overall incidence of severe (WHO Grade 3-4) oral mucositis among placebo patients of 67% in one study and 69% in the other[7,8]. Oral mucositis prevention and management remains a substantial unmet need.

Patients with HNC may also suffer the additional complications of short- and long term xerostomia, taste change, and trismus related to post-radiation fibrosis. McBride et al. demonstrated a significant improvement in rates of xerostomia, shorter durations of PEG tube dependence when comparing intensity-modulated radiation therapy (IMRT) vs conventional radiation therapy [9]. Even though they found a significant early benefit, late stage dysphagia persisted in 7-10% of patients at 2-4 years out had grade 2 dysphage or higher with IMRT patients having a significantly higher rate of cervical esophageal strictures[9].
Fatigue is a well-known side effect of radiation with some indication that circulating cytokines and chemokines are responsible for this phenomena. Increase in interleukin (IL-1), tumor necrosis factor (TNF) and C-reacitve protein (CRP) have been found in patients with profound fatigue[10]. Others have not been able to make association with inflammatory markers. Xiao et al. found a strong correlation to fatigue and inflammatory markers with gene transcripts related to NFkB. We propose in this study to look at an entire panel of inflammatory markers, utilizing Luminex technology with the Milliplex MAP Human Cytokine/chemokine Magnetic kit I (41 analytes) (sCD40L, EGF, Eotaxin, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, TGF-α, TNF-α, TNF-β, VEGF). Another possible mechanism that has not been described is exosomes/microvesicle communication that can be found systemically released by tissue under stress.

Exosomes are small extra-cellular vesicles produced by all cells[11] and present in almost all biological fluids including blood, urine, ascites, CSF, serum and plasma, and in the culture medium of cell cultures[12]. Currently exosomes are

defined as 40-150 nm diameter vesicles of endocytic origin, similar in size to viruses, with a bilayered lipid membrane, a cup shaped morphology, and densities ranging between 1.13-1.19 g/mL[12].

Exosomes have recently emerged as important mediators in cell communication due to their enriched content in genetic material like mRNAs and non-coding RNAs[12]. The biogenesis of exosomes which involves the endosomal compartment sets them apart from other extracellular vesicles[11]. Their molecular profile partly, but not completely, resembles that of the parental cell. The precise function of exosomes remains unknown[13]. The leading theory is that exosomes are an integral part of a complex, well-organized, form of information delivery that operates at short and long distances[11]. The content and number of exosomes generated likely change depending on whether cells are experiencing different stressors or stimuli[13]. Tumor-derived exosomes transfer signals and convey information locally within the tumor microenvironment as well as to distant tissues and organs to facilitate tumor growth and metastasis. They are present in the circulation and have ready access to all parts of the body[11].

Cytokines/chemokines are well established as molecules that convey a phenotype of tissue behavior or biologic mechanism. They likewise are found in all bodily fluids and are secreted in times of stress.

1.3 Preclinical Data:

Metformin and its potential as radioprotective agent

Metformin (N, N-dimethylbiguanide) is a biguanide that is best known for its use as first line therapy for type II diabetes patients[14]. Metformin specifically inhibits the complex I (NADH:ubiquinone oxidoreductase) of the mitochondrial electron transport chain decreasing cellular respiration and the rate of ATP formation[15,16]. This triggers the activation of the energy sensor AMP-activated protein kinase (AMPK) that regulates cell metabolism and shifts it towards an energy-sparing state[17]. This leads to reduced hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity thereby increasing peripheral glucose uptake and utilization without causing hypoglycemia. It is an FDA approved medication for the management of type 2 diabetes mellitus with extensive safety data. In general, clinically significant responses are not seen at doses <1500 mg daily; however, a lower recommended starting dose and gradual increased dosage is recommended to minimize gastrointestinal symptoms. Extensive preclinical data now also support the effectiveness of metformin as an antineoplastic and radioprotective agent[18].

Epidemiologic Data for Metformin's Efficacy for Radioprotection and Cancer Prevention:

Retrospective studies have shown that diabetics treated with metformin have a cancer risk reduction of approximately 40% compared to diabetics not treated with metformin[19,20]. Evans et al reported that the risk of subsequent cancer diagnosis was reduced in patients with type 2 diabetes who received metformin (with an odds ratio of 0.85 for any metformin exposure versus no metformin exposure)[21]. The protective effect increased with greater metformin exposure (measured as total dose prescribed or total duration of use).
Current evidence from epidemiologic studies suggests that metformin has clinical activity in breast cancer. Hadad et al 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)[22]. Similarly, Niraula et al 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[23]. Multiple clinical trials are currently evaluating the effect of metformin in combination with standard treatment in a variety of malignancies including breast, colorectal, pancreatic, lung, gynecologic, and prostate cancer.
Anti-tumor effects by metformin are contrasted by several reports supporting the hypothesis that metformin may serve as a radioprotectant that can help to protect normal tissues against the radiation toxicity. At present, the only prophylactic radioprotector that has been approved by the FDA is amifostine, which is used for radioprotection against xerostomia by radiation exposure in the treatment of head and neck cancer[24]. Metformin in conjunction with N-acetyl-cysteine or captopril resulted in a 2.4-2.6 fold enhanced survival in mice exposed to 7Gy of radiation as well as survival of cell cultures. Miller argues that the wide use and tolerability of metformin with an animal model that demonstrates enhanced survival should be considered 1st line in radiation countermeasures in the event of a radiation exposure. Based on the observations of several preclinical studies, Bikas et al conducted a 2016 retrospective study that showed that metformin significantly attenuated the I-131-induced decrease in white blood cells post-treatment and that patients treated concomitantly with metformin returned to their baseline values sooner[25].

Our current animal model looking at ameliorating the effects of radiation utilizes a thiol modifying agent RTA 408 and metformin. In unpublished data the group drinking metformin dissolved in water is healing wounds at a significantly faster rate then the control animals.

<u>Mechanism of Action for Metformin:</u>
Metformin inhibits oxidative phosphorylation by downmodulating the activity of the complex I enzyme NADH dehydrogenase. The inhibition of mitochondrial complex activity may contribtue to ROS metabolism and the activation of AMP-

activated protein kinase (AMPK) by metformin. AMPK is a central cellular energy sensor, activation of which leads to suppression of many of the processes highly dependent on ample cellular adenosine triphosphate supply, including gluconeogenesis, protein and fatty acid synthesis and cholesterol biosynthesis, promoting catabolic processes such as fatty acid beta oxidation and glycolysis[26]. AMPK activation potentially promotes survival after radiation, especially in a low nutrient environment found in human tumors[27]. By inhibiting the formation of ROS, metformin may further protect tissues or cells against DNA damage and mutations[28].

Preclinical studies and clinical trials support the view that metformin has anticancer properties[29-32] although the mechanism(s) underlying this effect are subject to debate. The purported mechanisms are numerous and include OXPHOS complex I inhibition, AMPK activation and insulin growth factor signaling[14,22,23,33]. Several groups have shown that metformin's ability to limit tumor growth *in vivo* is dependent on mitochondrial complex I[34]. Complex I inhibition blocks mitochondrial-dependent production of reactive oxygen species (ROS) and adenosine triphosphase (ATP)[35,36]. Catabolite access may determine susceptibility to metformin anti-tumor effects as some cancer cells grown in the absence of glucose and presence of glutamine are more affected by metformin treatment than cells grown in the presence of glucose[29] . Metformin sensitivity is further determined by glucose availability and overall oxidative phosphorylation (OXPHOS) capacity[30]. The decrease in ATP production results in the activation of the liver kinase B1 (LKB1) – adenosine monophosphate-activated protein kinase (AMPK) signaling pathway[26-28]. Activation of this pathway usually occurs during times of hypoxia and nutrient deprivation, and reciprocally, it can be suppressed in times of "over nutrition" and hyperglycemia. AMPK is a key energy sensor that regulators metabolism in an attempt to maintain energy homeostasis[31]. The end result of blocking the LKB1-AMPK signaling pathway is a down-regulation of energy consuming biosynthetic processes including gluconeogenesis, protein and fatty acid synthesis and cholesterol biosynthesis, and promotion of catabolic processes such as fatty acid beta oxidation and glycolysis[32]. Metformin may also have activity that is independent of LKB1. In LKB1 deficient cells, metformin is still able to affect the intracellular energy state[33]. Metformin also alters the mitochondrial redox state by inhibiting glycerophosphate dehydrogenase[34]. Metformin reduces the mitochondrial citric acid cycle and induces aerobic glycolysis as well[35].

Collectively these considerations highlight that metformin may have utility as a selective radiation protector of normal but tumor cells.

1.4 Clinical Data

Summary of results from clinical studies:

Studies with metformin in cancer patients are abundant including all tumor sites. Our group has recently finished an investigator run trial looking at evidence of OXPHOS metabolism pre and post metformin.

We have shown that head and neck squamous cell carcinomas (HNSCC) have high mitochondrial OXPHOS metabolism in highly proliferative cells[29]. Also, there is high MCT4 expression in HNSCC cancer associated fibroblasts (CAFs) and in carcinoma cells with low proliferation rates[29]. 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[29].

Bikas et al. retrospectively studied the radioprotective effects of Metformin on RAI treatment in differentiated thyroid cancer (DTC) patients by looking at complete blood count (CBC). The study used two arms. The Metformin group consisted of 40 diabetic patients with DTC taking Metformin while the control group consisted of 39 patients with DTC, combination of diabetic or not diabetic, not taking Metformin at the time of RAI treatment. Blood parameters included hemoglobin, red blood cell count, white blood cell count, absolute neutrophil count, absolute lymphocyte count, and platelet count. CBC was recorded at one month, six months, and twelve months. Patients treated with Metformin post RAI treatment showed less of a decrease in white blood cell (WBC) count. The differences in WBC count between the two groups was highly statistically significant at all time points ($P< 0.0001$, $P<0.0027$, and $P< 0.0001$, respectively). The most prominent decrease in WBC was in absolute neutrophil count. WBC levels of patient in the Metformin group were also shown to recover to baseline levels more quickly[25].

Metformin not only likely has radioprotective effects but may also have anticancer effects as well which could impact effect of radioactive Iodine treatment. Retrospective studies have shown that diabetics treated with metformin have a cancer risk reduction of approximately 40% compared to diabetics not treated with metformin[30,31]. Other studies have also shown a reduction in the frequency of cancer with metformin use[32]. Evans et al [33] reported that the risk of subsequent cancer diagnosis was reduced in patients with type II diabetes who received metformin (with an odds ratio of 0.85 for any metformin exposure *versus* no metformin exposure). The protective effect increased with greater metformin exposure (measured as total dose prescribed or total duration of use).

Current evidence from epidemiologic studies suggests that metformin has clinical activity in breast cancer. Hadad et al[22] 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[23] 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[14,22,23]. 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.

Metformin reduces mitochondrial OXPHOS metabolism and hence we expect it to reduce mitochondrial metabolism in carcinoma cells. We want to investigate metformin's effects on epithelial-stroma metabolic coupling and its ability to revert the high OXPHOS metabolism in carcinoma cells and revert the stroma to a less tumor permissive state in various cancers.

Secondary end points will look at quality of life metrics including the EORTC QLQ – H&N35, mucositis score, the Multidimensional fatigue inventory and the Xerostomia Questionnaire (XQ) all of which have been validated and used in multiple clinical studies to-date (Appendix).

1.5 Study Therapy

Description of the experimental product:

Metformin is a biguanide oral antidiabetic drug. It inhibits hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity thereby increasing peripheral glucose uptake and utilization without causing hypoglycemia. It is an FDA approved medication for the management of type 2 diabetes mellitus with extensive safety data. In general, clinically significant responses are not seen at doses <1500 mg daily; however, a lower recommended starting dose and gradual increased dosage is recommended to minimize gastrointestinal symptoms. Metformin's main effect on cells is to decrease mitochondrial oxidative phosphorylation (OXPHOS) complex I activity. Metformin treatment does not induce hypoglycemia. Metformin may represent a novel radioprotectant therapy.

1.6 Dose Rationale and Risk/Benefits

Summary of known and potential risks and benefits, if any, to subjects:

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[27]. Minor gastrointestinal upset is the most common toxicity, leading to cessation of therapy in less than 5% of individuals. Metformin does not induce hypoglycemia. The possible societal benefits are large since this will allow us to learn about the pharmacodynamic effects of metformin in normal tissue protection during radiation.

Description and justification for the route of administration, dosing regimen and treatment period:
Metformin will be administered orally since this is the route of administration currently approved by the FDA. Beginning one-two weeks prior to planned external beam radiation therapy, the drug will be initiated at a dose of 500mg orally daily for 3 days and will then be increased to 500 mg twice daily. If tolerated, metformin will be further increased to 1000mg twice daily after day 6. Subjects will maintain 1000 mg twice a day dosing until two weeks post radiation.

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)[28, 29]. The time of planned exposure to metformin will be 8-10 weeks. We will allow a window of an additional 2 weeks in the event that there are delays in the treatment scheduling but no patients will receive metformin for more than 12 weeks.

Rules for dose modification: Toxicity monitoring, dose modification and treatment of complications:

Particular attention will be paid in the first three days of treatment. Patients will take 500mg/day for 3 days. From day 4, 500mg twice daily and then in 3 days (Day 6) dose escalation to 1000mg twice daily will be achieved. A phone call on the day of 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 side effects occur during the study[31].

In the event that an Iodinated IV contrast dye load for CT scanning is required at some point during the metformin treatment period, the drug will be held 48 hours in advance of administration of IV dye, as is the standard practice among patients taking metformin. This measure is generally considered to be conservative as the risk of lactic acidosis in patients taking metformin has not been shown to be higher than that of the general population after IV contrast dye in recent literature. Nevertheless, this measure is in compliance with current

American College of Radiology guidelines[37]. Metformin will be resumed at the last dose the subject was receiving prior to being held and if escalation was planned as previously described.

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. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov.

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 the treatment will be discontinued definitively.
Grade 3 toxicity (probably or definitely drug related): Treatment will be interrupted and reassessment of toxicity will be performed daily. If toxicity is reduced to grade 1 or lower dose will be reduced by 50%. If toxicity is not resolved after 3 days the treatment will be discontinued definitively.
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), concomitant loperamide will be provided and with grade 2 diarrhea the dose will be reduced by 50% until grade 1 or lower.

Risk benefit analysis

The risks using metformin are small in this protocol with strict inclusion and exclusion criteria. It is unknown if metformin improves outcomes in HNSCC and this study is not designed to evaluate efficacy of metformin in HNSCC, but this study is designed to evaluate whether the sequelae of treatment from external beam radiation are mitigated by use of metformin. Hence, the potential direct benefits to subjects in this study include the possibility of decreased, xerostomia, dysphagia, fatigue and other sequelae of RAI. Further, the possible societal benefits are large since this will allow us to learn about the pharmacodynamic effects of metformin in radiation treatment, tolerability and safety profile. Metformin is a widely used drug with an extensive safety record. In order to further minimize the risk of toxicity, strict exclusion criteria will be applied. This trial could lead to a better understanding of the properties of the cancer microenvironment. The hypothetical risk of loss of confidentiality is minimized by the layers of security in place as detailed above.

Potential risks:

There is a potential risk of development of side effects related to metformin administration. Most commonly reported side effects in 1-5% of patients are

nausea, vomiting, and abdominal pain. An extremely low risk of lactic acidosis is present (0.001%) which is minimized by excluding patients with renal dysfunction, hepatic dysfunction, cardiac impairment, pulmonary impairment, or excessive alcohol use. There is also a risk of loss of confidentiality which will be minimized as outlined in section 0.

Potential benefits:

Metformin may provide a radioprotective effect and mitigate the sequelae of external beam radiation. Furthermore, metformin may alter tumor metabolism and improve outcomes in various cancers. This study is designed to evaluate whether the sequelae of radiation are mitigated by metformin and thus some patients may benefit from this study by taking metformin. With regards to cancer benefit, this study is not designed to evaluate efficacy in cancer treatment and it is unknown if metformin improves outcomes in cancer.

2.0 STUDY OBJECTIVES

1. To assess the capacity of metformin to alter the cytokine and exosome profiles.
2. To assess the capacity of metformin to mitigate known side effects of external beam radiation therapy including inflammation, mucositis, dysphagia, xerostomia, and fatigue.

2.1 Primary Objective:

We have chosen a cytokine/chemokine profile analysis as a surrogate marker of trending inflammatory reaction. This response drives the severity of mucositis/xerostomia and acute radiation toxicity. The blood and saliva counts will be compared in the pre-treatment sample, during treatment samples and post treatment samples. Luminex technology with 40 cytokines markers will be used (Luminex technology with the Milliplex MAP Human Cytokine/chemokine Magnetic kit I (41 analytes) (sCD40L, EGF, Eotaxin, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, TGF-α, TNF-α, TNF-β, VEGF). Blood and saliva samples will be drawn before, during and immediately after treatment and every three months thereafter for one year. All blood samples will be taken by a phlebotomist who will be blinded as to whether the patient is in the experimental (radiation + metformin) or control (radiation + placebo) groups.

2.2 Secondary Objective:

- Assess safety and tolerability of metformin treatment in subjects undergoing external beam radiation treatment for head and neck cancer.

- To determine the effect of metformin treatment on symptoms of **xerostomia** as assessed by the Xerostomia Questionnaire (XQ)
- To determine the effect of metformin treatment on symptoms of **mucositis** as assessed by WHO classification
- To determine the effect of metformin treatment on symptoms of **dysphagia** as assessed by the MD Anderson Dysphagia Inventory
- To determine the effect of metformin treatment on symptoms of **Fatigue** as assess by the Multidimensional fatigue inventory (MFI).

2.3 Primary Study Endpoints

a) Cytokine/chemokine profile
b) Exosome profile.

2.4 Secondary Study Endpoints

Measures of

a) Mucositis
b) Dysphagia
c) Xerostomia
d) Fatigue.

2.5 Primary Safety Endpoints

Toxicity will be evaluated using the most recent version (version 3) of the NCI toxicity criteria, i.e. the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov. *Any grade 3 or 4 SAE will require immediate notification to the DSMB and IRB. Metformin will be held for any grade 3 or 4 SAE.*

3.0 STUDY DESIGN

Purpose of the study: To assess the radioprotective effect of metformin in subjects with head and neck cancer by evaluating both the ability of metformin to mitigate known side effects of radiation therapy as well as alter the exosome profile in the serum.

3.1 General Design

Type of trial: Pilot Feasibility trial

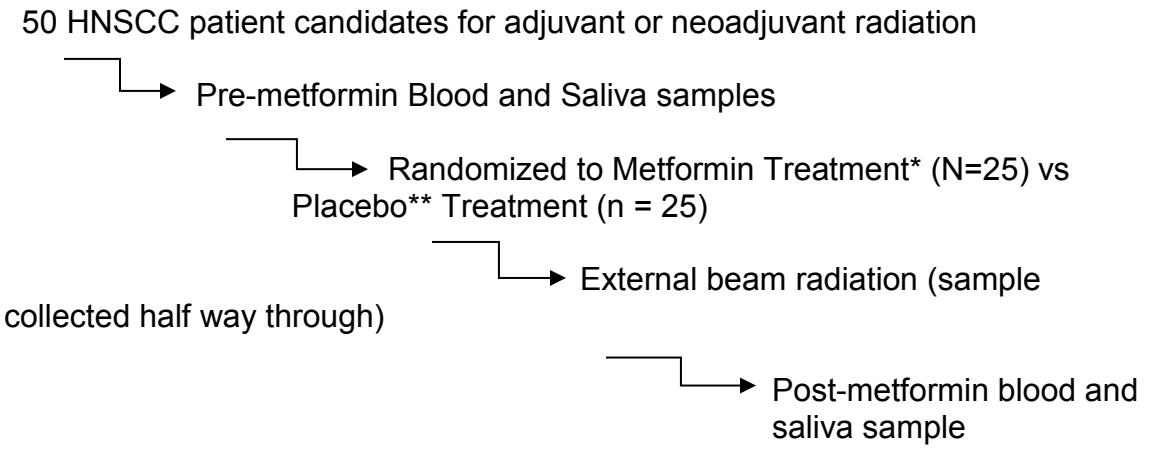
Planned accrual period: 24 months

Planned follow-up period: 12 months

Planned enrollment: Total of 50 patients

Schema of the trial design, procedures, and stages:

Patients with a head and neck region biopsy and diagnosis of HNSCC cancer who are planned to undergo definitive treatment by definitive radiation or up front surgery followed by adjuvant radiation. After informed consent is signed, patients will be administered metformin with dose escalation as tolerated* until time of radiation.



*Metformin (500 mg/day for 3 (+2 days) days followed by 500 mg twice daily for 3 days followed by 1000mg twice daily from day 6 (+2 days). Subjects will maintain 1000 mg twice a day dosing until two weeks post radiation.)
** Placebo (1 tab taken BID) No ramp up phase

Participant's Follow-up. The participant's medical records will be reviewed every 3 months for 12 months to assess: Mucositis, xerostomia, fatigue and dysphagia

4.0 SUBJECT SELECTION AND WITHDRAWAL

4.1 Inclusion Criteria

1. Diagnosis: Subjects with a diagnosis of head and neck cancer biopsy proven, and who are candidates for radiation therapy
2. Age: Subjects must be ≥ 18 years of age and ≤90 years old.
3. Informed Consent: All subjects must be able to comprehend and sign a written informed consent document.

4.2 Exclusion Criteria

1. Subjects who are pregnant or may become pregnant during metformin administration. Pregnancy testing will be done in conjunction with preradiation protocols.
2. Subjects on metformin for any reason during the preceding 4 weeks.
3. Diabetic subjects are eligible if they are not taking metformin or insulin

4. 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.
5. Patients with plasma creatinine level greater than 1.3 mg/dL.
6. Patients with plasma bicarbonate less than 22 mEq/L or history of lactic or any other metabolic acidosis.
7. Patients with history of congestive heart failure.
8. Patients with myocardial ischemia or peripheral muscle ischemia.
9. Patients with sepsis or severe infection.
10. Patients with history of lung disease currently requiring any supplemental oxygen treatment.
11. Patients scheduled for radiation less than 6 days from enrollment
12. Patients with history of hepatic dysfunction or hepatic disease and abnormal liver function tests. Patients who have a history of hepatic dysfunction or hepatic disease and normal liver function tests will be eligible to participate.
13. Patients with a current history (in the past 30 days) of heaving drinking which is defined in accordance with CDC definition as more than 8 drinks per week for women and more than 15 drinks per week for men. A standard drink contains .6 ounces of pure alcohol. Generally, this amount of pure alcohol is found in 12-ounces of beer, 8-ounces of malt liquor, 5-ounces of wine, 1.5-ounces or a “shot” of 80-proof distilled spirits or liquor (e.g., gin, rum, vodka, or whiskey). While on study, patients should limit their alcohol consumption to no more than 8 drinks per week for women and no more than 15 drinks per week for men. Patients who feel they cannot comply with this recommendation are not eligible

All medications are permitted except those that are contraindicated with metformin 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. The following is a list of medications identified as class C (monitor therapy) and class D (consider therapy modification) when treatment with metformin is considered:

Class C:	Class D:
Carbonic anhydrase inhibitors	Cimetidine
Cephalexin	Iodinated contrast agents
Corticosteroids (orally inhaled)	Somatropin
Corticosteroids (systemic)	
Dalfampridine	
Dofetilide	
Glycopyrrolate	
Lamotrigine	
Luteinizing hormone-releasing hormone analogs	

Pegvisomant
Trospium

4.3 Gender/Minority/Pediatric Inclusion for Research

This protocol will include women, minorities and pediatric cases (participants between 18 and 21 years). African Americans have a higher incidence of head and neck cancer and hence minority subjects are expected to be accrued at a higher rate than white subjects as compared to the population that Thomas Jefferson University Hospital serves. There is nearly a 3:1 predominance of males to females in this diagnosis. We will review accruals to this trial quarterly in the Head and Neck MDG and review the number of males and females accrued. If this deviates from the expected ratio we will review our actual patient ratio for that time and determine a remediation plan within the MDG to ensure accrual of women.

4.4 Subject Recruitment and Screening

50 subjects will be recruited and we expect to recruit subjects between the ages of 18 and 90 with multiple comorbidities. Subjects will be recruited from the investigator or sub-investigator clinical practices. No advertisement will be conducted. Screening requirements include serum measurement of creatinine, bicarbonate, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase.

4.5 Early Withdrawal of Subjects

4.5.1 When and How to Withdraw Subjects

Withdrawal criteria and procedures specifying when and how to withdraw subjects from the trial:

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 administration will occur and patients will be required to return remaining metformin 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 from the subject to record survival and progression for 2 years after enrollment. It is a high priority to try to obtain survival and progression free survival data on all subjects.

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

4.5.2 Data Collection and Follow-up for Withdrawn Subjects

Survival data will be collected for subjects that withdraw prematurely as well as progression of disease information. If a subject withdraws consent to participate in the study, permission will be sought from the subject to record survival and progression for 1 year after enrollment. It is a high priority to try to obtain survival and progression free survival data on all subjects lost to follow-up. Subjects will be called by phone at least on three occasions, phone calls at least twice will be carried out to the next-of-kin and certified letters will be sent twice. If follow-up is not obtained after the previously listed attempts have been carried out the subject will be considered lost to follow-up.

5.0 STUDY DRUG/THERAPY

5.1 Description

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.

5.2 Treatment Regimen

Metformin is the therapeutic agent in the protocol. The initial starting dose will be 500mg orally daily for 3 (+2 days) days which then will be increased to 500 mg twice daily and, if tolerated, further increased to 1000mg twice daily after day 6 (+ 2 days). Patients will maintain 1000 mg twice a day dosing until 2 weeks after completing their radiation. This dose schedule has been shown to be well tolerated**.**

The placebo will be dosed as 1 tab BID and will not have a dose escalation.

5.3 Risks

Summary of known and potential risks and benefits, if any, to subjects:

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 and in those age 80 years or older. 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[27]. Minor gastrointestinal upset is the most common toxicity, leading to cessation of therapy in less than 5% of individuals. Metformin does not cause hypoglycemia. The study is designed to assess as the primary end-point the effects of metformin on cytokines with short term administration and not to assess if metformin improves outcomes in head and neck cancer and hence it is unlikely to improve the clinical outcome of the subjects enrolled in this study.

Rules for dose modification: Toxicity monitoring, dose modification and treatment of complications:

Particular attention will be paid in the first three days of treatment. Patients will take 500mg/day for 3 days (+2 days). From day 4, 500mg twice daily and then in 3 days (+2 days) 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 of 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[31].

Metformin will not be started if CT scan with intravenous contrast is scheduled. 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[37].

Toxicity will be evaluated using the most recent version (version 3) of the NCI toxicity criteria, i.e. the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov.

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.

5.4 Method for Assigning Subjects to Treatment Groups

Randomization of 1:1 will be assigned at the time of accrual. A randomization schedule will be developed by the study statistician using the method of random permuted blocks. Randomization assignments will be loaded into REDCap and subjects will be randomized by accessing assignments through the REDCap randomization facility.

5.5 Preparation and Administration of Study Drug/Therapy

Metformin tablets will be provided by the Department of Otorhinolaryngology and will be coordinated through the Thomas Jefferson University Hospital Investigational Drug Service (IDS) Pharmacy. Drug will be stored in locked cabinet until given to the participants. An 8 week supply of metformin will be provided to subjects and the drug will be self-administered by the participants.

5.6 Subject Compliance Monitoring

There will be a pill bottle with the appropriate number of metformin tablets distributed to the patients upon enrollment into the trial. The pill bottle will be accompanied with detailed instructions on the proper dosage/number of tablets to take daily as noted above. Upon arrival for post-radiation follow-up, the bottle will be collected by our trial coordinator and the contents will be evaluated for compliance.

5.7 Prior and Concomitant Therapy

All medications are permitted except those that are contraindicated with metformin 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. See section 4.2 for more information.

5.8 Packaging

The study drug will be provided by the Department of Otorhinolaryngology and will be coordinated through the Thomas Jefferson University Hospital Investigational Drug Service (IDS) Pharmacy. The study drug will be packaged in 1 bottle containing 500mg tablets for a maximum of 8 weeks.

The bottle will be labeled “take one tablet daily for three days then one tablet twice daily for three days then two tablets twice daily until two weeks after radiation therapy ends”.

5.9 Blinding of Study Drug

The bottle will be non-descript and contain either metformin or placebo depending on randomization. Patients will be blinded to which pill (metformin or placebo) they will receive. Pill distribution will be completed by the pharmacist who will not be blinded. Principal Investigator and care providers making assessments will not be blinded.

5.11 Placebo

Placebo will be given as 500mg twice daily. Placebo will be in capsule form. It 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.

The placebo will be packaged in bottles containing 500mg capsules. The bottle will be labeled “take one tablet twice daily until two weeks after radiation therapy ends”.

5.12 Receiving, Storage, Dispensing and Return

5.12.1 Receipt of Drug Supplies

The study drug will be provided by the Department of Otorhinolaryngology and will be coordinated through the Thomas Jefferson University Hospital Service (IDS) Pharmacy. Upon receipt of the study treatment supplies, inventory must be performed and a drug receipt log will be 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 the study sponsor of any damaged or unusable study treatments that are supplied to the investigator’s site.

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.

5.12.2 Storage

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. 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.

5.12.3 Dispensing of Study Drug

The study drug will be dispensed to the participants in 1 bottle containing 500mg tablets for a maximum of 8 weeks. The bottle will be labeled “take one tablet daily for three days then one tablet twice daily for three days then two tablets twice daily until two weeks after radiation therapy ends” for metformin or bottle will be labeled “take one tablet twice daily until two weeks after radiation therapy ends”. The amount of pills dispensed to every participant will be logged in the accountability logs by the pharmacist. The participant will be instructed to bring the bottle and all the pills that were not taken the day of the visit two weeks after the completion of radiation. Regular study drug reconciliation will be performed only at the end of the study.

5.12.4 Return or Destruction of Study Drug

Regular study drug reconciliation will be performed at the end of the study. Drug that was assigned, drug consumed, and drug remaining will be logged in the drug accountability form and will be signed and dated.

At the completion of the study, there will be a final reconciliation of drug purchased, 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 destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6.0 STUDY PROCEDURES

A schedule of events can be found in Appendix A.

6.1 Study Visit Schedule

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.

Visit 1: Subjects that meet the inclusion criteria after reviewing the initial biopsy, laboratory data and clinical chart will be approached the day of their follow up after initial biopsy. The study coordinator will meet with the prospective candidates and go over the protocol, answer questions and obtain informed consent. If the subject is agreeable to the study, a physical exam will be performed, blood and saliva will be collected, and participant will be randomized.

Metformin/placebo 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. Participants will be instructed to call with any side effects or adverse events that occur in the interim of the follow up phone calls. The exact date to start the metformin will be determined this visit based on the planned start of radiation. Subject will be instructed to start taking the metformin/placebo at any point from 1 to 7 days from the start of radiation.

Quality of life measures including the EORTC QLQ – H&N35, mucositis score, Multidimensional fatigue inventory and the Xerostomia Questionnaire (XQ) will be administered on an IPad through the website (https://az1.qualtrics.com/jfe6/preview/SV_87y9IyJ0sFo8oJv*).* Patients will be identified only by an ID number.

Visit 2: Subjects will come in during the 3rd week of their radiation for blood draw, saliva collection, physical exam, quality of life questionnaires and tolerability assessment. An inventory of medication will also be taken to assess compliance. Location of radiation is not dictated by this protocol.

Visit 3: Two weeks after completion of radiation. Subjects will be approached for bottle and pill reconciliation as well as follow up of side effects and adverse events. Blood and saliva will be collected, and MD Anderson Dysphagia Inventory, Multidimensional fatigue inventory (MFI-20) and the Xerostomia Questionnaire (XQ) will be administered will be administered on an IPad through the website (https://az1.qualtrics.com/jfe6/preview/SV_87y9IyJ0sFo8oJv). Patients will be identified only by an ID number.

Follow-up: Every 3 months during the 1st year post treatment subjects will have their regularly schedule appointments at which we will draw blood and saliva and administer quality of life surveys. The participant's medical records will be reviewed every 3 months for 12 months.

6.2 Definition of Dose-Limiting Toxicities and early stopping rules:

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. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov.

Grade 4 toxicity (probably or definitely drug related): Treatment will be permanently discontinued.

6.3 Dose Delays and Dose Modifications

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. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov.

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 of toxicity development the treatment will be interrupted definitively.

Grade 3 toxicity (probably or definitely drug related): Treatment will be interrupted. Toxicity will be evaluated daily and if toxicity improves to grade 1 or lower dose will be reduced by 50%. If toxicity is not improved or resolved or reduced to grade 1, treatment will be interrupted definitively.

Grade 4 toxicity (probably or definitely drug related): Treatment will be permanently discontinued.

In case of grade 1 or 2 diarrhea (the most frequent side effect) a concomitant administration of loperamide will be provided.

6.4 Laboratory Procedures/Evaluations

6.4.1 Clinical Laboratory Evaluations

To assess changes in cytokines and exosomes, patients will have two 7ml purple top tubes drawn in the office as well as collection of 5ml of saliva. To assess the effects of metformin on serum and salivary exosome profiles, samples will be collected in person by office nursing staff at follow-up visits to the Department of Otolaryngology and transported directly to Dr. Rodeck's lab.

6.4.2 Special Assays or Procedures

Exosome profiles of serum and saliva samples will be isolated and analyzed in the laboratories of Drs. Rodeck and Harshyne using previously established protocols.

6.4.3 Specimen Preparation, Handling, and Storage

Blood and saliva will be collected in the Department of Otolaryngology, de-identified and analyzed in the labs of Dr. Rodeck and Harshyne.

7.0 STATISTICAL PLAN

7.1 Analysis for Primary Outcome

Mixed effects linear regression will be used to model longitudinal measurements of each cytokine. Measurement time will be treated as a categorical variable and fixed effects in the model will include randomization assignment, time, and randomization by time interaction. A linear contrast will be used to estimate the difference in randomization groups with respect to mean change from baseline to

post-treatment. Significance of this difference will be calculated for each cytokine and p-values will be adjusted using the Benjamini and Hochberg procedure to control the false discovery rate at 5%. Cytokine levels will likely need to be log-transformed prior to analysis to meet model assumptions.

Longitudinal measurements of exosomes will also be modeled using mixed effects linear regression in the same fashion. This analysis is treated as separate from the cytokine questions and the p-value will not be adjusted.

7.2 Analysis for Secondary Outcomes

Analysis of secondary outcomes will be primarily descriptive. The distribution of swallowing function and mucositis scores will be summarized by arm using means, medians, standard deviations, and ranges. The objective response rate will be estimated by arm along with an exact 95% binomial confidence interval.

7.3 Subject Population(s) for Analysis:

All randomized participants with any data will be included in the analysis.

7.4 Sample Size Justification

50 subjects will be randomized 1:1 to either metformin or placebo resulting in 25 subjects per group. The standardized difference in means between groups (effect size) that can be detected with 80% power was calculated for various assumptions of the number of cytokines with true differences above the detectable difference using the method of Jung (2005). With at least 5 cytokines out of 40 being different, we have 80% power of detecting a difference for each test if the true difference is at least 1.1 standard deviations.

Detectable difference (D) by number of cytokines with true difference>D assuming 80% power for each test.

Number of cytokines with true difference >D (K)	Detectable difference in means (D)	Single test alpha	Probability of detecting all K
5	1.1	0.0060	0.33
10	1.0	0.014	0.11
15	0.9	0.025	0.035
20	0.8	0.042	0.012
25	0.8	0.070	0.0038

For the exosome analysis, we have 80% power to detect an effect size of 0.81 (difference in means of 0.81 standard deviations) or greater using a two-sided test with alpha=0.05.

Ref: Jung, S.-H. 2005. Sample size for FDR-control in microarray data analysis. Bioinformatics: Vol. 21 no. 14, pp. 3097-3104. Oxford University Press.

8.0 SAFETY AND ADVERSE EVENTS

8.1 Definitions

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events are classified as serious or non-serious.

A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator will notify the sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor will also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be

reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period will be recorded.

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

8.3 Unblinding Procedures:

At the completion of the accrual period subjects will remain blinded until after the primary and secondary endpoints have been collected.

8.4 Stopping Rules:

Grade 4 toxicity (probably or definitely drug related): Treatment will be discontinued definitively.

Participants will be discontinued from study drug as per physician discretion

8.5 Data and Safety Monitoring Plan

The safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the compliance and implementation of the SKCC data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events by both the PI and the SKCC DSMC.

8.5.1 Medical Monitoring and AE/SAE Reporting

Every SKCC investigator initiated protocol includes requirements for reporting of adverse events based on CTC 4.0. All events are reported to the IRB and Medical Monitor using a password protected web-site. In addition all unexpected and serious adverse events (SAEs) are reported to the TJU IRB and to the Food and Drug Administration (FDA) if applicable. The investigator is required to submit all unexpected and serious adverse events to the TJU IRB and the Medical Monitor within the timeframes outlined in the below table. All AE/SAEs will be reported to the DSMC at the quarterly DSMC review meetings. ***Fatal adverse events related to treatment which are unexpected must be reported within 24 hours to the TJU IRB and the DSMC. Fatalities not related to the study drug/device must be reported within 5 days***

A summary of the reporting requirements for SKCC investigator-initiated studies are presented below.

	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 1 - 48 Hours (Death: 24 Hours) Phase 2 - 5 Working Days
Possible Probable 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 Hour (Death: 24 Hours)	Phase 1 - 48 Hours Phase 2 - 5 Working Days	48 Hours (Death: 24 Hours)	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase 1 and Phase 2 - 48 Hours (Death: 24 Hours)

NOTE: This table is based on the NCI AE/SAE reporting Guidelines and the TJU IRB Policy and Procedures. Please follow the individual protocol AE/SAE reporting guidelines if more stringent reporting procedures are specified

8.5.2 Data and Safety Monitoring Committee

Data and Safety Monitoring Committee (DSMC) is the Data and Safety Monitoring Board (DSMB) for the SKCC. The DSMC is a multidisciplinary committee charged with overseeing the monitoring of safety of participants in clinical trials, and the conduct, progress, validity, and integrity of the data for all clinical trials at the Thomas Jefferson University SKCC. The committee meets

quarterly to review the progress and safety of all active research protocols that are not monitored by another safety and data monitoring committee or board.

- The DSMC meets quarterly. Additional DSMC meetings are scheduled based on the nature and number of trials being monitored over a specified time period. The DSMC meets (by conference call) within 24 hours following the notification of an unexpected adverse event felt to be related to the study drug.
- Prior to each DSMC meeting, each board member, is provided a printout of all reported AEs and SAEs occurring during the reporting period for this clinical trial. The principal investigator provides a detailed and comprehensive narrative assessment of current adverse events to date, indicating their possible significance and whether these toxicities have affected the conduct of the trial. DSMC members are provided with the principal investigator's assessment, a written report summarizing adverse events, safety data, and activity data observed during the specified time period described in each protocol, as well as recommendations from the Medical Monitor. A review of outcome results (response, toxicity and adverse events) and factors external to the study (such as scientific or therapeutic developments) is discussed, and the Committee votes on the status of each study.
- A summary of the board's action is sent to each investigator, the PRC and TJU IRBs. The DSMC actions may include recommendations/requirements that will lead to improved patient safety and/or efficacy, significant benefits or risks that have developed, or other changes determined to be necessary. The DSMC may also take note of slow accrual or lack of scientific progress, and refer such issues to the PRC. The DSMC provides the investigator with the rationale for any decision made.

9.0 DATA HANDLING AND RECORD KEEPING

9.1 Confidentiality

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

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

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

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. The source documents for the trial will include: hospital records, clinical and office charts, laboratory notes, pharmacy dispensing records, pathology records, adverse events and SAE forms, phone interview logs.

9.3 Case Report Forms

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

9.4 Records Retention

All study essential documents will be retained by the investigator for 2 years after the completion of the study.

10.0 STUDY MONITORING, AUDITING, AND INSPECTING

10.1 Study Monitoring Plan

The investigator will allocate adequate time for monitoring activities. The Investigator will also ensure that compliance or quality assurance reviewers are given access to all 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.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The

investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

10.2.1 Independent External and Internal Audits

In addition to review by the DSMC, all studies initiated by SKCC investigators are audited by an independent auditor once they have achieved 10% of target accrual. However, a study can be audited at any time based on recommendations by the IRB, DSMC, PRC and/or the Director of Clinical Investigations, SKCC. Studies are re-audited once they have achieved 50% of target accrual. Special audits may be recommended by the IRB, DSMC or PRC based on prior findings, allegations of scientific misconduct and where significant irregularities are found through quality control procedures. Any irregularities identified as part of this process would result in a full audit of that study.

In addition to the audits at 10 and 50%, the CRO randomly audits at least 10 percent of all patients entered into therapeutic SKCC trials and other trials as necessary, on at least a bi-annual basis, to verify that there is a signed and dated patient consent form, the patient has met the eligibility criteria, and that SAEs are documented and reported to the TJU IRB.

All audit reports are submitted to the DSMC for review and action (when appropriate). A copy of this report and recommended DSMC action is sent to the PRC and TJU IRB. The committee regards the scientific review process as dynamic and constructive rather than punitive. The review process is designed to assist Principal Investigators in ensuring the safety of study subjects and the adequacy and accuracy of any data generated. The TJU IRB may, based on the DSMC and auditor's recommendation, suspend or terminate the trial.

11.0 ETHICAL CONSIDERATIONS

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

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator before commencement of this study.

All subjects for this study will be provided a consent form that is compliant with local and federal regulations, describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12.0 STUDY FINANCES

12.1 Funding Source

The study is funded by the Department of Otorhinolaryngology.

12.2 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.) will 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).

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Appendix A: Schedule of Events

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Study Procedures	Screening	Visit 1	Visit 2 (During 3rd week of RT)	End of Treatment (Visit 3)[E]	Follow-up[F]
Informed consent		X			
Inclusion/Exclusion Criteria	X	X			
Demographics, Medical history	X	X			
Physical examination, vitals, weight, blood pressure		X	X		
Randomization		X			
Quality of Life Questionnaires[D]		X	X	X	X[C]
Pathology Report histologic confirmation of disease if available	X				
Concomitant meds	X	X			
Toxicity and AE Assessment		X	X	X	X
Treatment/ Intervention					
Metformin[G]		X	X		
Placebo[H]		X	X		

Radiation Treatment		X	X		
Correlative Studies					
Blood sample collection[A]		X	X	X	X[C]
Saliva sample collection[B]		X	X	X	X[C]

A. A Two 7ml purple top tubes are to be drawn at each time point and transported directly to Dr. Rodeck's laboratory.

B. B 5ml of saliva is to be collected at each time point and transported directly to Dr. Rodeck's laboratory.

C. C Blood samples, saliva samples, and quality of life questionnaires will be collected every 3 months for the first year post treatment. Collection will occur at participant's regularly scheduled appointments.

D. D Quality of life surveys include: EORTC QLQ – H&N35, mucositis score, Multidimensional fatigue inventory and the Xerostomia Questionnaire (XQ). All questionnaires will be administered online via an IPad provided at the visit.

E. E Visit will be 2 weeks after the completion of radiation therapy.

F. F Patients will be seen at regularly scheduled appointments every 3 months for the 1st year post treatment. Participant's medical records will be reviewed every 3 months for 12 months

G. G The initial starting dose of metformin will be 500mg orally daily for 3 (+2 days) days which then will be increased to 500mg twice daily and, if tolerated, further increased to 1000mg twice daily after day 6 (+2 days). Patients will maintain 1000mg twice daily dosing until 2 weeks after completion of radiation. If patient must have CT scan with intravenous contrast, metformin will be held and participant will be instructed to start metformin the day following the scan.

H. H Placebo will be given as 500mg tablets twice daily with no dose escalation