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Pilot study of metformin to mitigate sequelae of radioactive iodine treatment for well-differentiated thyroid cancers.

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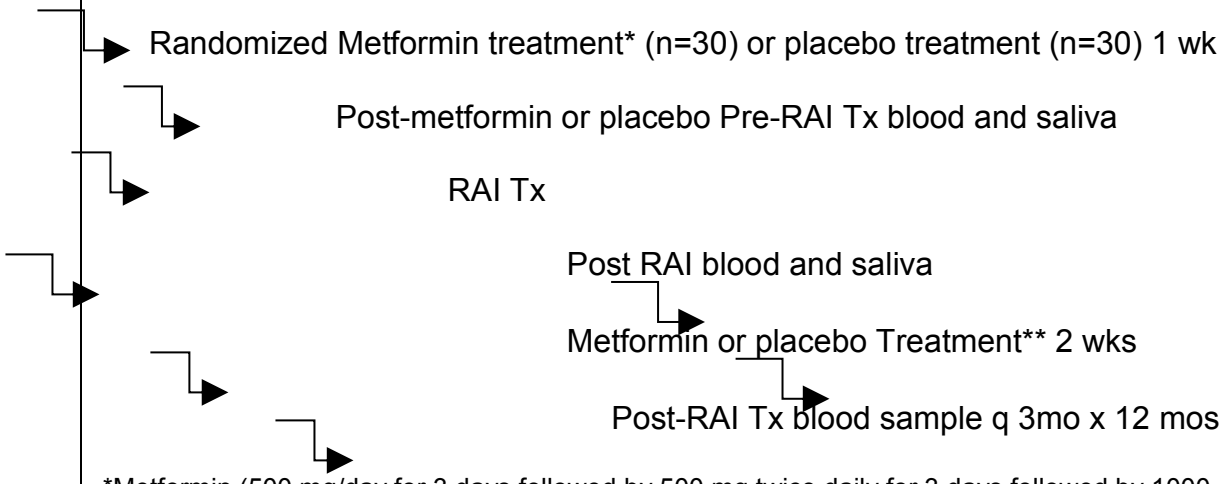
List of Abbreviations

AKT: also named protein kinase B
ALC: absolute lymphocyte count
AMPK: adenosine monophosphate protein kinase
ANC: absolute neutrophil count
ATP: adenosine triphosphate
CAFs: cancer associated fibroblasts
cAMP: cyclic adenosine monophosphate
CBC: complete blood count
HOMA: homeostatic model assessment
HNSCC: head and neck squamous cell carcinoma
IHC: immunohistochemistry
Ki67: Kiehl-67 antibody
LKB1: liver kinase B1
lncRNA: long noncoding RNA
miRNA: microRNA
mTOR: mammalian target of rapamycin
OXPHOS: oxidative phosphorylation
PDE3B: phosphodiesterase 3B
PET: positron emission tomography
PI3K: phoshoinositide 3 =kinase
PTC: papillary thyroid cancer
RAI: radioactive iodine
RBC: red blood-cell count
ROS: reactive oxygen species
WBC: white blood-cell count

Study Summary

Title	Pilot study of metformin in thyroid cancer patients undergoing radioactive iodine treatment.
Short Title	*Metformin in radioactive iodine treatment*
Protocol Number	JT#9411/IRB#16D.564
Phase	Pilot
Methodology/ Study Design	Preliminary Efficacy trial
Study Duration	60 months
Study Center(s)	Single Center - Jefferson
Objectives	To assess the effect of metformin on radioprotection in normal tissue for subjects undergoing radioactive iodine treatment. We will evaluate the ability of metformin to attenuate the myelosuppression and decrease xerostomia, xerophthalmia, and dysgeusia.
Number of Subjects	32
Diagnosis and Main Inclusion Criteria	Subjects with a biopsy proven diagnosis of differentiated thyroid cancer, and who have a scheduled appointment for definitive resection of the tumor followed by treatment with radioactive iodine at TJUH are eligible to participate.
Study Therapy, Dose, Route, Regimen	Metformin is the therapeutic agent in the protocol. The initial starting dose will be 500 mg 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. Subjects will maintain 1000 mg twice a day dosing or highest tolerated 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 500 mg orally daily for 3 days which then will be increased to 500 mg twice daily and, if tolerated, further increased to 1000 mg twice daily after day 6. Subjects will maintain 1000 mg twice a day dosing until two weeks post radioactive iodine treatment. The participant's medical records will be reviewed every three months, surveys administered, and CBC repeated over the course of one year according to the schedule outlined below
Reference therapy	None

Statistical Methodology	The primary objective of the study is to estimate the impact of metformin on complete blood count and serum exosome profile following radioactive iodine treatment. All patients from whom samples are obtained both pre- and post-treatment will be included in the primary analyses. Blood counts will be compared between the RAI/Metformin group (N=16) and the placebo control RAI group (N=16). The estimated mean difference and associated 95% confidence interval will be calculated. Analysis of secondary outcomes will be primarily descriptive and related to taste disturbance, xerostomia, and xerophthalmia.
Schema	32 patients with thyroid cancer who undergo primary surgical resection and subsequently require RAI treatment. Preoperative blood and saliva Thyroidectomy Recovery for approx 6-8 weeks Postoperative Pretreatment Metformin Blood and Saliva  *Metformin (500 mg/day for 3 days followed by 500 mg twice daily for 3 days followed by 1000 mg twice daily from day 6 until RAI tx) **Metformin (1000 mg twice daily for two weeks following RAI tx)

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

Primary:

- To estimate the impact of metformin on radiation-induced myelosuppression in patients treated with RAI after surgery for differentiated thyroid cancer.
- To estimate the effects of metformin on serum and salivary exosome profile in patients with well-differentiated thyroid cancer treated with total thyroidectomy and radioactive iodine.

Secondary:

- To assess safety and tolerability of metformin treatment in subjects with thyroid cancer.
- To assess the effect of metformin therapy on RAI-induced xerostomia, xerophthalmia, and dygeusia as measured by Xerostomia Questoinnaire (XC), the modified Vanderbilt Head and Neck Cancer Symposium Survey (version 2.0), University of Washington Quality of Life Questionnaire, and EORTCQLQ-H&N35

Correlatives:

- To assess the results of the radioactive iodine treatment in patients treated with metformin compared those not treated based on the outcomes of the radioactive iodine scan.

1.2 Background and Rationale

Differentiated thyroid cancer (DTC) is the most frequently diagnosed primary endocrine-related malignancy, accounting for 3.8% of all new cancer cases in US. The majority of cases are papillary and follicular and 10-year overall survival is excellent, exceeding 90%. (Clement 2015) Treatment for DTC consists of total or near-total thyroidectomy, followed by administration of radioactive iodine (I-131) for remnant ablation or residual disease. (Clement 2015)

I-131 has been used in therapy for well-differentiated thyroid cancer for over 50 years. The use of RAI is an essential adjuvant treatment strategy after thyroidectomy in patients with DTC. The 2009 ATA guidelines recommend I-131 ablation in high-risk patients and selected low-risk patients (1-4 cm thyroid cancer confined to thyroid and high-risk features predicting an intermediate to high-risk of recurrence or death from thyroid cancer) (Cooper 2009). The primary objectives of RAI treatment include eliminating residual normal and tumoral thyroid cells as the absence of normal residual

thyroid tissue improves the sensitivity of subsequent serum thyroglobulin levels as a tumor marker during follow-up for recurrence (Van Nostrand 2009). The eliminated normal residual thyroid tissue can no longer compete to take up a portion of administered RAI thus increasing sensitivity of detection of locoregional and/or metastatic disease on follow-up RAI whole body scans. Additionally, the absence of intense uptake in the thyroid bed that would otherwise obscure other foci of adjacent uptake allows post-treatment scans to reveal additional disease that was not previously identified.

RAI therapy is usually carried out with high doses of iodine-131, and, in cases of recurrent or progressive iodine-avid metastatic disease, is often repeated to get favorable response. RAI therapy is performed following either thyroid hormone withdrawal or preparation with recombinant human thyroid stimulating hormone (rhTSH) using whole-body and blood clearance studies. For successful remnant ablation in low-risk and high-risk patients, a minimum activity of 30-100 mCi/1.1-3.7GBq and 100-200mCi/3.7-74 GBq is recommended (Clement 2015, Cooper 2009)

RAI is generally well tolerated. Complications are rarely life-threatening but can negatively affect patients' quality of life. (Fard-Esfahani 2014) Side effects of I-131 occur in many areas and organs systems. They are most often categorized by time of occurrence after therapy. Early effects are those that begin immediately after therapy until around 10 days post-treatment. Intermediate effects occur during the remainder of the first year after therapy and late effects can be experienced any time during the long-term follow-up period. (Van Nostrand, 2009)

Within the head and neck, salivary gland dysfunction is the most frequently described complication after I-131 therapy. (Clement 2015) Usually salivary gland dysfunction occurs early and is transient, but it may also persist or appear late. Xerostomia, occuring in 2-55% of patients, is generally the result of radiation-induced sialoadenitis. (Van Nostrand) Obstruction of Stensen's and/or Wharton's duct due to inflammation also leads to pain and swelling and can ultimately result in ductal narrowing secondary to fibrosis. Ensuing hyposalivatoin can become chronic and leads to problems with oral hygiene, dental caries, and candidiasis.

Salivary glands concentrate iodide by a factor of 30-40 compared to blood and, as a result, the dose to salivary gland during first 12 hours after administration could be as high as 6Gy. (Fard-Esfahani 2014) Effect on salivary gland function is dose-related and more prominent in the parotid glands. Evaluation of volume in salivary glands using sonography shows significantly increased volume in the parotid glands after RAI dependent on received activity and time from irradiation. Occasionally radiation

sialoadenitis will result in facial nerve paralysis secondary to compression by a swollen parotid gland.

A review by Clement et al of 683 pts found that the prevalence of objective salivary gland dysfunction after I-131 ranged from 37-72%. In general, higher cumulative activities of I-131 are associated with increased risk of salivary gland dysfunction. Symptomatic salivary gland dysfunction correlated well with cumulative I-131 activity. (Clement 2015)

Treatment with I-131 can also result in disturbed nasolacrimal gland function and xerophthalmia resulting from inflammation of lacrimal gland. Recent studies have shown evidence of xerophthalmia in 16-33% of patients lasting for up to 2 years. (Van Nostrand 2009) A recent review reported that an abnormal Shirmer's test was found significantly more frequently in patients exposed to I-131 compared to unexposed patients.(Clement 2015) RAI uptake by nasolacrimal duct mucosa leads to subsequent inflammation, edema, and fibrosis and can be responsible for lacrimal duct obstruction and resulting chronic epiphora. (Fard-Esfahani 2014). Conjunctivitis has also been reported in 23% of patients. (Van Nostrand 2009)

Leukopenia, thrombocytopenia, and anemia are major risks of I-131 therapy. (Van Nostrand 2009) Of the known potential adverse events of I-131 therapies, bone marrow suppression has been considered the most important. (Bikas 2016) More than 20 publications have described bone marrow suppression secondary to I-131 therapy, though most are underpowered. Bone marrow suppression is mostly transient and results in a decreased in white blood cell and platelet count for up to 6-10 weeks. Total incidence is about 25% with leukocyte and platelet count nadirs 1-2 months after RAI administration (Fard-Esfahani 2014) Occasionally it results in increased susceptibility to infection or bleeding if the marrow dose exceeds about 200 cGy. (Fard-Esfahani 2014)

Of those patients receiving high-activity therapies, 38% have grade 1 bone marrow suppression as defined by the World Health Organization (WHO). Previous research has shown that even a single dose of RAI can cause changes in the peripheral CBC. (Padovani 2014) The degree to which an individual is affected is influenced by many factors including the patient's clearance rate, the total cumulative prescribed activity of I-131, the frequency of therapy and interval between treatments, the patient's bone marrow reserve and the degree to which bone metastases are present. Interpretation of the hematologic effects of RAI therapy is complicated by the fact that many patients who require high cumulative activities of RAI also receive external beam radiation therapy (EBRT) to treat metastatic lesions to the bone, which carries its own hematopoietic toxicity (Padovani 2014)

First reported in 1963 by Haynie et al, transient leukopenia, anemia, and thrombocytopenia and occasional persistent anemia were noted after repeated RAI doses administered at 3-month intervals with traditional thyroid hormone (TH)

withdrawal. (Padovani 2014, Haynie 1963) More recent studies confirm what appear to be transient effects on blood counts measured in the first few weeks after RAI therapy, which some studies have shown to be less evident with an rhTSH preparation as compared to traditional TH withdrawal. To minimize BM suppression, Benua et al suggested not exceeding 200 cGy. (Van Nostrand 2009)

Tumor Derived Exosomes

Exosomes are small extra-cellular vesicles produced by all cells and present in almost all biological fluids including blood, urine, ascites, CSF, serum and plasma, and in the culture medium of cell cultures. (Whiteside 2016, Silva 2015) 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 (Silva 2015).

Exosomes have recently emerged as important mediators in cell communication due to their enriched content in genetic material like DNA, mRNA, and non-coding RNAs such as microRNAs (miRNA). (Silva 2015) MicroRNAs are small (19-24bp) endogenous RNAs that regulate gene expression at the posttranscriptional level by targeting mRNA transcripts. (Silva 2015)

The biogenesis of exosomes, which involves the endosomal compartment, sets them apart from other extracellular vesicles (EV). (Whiteside 2016) Their molecular profile partly, but not completely, resembles that of the parental cell. Exosomes are composed of a lipid bilayer and at any given point can contain all known molecular constituents of a cell. (Kalluri 2016) Similarities in the molecular and genetic profile of the parent cell are usually present, and this has given rise to the concept of exosomes as putative tumor biomarkers. (Whiteside 2016)

The precise function of exosomes remains unknown. (Kalluri 2016) The leading theory is that exosomes are an integral part of a complex, well-organized, and evolutionarily conserved (present in bacteriea, plants, animals, man) form of information delivery that operates at short and long distances. (Whiteside 2016) They carry proteins, lipids, and nucleic acids in functionally active form and can regulate gene expression in recipient cells, thus determining their behavior. (Whiteside 2016) The discovery that their contents can be transferred to a recipient cell via fusion to mediate phenotypic alterations supports this notion that exosomes are dynamic mediators of intercellular communication (Kalluri 2016) Although some models of exosome uptake in target cells have been proposed, there is no consensus regarding the definition of the mechanisms. (Silva 2015)

The content and number of exosomes generated likely change depending on different stressors or stimuli cells experience. (Kalluri 2016) Exosome levels in plasma and other body fluids of patients with cancer are frequently elevated compared to

normal proliferating cells. (Whiteside 2016) Developing tumors become the main driver of cellular interactions and enhanced exosome biogenesis likely plays a particularly important role. (Whiteside 2016) Exosomes produced and secreted by tumor cells have been largely described as promotors of tumor progression. They manipulate the local and distant biological environment by converting normal physiological processes to those favoring tumor growth and dissemination in part by silencing antitumor responses, supporting new vessel growth, and promoting survival of tumor cells. (Silva 2015, Whiteside 2016) Exosome-mediated transfer of oncogenes and oncogenic signals from one tumor cell to another or from tumor cells to normal cells is well documented in the literature (Whiteside 2016) Intercellular transfer of molecules containing oncogenic mutations to normal or malignant recipient cells includes activated oncoproteins, their transcripts, oncogenic DNA sequences and oncogenic mRNAs, and leads to reprogramming of cellular pathways, especially those responsible for growth factor production (Whiteside 2016) The ability of oncosomes to carry and deliver oncogenic signals to target cells, sustain autocrine growth-promoting pathways in parent tumor cells, and modify functions of stromal cells in the TME supports the conclusion that tumor-derived exosomes may play a critical role in oncogenic transformation (Whiteside 2016) Tumor-derived exosomes have been shown to play a role in promoting angiogenesis. They are present in the circulation and have ready access to all parts of the body. (Whiteside 2016) Thus, tumor-derived exosomes have been dubbed “oncosomes” because of evidence for the presence in their cargo of oncogenes and factors promoting tumor growth (Whiteside 2016)

It has been suggested that stress, including hypoxia prevalent in the TME accounts for this copious exosome secretion by tumor cells (Whiteside 2016) Hypoxic conditions in the growing tumor induce malignant cells to increase production of exosomes (Whiteside 2016) In more advanced cancers, expanding tumor cells which have escaped immune surveillance produce exosomes which carry an immunosuppressive cargo and become active participants in the tumor escape from the host immune system (Whiteside 2016) One of the clinically undesirable effects of tumor-derived exosomes that merits attention is their ability to horizontally transfer drug resistance (Whiteside 2016) Recent studies indicate that this drug-resistance is, in part, attributable to packaged miRNAs transferred intercellularly transfer by exosomes from drug-resistant to sensitive cancer cells. (Whiteside 2016)

On the other hand exosomes can also program the immune system in order to evoke an anti-tumor response by the organism (Silva 2015) It has been shown that exosomes possess antitumor function and can act to restrain disease progression. (Kalluri 2016) The duality of roles makes clear that the network of interactions created by exosomes is complex and of utmost importance for a better understanding of the carcinogenic process (Silva 2015)

Exosomes and their contents have emerged as a potential source to detect cancer and provide information on potential regulatory drivers of tumor progression and

metastasis (Kalluri 2016) Cancer cells secrete millions of exosomes in order reprogram their surroundings to a tumor-promoting microenvironment. (Whiteside 2016) Tumor-derived exosomes represent only a fraction of total exosomes present in the plasma, however, and this fraction may change depending on tumor progression. (Whiteside 2016) Tumor-derived exosomes represent a substantial part of the plasma vesicular content, and their molecular and genetic profiles change in the course of disease or therapy (Whiteside 2016) As they are readily accessible in nearly all body fluids, it has been suggested that cancer exosomes may in fact serve as a “liquid biopsy” to aid in the diagnosis of malignancies, including prostate, pancreas, breast, and ovarian cancers, glioblastoma and melanoma. (Kalluri 2016) Exosomes may also serve to unravel new therapeutic targets and predict therapeutic responses. (Silva 2015)

Lipids and metabolites in cancer exosomes may offer insights into cancer detection and biology, however more precise knowledge is evolving with respect to the utility of proteins and nucleic acids. (Kalluri 2016) DNA in exosomes may provide information about cancer-specific mutations. (Kalluri 2016) Various RNAs including mRNA and noncoding RNAs such as microRNAs (miRNA) and long non-coding RNA (lncRNA) in exosomes are functional and can be transferred to recipient cells and translated into functional proteins thereby impacting the transcriptome of recipient cells. (Kalluri 2016) mRNAs carried by exosomes are known to be involved in critical cellular activities such as cell cycle regulation, chromosome segregation, proliferation, and migration. (Whiteside 2016) MicroRNAs are estimated to regulate the translation of nearly 60% of protein coding genes as they are involved in the regulation of many processes including differentiation, apoptosis, and development (Silva 2015) Deregulation of the expressions of certain miRNAs in the cell has been consistently observed during various pathologies including cancer. Studies report that transfer of exosomes associated with miRNAs to recipient cells occurs, which results in altered gene expression and functional effects. (Silva 2015)

Thus far, cancer-derived exosomes have been shown to induce changes in the morphology, protein expression, patterns of growth, and cytokine profile. Examination of stromal fibroblasts in the TME of various tumors suggests that they increase production of collagen and hyaluronic acid. (Whiteside 2016) Exosomes released by tumor cells also directly affect molecular and genetic programs in endothelial cells thus promoting the process of neovascularization. (Whiteside 2016) Within bone marrow, exosome-mediated alterations have been shown to interfere with the hematopoietic cell development, differentiation, and function. (Whiteside 2016) Peinado et al have demonstrated in mice that exosomes from metastatic melanoma cells can enhance tumorigenesis by recruiting bone marrow-derived cells to initiate a pre-metastatic niche. (Silva 2015) In ovarian cancers, studies of the levels cancer-specific exosomal miRNAs have indicated that malignant tumors could be distinguished from benign disease.

Exosomes have also been shown to participate in the formation of the pre-metastatic niche in an in vivo pancreas cancer model. (Silva 2015)

The microRNAs nearly universally present in exosomes have been recently described as good biomarkers easily accessible in circulation of cancer patients (Silva 2015) Enriched and specific miRNAs within exosomes may inform diagnosis and serve to monitor the progression of cancer (Kalluri 2016) The properties of tumor-derived exosomes place them in an ideal position to serve as promising surrogates of tumor-derived proteins and transcripts (Whiteside 2016) They represent an enriched source of carefully packaged biomarkers, proteins, lipids, and nucleic acids. Transcripts carried in exosomes from cancer patients' plasma could serve as biomarkers for non-invasive detection of malignant cells (Whiteside 2016) Measurements of mRNA expression levels in plasma exosomes may also be useful as surrogates of responses to immune therapies in patients with cancer (Whiteside 2016) Clinical efficacy of immune therapies may be influenced by tumor-derived exosomes (Whiteside 2016)

To date, the mechanisms tumor cells employ to regulate exosome secretion remain unknown, although emerging insights suggest that several distinct mechanisms may be involved and may depend on the cancer type and its aggressiveness. Accumulating data indicate that miRNA signatures for extracellular vesicles derived from plasma of patients with different cancers are distinct (Whiteside 2016) Multiple studies have shown that exosomes isolated from plasma of patients with different cancers, including glioblastoma, lung ca, and breast carcinoma, have distinct miRNA profiles (Whiteside 2016)

MicroRNA in the circulation has been demonstrated as potential biomarkers of recurrence in papillary thyroid cancer (PTC). (Lee 2015) Currently, measuring serum thyroglobulin (Tg) levels provides the simplest long-term surveillance method, however, this may not suitable for up to 25% of patients with PTC due to anti-Tg antibodies, non-total thyroidectomy, or lack of RAI ablation (Lee 2015) Circulating miRNAs are being investigated as an adjunct or alternative (Lee 2015) Previous studies Lee et al have found higher circulating levels miR-146b and miR-222, miRNAS related to thyroid cell proliferation, in patients with PTC when compared with normal glands. Subsequently, in vitro studies by Lee et al have confirmed the presence of miR-146b and miR-222 in exosomes released by both normal and PTC cells. (Lee 2015)

1.2.1 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 (1). Metformin specifically inhibits the complex I (NADH:ubiquinone oxidoreductase) of the mitochondrial electron transport chain decreasing cellular respiration and the rate of ATP formation (2) (3) (4). This triggers the

activation of the energy sensor AMP-activated protein kinase (AMPK) that regulates cell metabolism and shifts it towards an energy-sparing state (5). This leads to reduced hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity thereby increasing peripheral glucose uptake and utilization without causing hypoglycemia. It is an FDA approved medication for the management of type 2 diabetes mellitus with extensive safety data. In general, clinically significant responses are not seen at doses <1500 mg daily; however, a lower recommended starting dose and gradual increased dosage is recommended to minimize gastrointestinal symptoms. Extensive preclinical data now also support the effectiveness of metformin as an antineoplastic and radioprotective agent (6).

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 (7,8). Evans et al (9) 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). The protective effect increased with greater metformin exposure (measured as total dose prescribed or total duration of use).

Current evidence from epidemiologic studies suggests that metformin has clinical activity in breast cancer. Hadad et al (11) demonstrated biomarker evidence for anti-proliferative effects of metformin in women with breast cancer by decreasing Ki67 and messenger RNA expression for PDE3B (critical regulator of cAMP levels that affect activation of AMPK). Similarly, Niraula et al (12) showed short-term preoperative metformin with a dosing schedule of 500mg three times daily was well tolerated and resulted in clinical and cellular changes consistent with beneficial anti-cancer effects with increased insulin sensitivity by HOMA in subjects and decreased proliferation and increased apoptosis in carcinoma cells. 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 (13, 14, 15).

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 (Miller 2014) 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. (Bikas 2016)

Mechanism of Action for Metformin:

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 (DOI: 10.1111/jdi.12328). 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[14]. AMPK activation potentially promotes survival after radiation, especially in a low nutrient environment found in human tumors. (Muaddi 2013) By inhibiting the formation of ROS, metformin may further protect tissues or cells against DNA damage and mutations. (Xu 2015)

Preclinical studies and clinical trials support the view that metformin has anticancer properties (17) (18) (19) (20) 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 (21) (22) (23) (24). Several groups have shown that metformin's ability to limit tumor growth *in vivo* is dependent on mitochondrial complex I (25). Complex I inhibition blocks mitochondrial-dependent production of reactive oxygen species (ROS) and adenosine triphosphase (ATP) (26-28). Catabolite access may determine 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 regulates 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 not tumor cells.

1.2.2 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[17]. Also, there is high MCT4 expression in HNSCC cancer associated fibroblasts (CAFs) and in carcinoma cells with low proliferation rates[17]. 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[17].

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 (Bikas 2016).

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[18, 19]. Other studies have also shown a reduction in the frequency of cancer with metformin use [20]. Evans et al [21] 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).

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[22, 24, 25]. 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 [26]

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 Vanderbilt Head and Neck Cancer Symptom Survey, University of Washington Quality of Life Questionnaire, EORTCQLQ-H&N35, and the Xerostomia Questionnaire (XQ) all of which have been validated and used in multiple clinical studies to-date.

1.3 Study Therapy

Description of the experimental product:

Metformin (N, N-dimethylbiguanide) is a biguanide that is best known for its use as first line therapy for type II diabetes patients (1). Metformin specifically inhibits the complex I (NADH:ubiquinone oxidoreductase) of the mitochondrial electron transport chain decreasing cellular respiration and the rate of ATP formation (2) (3) (4). This triggers the activation of the energy sensor AMP-activated protein kinase (AMPK) that regulates cell metabolism and shifts it towards an energy-sparing state (5). This leads to reduced hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity thereby increasing peripheral glucose uptake and utilization without causing hypoglycemia. It is an FDA approved medication for the management of type 2 diabetes mellitus with extensive safety data. In general, clinically significant responses are not seen at doses <1500 mg daily; however, a lower recommended starting dose and gradual increased dosage is recommended to minimize gastrointestinal symptoms. Extensive preclinical data now also support the effectiveness of metformin as an antineoplastic and radioprotective agent (6).

1.4 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 RAI, allowing for radioprotection against myelosuppression, xerostomia, and other sequelae of radiatioactive iodine treatment. More broadly, this will allow us to gain new information on metformin's effect on mitigation of side effects of radiation, tolerability, and safety profile in a population of subjects with various cancers.

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 week prior to planned RAI 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, and, if tolerated, further increased to 1000mg twice daily after day 6. Subjects will maintain 1000 mg twice a day dosing until their scheduled radioactive iodine treatment. Following radioactive iodine treatment, subjects will resume oral intake of metformin for two weeks at 1000 mg twice a day dosing

There are currently multiple ongoing studies using doses from 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 3 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 35 days.

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 500 mg/day for 3 days. From day 4, 500 mg twice daily and then in 3 days dose escalation to 1000 mg 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[19].

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 stopped the day before IV dye administration, as is the standard practice among patients taking metformin. However, IV contrast dye generally precludes treatment with RAI as the iodinated contrast competes with RAI and thus limits its effect. Therefore, it is extremely unlikely that any patient will be receiving iodinated contrast at the time of the study. Furthermore, 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[30, 31]. Metformin will be resumed the day after RAI treatment
completion 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 cancer but this study is designed to evaluate whether the sequelae of treatment with RAI are mitigated by use of metformin. Hence, the potential direct benefits to subjects in this study include the possibility of decreased myelosuppression, xerostomia, 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 RAI 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 also 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 diarrhea, 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 the Risk Management Procedures section.

Potential benefits:

Metformin may provide a radioprotective effect and mitigate known sequelae of RAI. Furthermore, metformin may alter tumor metabolism and improve outcomes in various cancers. This study is designed to evaluate whether the sequelae of RAI 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. The study may have societal benefits by allowing us to learn more about the properties of metformin which could help future cancer patients.

2.0 STUDY OBJECTIVES

1. To estimate the capacity of metformin to mitigate radioactive iodine therapy-induced myelosuppression as assessed by CBC
2. To assess the capacity of metformin to mitigate known side effects of radioactive iodine therapy including xerostomia, xerophthalmia, and dysgeusia as assessed by standardized questionnaire
3. To analyze changes in serum and salivary exosome profiles following a trial of metformin administration in patients receiving radioactive iodine treatment

2.1 Primary Objective:

We have chosen complete blood count (CBC) as our primary endpoint and serum and salivary exosome profiles as secondary endpoints. To evaluate if treatment with metformin inhibits RAI-induced myelosuppression and induces faster recovery of white blood cell count to baseline values, the blood counts will be compared in the pre- and post-treatment samples. Blood parameters will include hemoglobin, red blood-cell count, white blood-cell count, absolute neutrophil count, absolute lymphocyte count, and

platelet count. Blood and salivary samples will be drawn before surgery, before and immediately after treatment with metformin and RAI, and every three months thereafter for one year.

All blood samples will be taken by a phlebotomist or trained personnel. A baseline blood sample, consisting of two tubes of 1 cc of whole blood each, will be drawn before thyroidectomy as part of standard procedure at patients' pre-operative testing appointment. Patients will recover for a period of 6-8 weeks and identical samples will be drawn within 72 hours of starting metformin. Then samples will be drawn within three days before and within three days after RAI treatment, at the conclusion of metformin treatment, and every three months thereafter for one year. Blood parameters will include hemoglobin, red blood-cell count (RBC), white blood-cell count (WBC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count. Assays will also be conducted on samples to assess metformin-induced changes in serum and salivary cytokines and growth factors, erythrocyte sedimentation rate (ESR), exosome evaluation, and metabolomics profile. 5 cc samples of patient saliva will be collected in specimen cups.

2.2 Secondary Objective:

- Assess safety and tolerability of metformin treatment in subjects undergoing RAI treatment for differentiated thyroid cancer
- To estimate the effect of metformin treatment on symptoms of xerostomia, xeropthalmia and dysgeusia as assessed by the Xerostomia Questionnaire (XQ), the modified Vanderbilt Head and Neck Cancer Symposium Survey (version 2.0), University of Washington Quality of Life Questionnaire, and EORTCQLQ-H&N35

3.0 STUDY DESIGN

3.1 General Design

Type of trial: Pilot Efficacy trial

Planned accrual period: 48 months

Planned follow-up period: 12 months

Planned enrollment: Total of 32 patients

Schema of the trial design, procedures, and stages:

Patients with a diagnosis of well-differentiated thyroid cancer who have undergone tumor resection and have indications for RAI treatment that meet our inclusion and exclusion criteria will be offered participation in this clinical trial. Patients who present to TJUH with a known diagnosis of differentiated thyroid cancer who plan to undergo surgical resection followed by radioactive iodine will be offered enrollment in the trial at the time of surgical scheduling. Patients who undergo lobectomy for atypia of undetermined significance/follicular lesion of undetermined significance or follicular neoplasm/suspicious for follicular neoplasm who are subsequently found to a malignancy will be offered enrollment at their first follow-up visit. After informed consent is signed and study subjects are randomized 1:1 to take metformin or placebo, patients will be administered metformin with dose escalation as tolerated until time of RAI treatment. There is no dose escalation for patients randomized to the placebo arm. Pre-operative and pre-RAI treatment CBC with differential will be compared to post-RAI treatment lab values. Standardized surveys assessing symptoms of xerostomia, sialadenitis, xerophthalmia and dysguesia will be completed.

Participants' Follow-up: Participants' will have blood and saliva samples drawn post-op before metformin administration, before and immediately following RAI treatment, after metformin, and then every three months for 12 months. They will complete surveys at each post-op visit, and their medical records will be reviewed every 3 months for 12 months to assess for symptoms of myelosuppression, xerostomia, xerophthalmia, and dysgeusia. This will allow us to assess the radioprotective effects of metformin post-RAI treatment.

Purpose of the study: To estimate the radioprotective effect of metformin in subjects with differentiated thyroid cancer by evaluating both the ability of metformin to mitigate known side effects of radioactive iodine therapy as well as alter the exosome profile in the serum.

3.2 Primary Study Endpoints

- complete blood count
- serum and salivary exosome profile

3.3 Secondary Study Endpoints

- Measures of
  a) xerostomia
  b) xerophthalmia
  c) dysgeusia

Correlative

- To assess the results of the radioactive iodine treatment in patients treated with metformin compared those not treated based on the outcomes of the radioactive iodine scan.

3.4 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.

4.0 SUBJECT SELECTION AND WITHDRAWAL

4.1 Inclusion Criteria

1. Diagnosis: Subjects with a diagnosis of differentiated thyroid cancer who have undergone total or near-total thyroidectomy and are candidates for I-131 treatment at TJUH are eligible to participate.
2. Age: Subjects must be ≥ 18 years of age and ≤ 90 years old.
3. Disease Status: Subjects must be diagnosed with differentiated thyroid cancer
4. Prior Therapy: Patients must have previously undergone or plan to undergo thyroidectomy. For those patients who have previously undergone surgery, pre-operative labs, including complete blood cell count with differential must be available.
5. Patients who have a negative urine pregnancy test prior to enrollment. This should be done as part of pre-admission testing prior to surgery (within 14 days of study enrollment).
6. 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 in accordance with radioactive iodine treatment guidelines.
2. Subjects on metformin for any reason during the preceding 4 weeks.
3. Diabetic subjects are eligible if they are not taking metformin, insulin or sulfonylureas.
4. Subjects who have received iodinated contrast dye. Metformin treatment can be started the day after subjects complete iodinated contrast treatment. If a CT scan with contrast is scheduled after screening and consent, the metformin treatment should be stopped the day before iodinated contrast administration. Metformin can be resumed on the day after last iodinated contrast was administered to the subject.
5. Patients with history of hepatic dysfunction or hepatic disease and abnormal liver function tests (previously documented alanine aminotransferase greater than 40 IU/dL and/or plasma aspartate aminotransferase greater than 45 IU/d). Patients who have a history of hepatic dysfunction or hepatic disease but whose most recent liver function tests have been documented as normal will be eligible to participate.
6. Patients with plasma creatinine level greater than 1.3 mg/dL.
7. Patients with plasma alkaline phosphatase greater than 190 IU/dL.
8. Patients with plasma bicarbonate less than 22 mEq/L or history of lactic or any other metabolic acidosis.
9. Patients with history of congestive heart failure.
10. Patients with myocardial ischemia or peripheral muscle ischemia.
11. Patients with sepsis or severe infection.
12. Patients with history of lung disease currently requiring any pharmacologic or supplemental oxygen treatment.
13. Patients with a current history (in the past 30 days) of heavy drinking which is defined in accordance with CDC definition as more than 8 drinks per week for women and more than 15 drinks per week for men. A standard drink contains .6 ounces of pure alcohol. Generally, this amount of pure alcohol is found in 12-ounces of beer, 8-ounces of malt liquor, 5-ounces of wine, 1.5-ounces or a “shot” of 80-proof distilled spirits or liquor (e.g., gin, rum, vodka, or whiskey). While on study, patients should limit their alcohol consumption to no more than 8 drinks per week for women and no more than 15 drinks per week for men. Patients who feel they cannot comply with this recommendation are not eligible.
14. Patients with a systemic disease that could affect their bone marrow or peripheral blood cells (e.g. systemic lupus erythematosus, human immunodeficiency virus infection, rheumatoid arthritis)
15. Patients who have received or will receive medication that could affect their hematologic state (tyrosine kinase inhibitors, cytotoxic chemotherapy)
16. Patients who are Non-English speaking

*Note: This is due to the nature of the study and the process for full translations of the study documents.

All medications other than those that may cause myelosuppression 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 (Patients taking class D medications who are unwilling/unable to switch to alternative therapy will be excluded):

Class C	Class D
ACE Inhibitors, Alpha-lipoic acid, Androgens, BuPROPrion, Carbonic Anhydrase Inhibitors, Cephalexin, Dalfampridine, Dofetilide, Glycopyrrolate (Systemic), Hyperglycemia-Associated Agents, Hypoglycemia-Associated Agents, LamoTRIgine, MAO Inhibitors, Ondansetron, Pegvisomant, Quinolone Antibiotics, Salicylates, Selective Serotonin Reuptake Inhibitors, Thiazide and Thiazide-Like Diuretics, Topiramate, Trimethoprim, Trospium, Vandetanib, Verapamil	Cimetidine, Dolutegravir, Iodinated Contrast Agents, Ranolazine

4.3 Gender/Minority/Pediatric Inclusion for Research

This protocol will include women, minorities and pediatric cases (participants between 18 and 21 years). The incidence of thyroid cancer among women in the United States is 20.3 per 100,000 compared to 6.8 per 100,000 among men making it almost three times as common among women. (Ryerson 2016) Thus, when enrolling patients, by the nature of this disease the study will include significantly more women than men. Differentiated thyroid cancer occurs less frequently in pediatric than in adult subjects. All women and pediatric patients between 18 and 21 who meet the inclusion criteria will be approached for inclusion in the trial.

Inclusion of women plan: we will actively monitor the enrollment of women in the study continuously throughout the enrollment phase. We will ensure that women are actively

recruited and that their enrollment in the study during that time frame reflects the 3:1 incidence ratio of the disease.

4.4 Subject Recruitment and Screening

32 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, and in patients with known hepatic dysfunction, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase should be measured.

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 to use data pertaining to the subject in the analysis as far as they participate and they will be removed from subsequent analyses. We will also attempt to obtain consent from the subject to record survival and progression for one year 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 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. Consent will also be sought from the subject to record survival and progression for one 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 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 2 weeks after completing their radiation. This dose schedule has been shown to be well tolerated**.**

See Section 5.11 for information on the placebo.

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 possible societal benefits are large since this will allow us to learn about the pharmacodynamic effects of metformin in RAI, allowing for radioprotection against myelosuppression, xerostomia, and other sequelae of radiatioactive iodine treatment, as well metformin's tolerability and safety profile in a cancer population. The study is designed to assess as the primary end-point the effects of metformin on radiation-induced bone-marrow suppression with short term administration and not to assess if metformin improves outcomes in 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 500 mg/day for 3 days. From day 4, 500 mg twice daily and then in 3 days dose escalation to 1000 mg twice daily will be achieved. This will be continued for two weeks following RAI treatment. 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[19].

Metformin will be started the following day if CAT scan with intravenous contrast is administered to decrease risk of lactic acidosis. This further mitigates this risk. 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[31]. CAT scan is not used routinely in patients with thyroid cancer as it is desirable to avoid iodinated contrast.

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 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. At the outpatient offices of the Department of Otolaryngology- Head and Neck Surgery, subjects will be randomly assigned to receive either a metformin or placebo. 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 the study coordinator using 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. A three week supply of metformin and placebo 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 or placebo 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. At the first visit following completion of metformin administration (approximately 2 weeks after RAI), the bottle will be collected by the 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.

5.8 Packaging

Metformin 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 3 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 the day before the RAI treatment. Resume two tablets twice daily the day after RAI treatment until the end of the bottle”.

5.9 Blinding of Study Drug

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

5.10 Receiving, Storage, Dispensing and Return

5.10.1 Receipt of Drug SuppliesThe 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. Upon receipt of the study treatment supplies, an 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 study sponsor of any damaged or unusable study treatments that were 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.

5.10.2 StorageDrug 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 package insert. Clinical supplies may not be used for any purpose other than that stated in the protocol.

5.10.3 Dispensing of Study DrugThe study drug will be dispensed to the participants in 1 bottle containing approximately 69 500mg tablets for a maximum of 3 weeks. The bottle will be labeled "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 the day before the RAI treatment. Resume two tablets twice daily the day after RAI treatment completion until the end of the bottle." 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 his surgery. Regular study drug reconciliation will be performed only at the end of the study.

5.10.4 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.

5.11 Placebo

Placebo will be given as 500mg capsules 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 approximately forty-two (42) 500mg capsules. The bottle will be labeled “take one capsule twice daily until the day before the RAI treatment. The day after RIA treatment completion, resume one capsule twice daily until the end of bottle.”.

6.0 STUDY PROCEDURES

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 for initial biopsy. Women of childbearing potential (WOCBP) should have a pregnancy test done as part of pre-admission testing prior to surgery (within 14 days of study enrollment). If a test was not done, it will be done at time of consent.
Visit 1: Subjects who meet 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, subject will be randomized and metformin or placebo will be dispensed. Instructions on how to take study drug will be given and times for follow up phone calls for tolerability and safety will be arranged with participants. 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 date of radioactive iodine (RAI) administration. One week prior to the radioactive iodine treatment, the first dose of metformin can start. Metformin or placebo will be stopped the day before RAI treatment is received.

Per standard of care, a pregnancy test will be done within 72 hours of starting radioactive iodine administration.

Quality of life measures will be assessed prior to start of metformin.

Quality of life measures will be administered on an IPad through a website (https://az1.qualtrics.com/jfe1/preview/SV_57qgtgkjaexOy9f). Participants will be

identified only by study ID number. Paper questionnaires will be provided if the forms cannot be completed electronically.

Metformin or placebo administration will resume the day after the last treatment with radioactive iodine and will be taken until the end of bottle (2 weeks after RAI treatment).

Visit 2: At the first visit following completion of metformin administration for two weeks after RAI, patients will be approached for bottle and pill reconciliation, review of compliance with appropriate lab draws, as well as follow up of side effects and adverse events.

Follow-up: A follow up phone call will be performed 2 weeks after the last dose of metformin for any other side effects or adverse events. Every 3 months during the first year post-treatment subjects will have their regularly scheduled appointments at which we will draw blood/saliva, administer quality of life surveys, and review medical records.

Quality of life measures will be administered on an IPad through a website (https://az1.qualtrics.com/jfe1/preview/SV_57qgtgkjaexOy9f). Participants will be identified only by study ID number. Paper questionnaires will be provided if the forms cannot be completed electronically.

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
Please see Section 5.3.

6.4 Laboratory Procedures/Evaluations

6.4.1 Clinical Laboratory Evaluations

To assess changes in CBC, patients will be provided prescriptions for lab draws to be performed at previously specified time intervals and instructed to have their results forwarded to the Department of Otolaryngology. 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 Sample Size Determination

The sample size was chosen to have adequate precision for estimating the effect size comparing groups with respect to change from pre-resection to post-resection. From data in Bikas et al, we estimate that the standardized effect size for comparing change from pre-resection to post-resection between groups will be at least 0.78 standard deviations. For a 95% confidence interval to have half-width of 0.78 SDs, 14 patients per group are required. Allowing for 10% attrition, we will recruit and randomize 32 patients (16 per group).

7.2 Statistical Methods

The primary objective of the study is to estimate the impact of metformin on change in complete blood count. Longitudinal measures of CBC will be modeled using mixed effects linear regression. Outcomes will be transformed as necessary (e.g., log transformed) prior to analysis. Fixed effects will include time (Pre-resection, Post-resection, 3 months post-resection, 6 months post-resection, 12 months post-resection), treatment (metformin vs. control) and treatment by time interaction. We will use a linear contrast to test the null hypothesis that change from pre-resection to post-resection is the same in the control and metformin groups. Secondary analyses will compare groups with respect to change from pre-resection to each of the follow-up times. Components of complete blood count will be analyzed with similar models.

Other longitudinally measured continuous outcomes will be modeled using mixed effects linear regression and longitudinally measured binomial and ordinal outcomes will be modeled using generalized linear mixed effects regression.

7.3 Subject Population(s) for Analysis:

All randomized participants with any data will be included in the analysis as randomized. Missing outcome data will not be imputed.

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 15 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. Once study is complete, PI will unblind patients to their randomization upon request.

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

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)

8.5.2 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

Appendix A: Schedule of Events

Study Procedures	Screening	Visit 1	Visit 2 (End of Treatment)[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[C]
Pathology Report histologic confirmation of disease if available	X			
Concomitant meds	X	X		
Toxicity and AE Assessment		X	X	X[I]
Pregnancy test[J]	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[C]
Saliva sample collection[B]		X	X	X[C]

A. Two 7ml purple top tubes are to be drawn at each time point and transported directly to Dr. Rodeck's laboratory.
B. 5ml of saliva is to be collected at each time point and transported directly to Dr. Rodeck's laboratory.
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. Quality of life surveys include: EORTC QLQ – H&N35, modified Vanderbilt Head and Neck Cancer Symposium Survey, University of Washington Quality of Life Questionnaire, and Xerostomia Questionnaire (XQ). All questionnaires will be administered online via an IPad provided at the visit.
E. Visit will occur following completion of metformin treatment for 2 weeks after RAI
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 24 months
G. The initial starting dose of metformin will be 500mg orally daily for 3 days which then will be increased to 500mg twice daily and, if tolerated, further increased to 1000mg twice daily after day 6. 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. Placebo will be given as 500mg tablets twice daily with no dose escalation.
I. Assessment for AEs will occur as a phone call performed 2 weeks after the last dose of metformin.
J. Pregnancy test to confirm eligibility should be done as part of pre-admission testing prior to surgery (within 14 days of enrollment) or done at time of consent. Pregnancy testing is done per standard of acre 72 hours prior to start of radioactive iodine administration.