

COVER PAGE

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M4OC-Prevent: Metformin for Oral Cancer Prevention

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SCHEMA

M4OC-Prevent: Metformin for Oral Cancer Prevention

Males and females \geq 18 years of age with oral leukoplakia or erythroplakia



Pre-study/Baseline Evaluation

Informed consent, medical history, concomitant medications, brief physical exam;
Baseline symptoms, performance status, urine pregnancy test, if applicable;
Examination of the oral cavity with lesion measurement and photography;
Biopsy lesion (or retrieve archival diagnostic tissue sample obtained within prior 6 weeks) and normal cheek mucosa;
Saliva collection (for research markers);
Fasting blood collection (for clinical labs and research markers);
Eligibility Evaluation



Intervention

Daily metformin for 12-14 weeks
(500 mg metformin ER per day for the 1st week, 1,000 mg per day for the 2nd week, then 2,000 mg per day for the remaining of the intervention)



Agent Dispensation Visit (clinic or virtual visit)

Initial supply of study agent;
Urine pregnancy test, if applicable and not done within the past 4 weeks



Interim Phone/email Contact

Prior to the scheduled agent start date and scheduled dosage increase dates to assess suitability for dose escalation;
Following each dose escalation and at Week 9 to assess compliance and for AE evaluation



Interim Visit

(Week 6)

Compliance and AE evaluation, conmeds, weight, vital signs, urine pregnancy test (if applicable);
Brief physical exam, assessment of changes in medical history;
Exam of oral cavity with lesion measurement and photography;
Return unused agent and receive a new agent supply



Post-Intervention Evaluation

(Week 12-14)

Compliance and AE evaluation, conmeds, weight, vital signs, urine pregnancy test (if applicable);
Brief physical exam, assessment of changes in medical history;
Exam of oral cavity with lesion measurement and photography;
Biopsy lesion and normal cheek mucosa;
Saliva collection (for research markers);
Fasting blood collection (for clinical labs and research markers);
Return unused agent



Follow-up

(2-4 weeks post-intervention)

AE review

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1. OBJECTIVES

The study is a phase IIa trial in individuals with oral leukoplakia or erythroplakia to explore repurposing metformin for oral cancer prevention.

1.1 Primary Objective

To determine the clinical response of oral premalignant lesions to 12-14 weeks of metformin intervention.

1.2 Secondary Objectives, in order of priority:

1.2.1 Histologic response to metformin intervention in the target lesion

1.2.2 Tissue-based biomarkers

1.2.2.1 Metformin effect on cell proliferation and its molecular targets in the target lesion and in the normal tissue

1.2.2.1.1 Marker of cell proliferation, Ki67

1.2.2.1.2 Molecular targets of metformin, including, in order of priority, pS6, pAKT^{S473}, p4EBP, pACC

1.2.2.2 Expression of dysregulated molecular mechanisms and organic cation transporter 3 (OCT 3) in the target lesion and in the normal tissue, including, in order of priority, EGFR, pEGFR, p53, PTEN, pERK, p16, and OCT3

1.2.2.3 Targeted analysis of cancer-associated genes in the target lesion and blood DNA

1.2.3 Serum and saliva based biomarkers

1.2.3.1 Metformin effect on serum metabolic markers (C-peptide, glucose and HbA1c)

1.2.3.2 Metformin concentrations in serum and saliva

1.2.3.3 Metformin effect on serum and saliva inflammatory and angiogenic cytokines, including interleukin (IL)-6, IL-8, growth-related oncogene-1 (GRO-1), and vascular endothelial growth factor (VEGF)

1.3 Exploratory Objectives

1.3.1 To characterize changes in the saliva microbiome before and after metformin intervention, including both the absolute microbial load and taxonomic composition

1.3.2 To evaluate the potential microbiome signatures that are correlated with treatment response

2. BACKGROUND

2.1 Study Disease

Clinical burden of HNSCC: Every year more than 500,000 cases of head and neck cancer, largely including cancers of the mucosal lining of the oral cavity, are diagnosed worldwide. In the United States, more than 41,000 new cases of oral cancer, mainly diagnosed as head and neck squamous cell carcinomas (HNSCC), were identified in 2013, and 7,900 deaths were predicted to occur [1]. Despite encouraging efforts to implement novel therapies, only a limited improvement has occurred in oral cancer patient survival rates in the last four decades, particularly in tongue and other oral cavity cancers that are often associated with tobacco use and alcohol consumption as the main risk factors [2]. Poor treatment outcomes are generally the result of delayed diagnosis and “field cancerization”, a unique term describing the synchronous or metachronous occurrence of multifocal potentially malignant lesions or secondary primary HNSCCs [3]. Clearly, prevention and early diagnosis are key to improving significantly the prognosis of HNSCC patients.

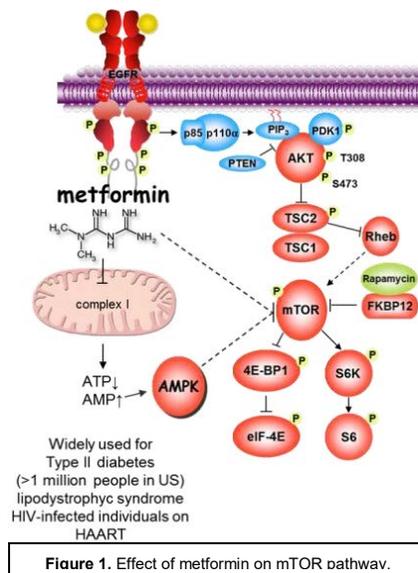
mTOR and HNSCC: Recent identification of the mammalian Target of Rapamycin Complex 1 (mTORC1)

signaling pathway as a highly prevalent molecular signature underlying HNSCC pathogenesis has provided opportunities to explore novel strategies aimed at halting disease progression [4-7]. The use of mTOR inhibitors is highly effective in decreasing tumor burden and metastasis and increasing survival in a large number of preclinical models, including human tumors xenografted into immune compromised mice as well as in a series of recently developed genetically-engineered and chemically-induced animal models resulting in skin and/or oral SCC lesions [5, 6, 8]. These studies and the realization that most HNSCCs exhibit genomic alterations converging in PI3K/mTOR activation [9] provided the foundation for multiple open clinical trials targeting mTOR in HNSCC. These include a presurgical study with a limited number of participants ([NCT01195922](#)) exploring the antitumor activity of rapamycin, which blocks mTORC1, in newly diagnosed HNSCC patients. This trial, which will be reported shortly, has been recently completed and presented in multiple meetings, with encouraging results in terms of objective responses (including a complete response) and limited toxicities. Overall, emerging evidence suggests the potential clinical benefit of mTORC1 inhibitors, such as rapamycin and its analogs (rapalogs), in the management of patients with fully established or recurrent HNSCC lesions. However, their potential immunosuppressive activity and other dose-dependent side effects, such as thrombocytopenia and hyperlipidemia, may raise safety concerns regarding the long term use of rapamycin and rapalogs as chemopreventive agents [10]. This is of particular relevance to patients diagnosed with potential premalignant oral lesions (OPL) that have a relatively low malignant transformation rate [11]. Therefore, long-term interventions with well-tolerated, low-cost drugs may offer a much needed therapeutic strategy designed to prevent HNSCC progression, before its loco-regional invasion and nodal metastasis.

2.2 Study Agent

Metformin: Metformin is an oral biguanide that is the most widely used drug for the treatment of type 2 diabetes mellitus.

Epidemiologic data suggest that metformin treatment may lower cancer risk and/or improve prognosis [12-15]. Compelling evidence demonstrates that in a variety of human cancer cell lines and animal models, metformin treatment reduces tumor cell growth in part by reducing mTORC1 activity [12-15]. These inhibitory effects appear to be controlled by the AMP-activated protein kinase (AMPK) signaling pathway, a key sensing mechanism of cellular bioenergetics [12] (**Figure 1**). As a mild inhibitor of mitochondrial complex I, metformin induces AMPK activation following the inhibition of oxidative phosphorylation, which leads to an increase in cellular AMP levels. AMP binds to AMPK, making AMPK a better substrate for phosphorylation by the upstream tumor suppressor serine/threonine kinase LKB1 [12]. Activated AMPK phosphorylates and activates TSC2, hence antagonizing the small GTPase Rheb and reducing mTORC1 activity [12]. Therefore, in malignancies where the mTORC1 pathway is frequently hyperactivated, such as HNSCC, targeting AMPK activation by metformin appears to be a therapeutic strategy that deserves further investigation. As described below, we have accumulated substantial preclinical information regarding the potential chemopreventive activity of metformin in HNSCC. Furthermore, recent retrospective population case-control cohort studies have demonstrated a decreased HNSCC risk in diabetic patients treated with metformin [16]. Metformin use also resulted in a better overall survival in diabetic patients diagnosed with laryngeal squamous cell carcinoma [17], which is highly related to HNSCC.



Oral premalignancy and chemoprevention: Carcinogenesis can be viewed as a multistep process occurring over lengthy periods of time that culminates in the acquisition of mutations and microenvironment changes that lead to the development of invasive disease. The rationale for chemoprevention is based on the

recognition that the early stages of carcinogenesis may be reversible and that prevention or delay of progression to invasive disease constitutes clinical benefit. Preinvasive tissue histologic abnormalities such as hyperplasia and dysplasia represent early to intermediate stages of the carcinogenic process. At a macroscopic level, these preinvasive changes present as oral premalignant lesions - leukoplakia or erythroplakia (white or red patches, respectively) that have variable rates of progression to cancer, ranging from 11-36% for leukoplakia to >50% for erythroplakia [18, 19]. Although it is not known with certainty that regression of an oral premalignant lesion truly equates to reduced risk for oral cancer, it is known that in a cohort of subjects with oral leukoplakia, approximately half of subsequent head and neck cancers develop from prior lesions [18]. Furthermore, in other organs such as the colon and breast, removal of preinvasive lesions (colorectal polyps or ductal carcinoma in situ, respectively) has been clearly shown to prevent subsequent invasive cancer. Thus, it is reasonable to hypothesize that regression of premalignancy is indicative of reduced cancer risk and premalignant lesions have typically been used as the endpoints for early phase chemoprevention clinical trials [20]. In the case of oral premalignancy, since half of the invasive cancers develop in areas outside of the index premalignant lesion due to field cancerization and the size and waxing and waning nature of many oral premalignant lesions preclude adequate surgical resection, systemic treatment to deliver preventive interventions to the entire exposed field is preferable to localized approaches. Any signal emerging from our proposed phase II preliminary efficacy chemoprevention trial would need to be further tested in a phase III definitive efficacy study prior to any recommendations for changes to current standard of care.

2.3 Rationale

In a recent study, the team led by Dr. Gutkind (NIDCR, NIH) developed a suitable model to study oral premalignancy, and used it to investigate the chemopreventive potential of metformin [21]. Specifically, they used an oral-specific carcinogenesis model induced by 4-nitroquinoline-1 oxide (4NQO) in the drinking water in immunocompetent non-diabetic C57Bl/6 mice [6]. 4NQO is a water-soluble chemical carcinogen that forms DNA adducts, causes adenosine for guanosine substitutions, and induces intracellular oxidative stress resulting in mutations and DNA strand breaks, all similar to the genetic alterations provoked by tobacco carcinogens, and hence 4NQO often serves as a surrogate for tobacco exposure [22]. By replicating common molecular changes observed in human HNSCC, including increased activity of mTORC1, they were able to control tumor progression in 4NQO-exposed mice by systemically administering rapamycin, a clinically relevant mTOR inhibitor [6]. Emerging studies from this and other groups suggest that this animal model may provide a predictable preclinical strategy to mimic human HNSCC carcinogenesis, which can be used to develop novel therapeutic approaches for HNSCC treatment. However, most mice exhibit 1-3 carcinomas together with multiple dysplastic lesions at the end of the carcinogen exposure, thus limiting the ability to examine the benefit of preventive agents unless they also display anticancer properties. Instead, they performed an optimization protocol to study the conversion of potential oral premalignant lesions (i.e., epithelial dysplasias) into squamous cell carcinomas, hence affording the opportunity to explore novel chemopreventive strategies to halt tumor progression [21].

In an initial study, Dr. Gutkind's lab showed that exposure to 4NQO for shorter time (14 weeks) causes primarily epithelial dysplasias, and that some of these lesions continue to progress into SCCs even after switching to regular water[21]. These findings suggested that 4NQO initially triggers genetic alterations in oral epithelial cells that result in the development of mainly premalignant lesions, which can then spontaneously progress into carcinomas. Of interest, the number of SCCs after 22 weeks with respect to the average number of potential premalignant lesions at the end of the carcinogen exposure is approximately 1:7, which is similar to the rate of conversion of human potential premalignancies into carcinomas [11]. Since oral epithelial transformation induced by 4NQO and those provoked by tobacco carcinogens are comparable at the tissue and cellular level, they next asked whether treating mice with metformin may prevent HNSCC progression. While the number and size of oral lesions in the control mice increased in a time-dependent manner, a significant reduction in these parameters was initially evident in the metformin group after 4 weeks of treatment (week 18) and remained lower until the conclusion of the experimental period (week 22).

By week 22, mice exposed to 4NQO predictably developed visible papillary-like oral tumors mainly localized on the tongue [6]. Conversely, the number and sizes of the visible oral lesions were significantly decreased in mice that were treated with metformin. Histological examination of all oral lesions at week 22 of control and metformin treated mice revealed a striking impact of metformin in tumor progression. In particular, metformin reduced the overall number of benign and malignant tumor lesions to less than half of those observed in the control group, and it nearly abolished the progression to SCCs, with a single malignant lesion within the group of treated mice, while most control mice exhibited one to two cancer lesions [21]. They also observed a significant decrease in the number of low and high grade dysplasias after metformin treatment. These results indicate that systemically administered metformin hampered the progressive increase in the number and size of oral lesions, suggesting a potential interference of the neoplastic transformation process induced by 4NQO exposure [21].

Metformin exerted a very limited impact on serum components, metabolic markers, and plasma levels of insulin and IGF-1. Instead, metformin decreased the basal proliferation of hyperplastic regions in the tongue of 4NQO treated mice. By the immunohistochemical analyses against the phosphorylated form of S6, pS6, they confirmed that pS6 immunoreactivity is clearly restricted to the upper non-proliferating parabasal epithelial cell layer, while basal and suprabasal cells lack positive staining in normal lingual mucosa derived from normal control mice and in 4NQO-induced hyperplastic lingual lesions [21]. In 4NQO-induced dysplastic lesions, a considerable number of basal and suprabasal cells were pS6 positive. In contrast, pS6 is clearly positive in most cells in oral SCCs, as previously documented in human HNSCC lesions and in the 4NQO-induced oral SCCs in mice. Thus, a progressive dysregulation of the mTORC1 pathway appears to occur early in the carcinogenic process, with basal proliferating epithelial cells exhibiting mTORC1 activation, a process that may then drive the malignant conversion of premalignant lesions. In this regard, in dysplastic lesions derived from 4NQO-exposed mice treated with metformin showed a highly significant reduction in the number of basal and suprabasal cells demonstrating positive pS6 reactivity [21]. Similar observations were noticed by immunofluorescence stainings in normal lingual mucosa and dysplastic lesions were performed against pS6 and keratin 5 (K5), a ubiquitous keratin commonly localized in the basal and suprabasal layers of the oral epithelium. Metformin treatment was able to significantly reverse the increase in pS6 reactivity observed in the basal cells of dysplastic lesions of control-treated 4NQO mice [21].

Overall, these results systematically demonstrate that metformin deters carcinogen-induced HNSCC development and progression, most likely through the inhibition of mTORC1 activity within a dysplastic epithelial niche occupied by proliferating basal and suprabasal cells. These observations have been recently extended to human tumor xenografts using HNSCC cells harboring mutations in the PI3K pathway (*PIK3CA* mutations) and in HNSCC cells derived from cancers associated with high risk human papillomavirus (HPV16, HPV) infection, which now account for roughly 20% of all human HNSCCs in the US and most parts of the world. Indeed, Dr. Gutkind's lab has recently shown that HPV-associated HNSCCs exhibit a remarkable activation of the mTOR pathway and that their derived xenografts are highly sensitive to the anti-tumor effect of mTOR inhibitors [23]. This laboratory has recently optimized the oral delivery of metformin to achieve a concentration in the blood similar to that reported for diabetic patients taking metformin for type 2 diabetes. They observed that at the appropriate doses, metformin reduces the tumor growth of HPV- and HPV+ HNSCC xenografts, concomitant with reduction of mTOR activity, supporting that precancerous lesions arising from high risk HPV infection may also benefit from metformin treatment as a chemopreventive strategy (manuscript under revision). Metformin is a very hydrophilic, membrane-impermeable compound that requires active transport to be incorporated into the cell. Therefore, its effects may depend on the expression and functional activity of organic cation transporters belonging to the SLC22A gene family. While its key metabolic effects in type 2 diabetic and polycystic ovary syndrome patients are likely

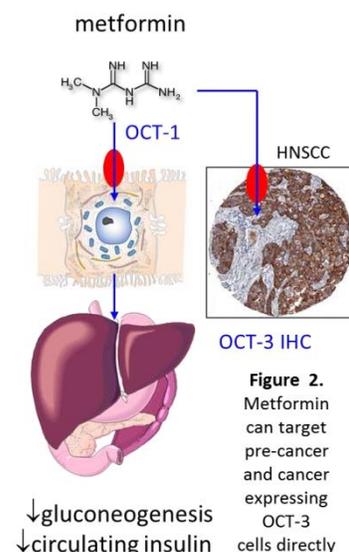
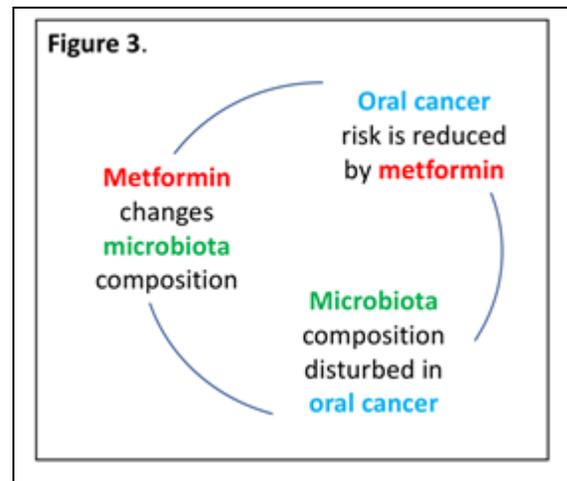


Figure 2.
Metformin can target pre-cancer and cancer expressing OCT-3 cells directly

dependent on the expression of OCT1 in the liver [24, 25], OCT1 is absent or minimally expressed in HNSCC cells [26]. Instead, HNSCC and potential OPL express OCT3/ SLC22A3 (a widely expressed organic cation transporter) [21, 26], and in a recently submitted study they showed that OCT3 is required for metformin activity in HNSCC cells (**Figure 2**). As OCT3 (as well as OCT1/ SLC22A1 and OCT2/ SLC22A2) exhibits genotypic variability affecting metformin pharmacokinetics and tissue uptake [21, 24-26], we will also explore SLC22A gene family pharmacogenetics in the enrolled patients.

In addition, recent studies showed that the increased glucose tolerance induced by oral metformin was caused by dramatic alterations in gut microbiota compositions [27]. By transplanting the gut microbiota from patients before and after metformin treatment into germ free mice, the authors were able to demonstrate that a metformin-modulated microbial community can directly affect host phenotype. Additionally, accumulating evidence suggests an individual's gut microbiota plays an active role in their response to cancer treatment.

Three independent, recent studies showed that epithelial cancer patients' response to PD-1 immunotherapy is dependent on the diversity of microbes present in their fecal samples [28-30]. Importantly, by transplanting a patient's microbiome into a mouse, the authors were able to replicate the patient's response to therapy, implying that the gut microbiota directly influence the response to cancer therapy. Furthermore, two independent studies have highlighted a role for the oral microbiome in patients with oral squamous cell carcinoma (OSCC). In one study, distinct functional profiles of the microbes in saliva were found across OSCC mutation categories [31]. Independently, a large scale prospective study showed that saliva microbial dysbiosis precedes OSCC onset [32]. Taken together, these three pieces of information form a compelling argument for the existence of an interplay among metformin, OSCC, and the oral microbiome (**Figure 3**) which will be explored in this study.



3. SUMMARY OF STUDY PLAN

The study is a phase IIa single-arm, open-label trial in individuals with oral leukoplakia or erythroplakia to explore the potential of metformin for oral cancer prevention. The study plans to accrue 26 eligible participants to initiate agent intervention. With a projected attrition rate of 20%, we anticipate to have at least 20 participants completing the agent intervention to provide study endpoint data. The plan duration of accrual is 24 months with the expected accrual rate of 1 per month among the three study sites.

Individuals with oral leukoplakia or erythroplakia will be recruited from local hospitals, Veterans Administration hospitals, pathology services, dental schools, dentist networks, and from advertising in local media.

Participants will undergo a Baseline Testing/Pre-Study Evaluation in which the informed consent form and medical records release and tissue release form will be signed. Participants will be assessed for study eligibility. Detailed inclusion and exclusion criteria are listed in sections 4.1 and 4.2. Participants will undergo a brief physical exam including chest, heart, abdomen, assessment of lower extremities for pedal edema, weight, height, vital signs (temperature, blood pressure, pulse) and performance status. They will also be evaluated for concomitant medications, medical history, baseline symptoms and signs, and assessment of tobacco and alcohol use. Participants will undergo an oral exam for lesion measurement, photography. Bi-dimensional measurements for all lesions will be recorded. All lesions that meet the size criteria ($\geq 8 \times 3$ mm) will be considered target lesions for clinical response assessment. Lesions that do not

meet the size criteria ($<8 \times 3 \text{mm}$) will be measured but recorded as non-measurable.

A biopsy will be performed on the lesion that meets the size criteria ($\geq 8 \times 3 \text{mm}$) or on the largest lesion, if multiple lesions that meet the size criteria. If smaller lesions meet the size criteria and are of clinical concern to treating physician, they may be biopsied. All biopsies will be sent for local pathology evaluation, followed by central pathology review. The lesion with the worst histology following the central pathology review will be selected as the target lesion for histologic response assessment and for tissue biomarker evaluation. If the participant underwent a pre-enrollment biopsy within 6 weeks prior to the screening visit, and the pre-enrollment biopsy has been histologically confirmed with adequate archival tissue for biomarker analysis, a biopsy of the lesion will not be obtained at the baseline clinic visit. Rather, archival tissue will be collected. In these participants, the same size criteria for eligibility will apply. The final histologic eligibility will be determined by a centralized pathology review.

Biopsy of visually normal cheek mucosa will be performed on all participants to evaluate tissue markers, including those who had a prior lesion biopsy that is qualifying. Saliva will be collected for metformin levels and cytokine biomarkers between 8 am and 12 noon, if feasible. Fasting blood will be collected for complete blood count with differential (CBC-diff), comprehensive metabolic panel (CMP), C-peptide, and HbA1c. Fasting blood will also be collected for serum metformin levels, serum cytokine biomarkers and genomic analysis of blood DNA. Urine pregnancy test will be performed for potentially fertile women. Participants may return within 7 days of the Baseline Visit for procedures not performed during the Baseline Visit. Participants will be provided with an AE Diary and instructions on how to complete the AE Diary.

After eligibility is confirmed, participants will be provided with an initial supply of metformin ER 500 mg tablets and an Intake Calendar (agent/medication log). The agent supply and Intake Calendar may be mailed to the participants, although potentially fertile women will need to have a negative urine pregnancy test within 4 weeks before provision of agent supply. Participants will be instructed on how to complete the Intake Calendar. The Calendar will be marked with agent start date and dosage increase dates. Participants will be instructed to take Metformin ER 500 mg per day for one week, followed by dose escalation to 1,000 mg per day for one week, followed by dose escalation to 2,000 mg/day for the remainder of the 3 month period. Suitability for dose escalation will be assessed via telephone (or email) contact prior to starting the intervention and each escalation as participants are eligible for dose escalation only in the absence of grade 2 or higher toxicities. Additional telephone (or email) contact will also be made following dose escalation. There will be an additional interim telephone (or email) contact at week 9 (± 3 days) to assess adverse events and encourage compliance. An interim clinic visit will occur after 6 (± 1) weeks of treatment. During this visit participants will undergo a brief physical exam of the chest, heart, abdomen, assessment of lower extremities for pedal edema, be assessed for changes in medical history, and will be evaluated for weight, vital signs. They will return unused pills. Unused pills will be counted and may be re-issued along with a new supply of study pills. AEs, concomitant medications, and compliance will be reviewed. A urine pregnancy test will be repeated, if applicable. Participants will also undergo an oral exam with lesion measurement and photography.

Participants will return after 12-14 weeks of agent intervention for post-intervention evaluation. This visit should be scheduled between 8 am and 12 noon, if feasible, because of the saliva collection. During this visit, participants will return unused pills and be evaluated for weight, vital signs, AEs, concomitant medications, and tobacco and alcohol assessment. They will undergo a brief physical exam of the chest, heart, abdomen, and assessment of lower extremities for pedal edema and be assessed for changes in medical history. Participants will undergo an oral exam for lesion measurement, photography. A biopsy will be performed on the selected target lesion for histopathology and tissue markers. If the target lesion is not visible post-intervention, a biopsy will be obtained at the site of the previous lesion biopsy. A biopsy of visually normal cheek mucosa and saliva collection will also be performed for tissue markers. Saliva will be collected for metformin levels and cytokine biomarkers. Fasting blood will be collected for complete blood count with differential (CBC-diff), comprehensive metabolic panel (CMP), C-peptide, and HbA1c. Fasting

blood will also be collected for serum metformin levels and serum cytokine biomarkers. Urine pregnancy test will be repeated, if indicated.

Participants will be contacted by telephone (or email) 2-4 weeks later for a final AE assessment of drug, and to be provided with final biopsy report. Participants will be reminded to return the AE Diary to the study office.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Participants with oral leukoplakia or erythroplakia with mild, moderate, or severe histologic dysplasia, or hyperplasia not associated with mechanical factors such as ill-fitted dentures.

4.1.2 Measurable disease – minimum lesion size of 8x3 mm before initial biopsy.

4.1.3 Age \geq 18 years.

4.1.4 Karnofsky performance status \geq 70%.

4.1.5 Participants must have normal organ and marrow function as defined below:

Leukocytes	\geq 3,000/microliter
Absolute neutrophil count	\geq 1,000/microliter
Platelets	\geq 100,000/microliter
Total bilirubin	\leq 1.5 \times institutional ULN
AST (SGOT)/ALT (SGPT)	\leq 1.5 \times institutional ULN

eGFR $>$ 40 mL/min using the Cockcroft-Gault equation
(<http://www.mdcalc.com/creatinine-clearance-cockcroft-gault-...equation/>).

4.1.6 Life expectancy $>$ 3 months.

4.1.7 Willing to use adequate contraception (barrier method, abstinence, subject has had a vasectomy or partner is using effective birth control or is postmenopausal) for the duration of study participation because the effects of metformin on the developing human fetus are unknown even though it is not teratogenic in rats and rabbits at 2-6 times the maximum recommended human daily dose.

4.1.8 Ability to take oral medication.

4.1.9 Ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 Patients with diabetes who are taking insulin or oral agents.

4.2.2 History of diabetic ketoacidosis.

4.2.3 Participants may not be receiving any other investigational agents within past 3 months.

- 4.2.4 History of allergic reactions attributed to compounds of similar chemical composition to metformin or prior use of metformin within the last year.
- 4.2.5 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, HIV-positive, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- 4.2.6 Oral carcinoma in situ.
- 4.2.7 History of chronic alcohol use or abuse defined as any one of the following: a) average consumption of 3 or more alcohol containing beverages daily in the past 12 months; b) consumption of 7 or more alcoholic beverages within a 24 hr period in the past 12 months.
- 4.2.8 HbA1c > 8%.
- 4.2.9 Pregnancy or nursing women.
- 4.2.10 Acute or chronic liver disease, evidence of hepatitis (infectious or autoimmune), cirrhosis or portal hypertension.
- 4.2.11 History of renal disease.
- 4.2.12 History of prior HNSCC unless curatively treated for ≥ 1 year.
- 4.2.13 Have received chemotherapy and/or radiation for any malignancy (excluding non-melanoma skin cancer and cancers confined to organs with removal as only treatment) in the past 2 years. Ongoing adjuvant hormonal therapy for breast cancer is allowed.

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.4 Recruitment and Retention Plan

Individuals with oral leukoplakia or erythroplakia will be recruited from local hospitals, Veterans Administration hospitals, pathology services, dental schools, dentist networks, and from advertising in local media.

The study team will provide a friendly and comfortable study setting for participants from initial contact through the completion of their study activities. Demands upon the subjects will be minimized to foster comfort while preserving the research goals. Wherever possible, flexibility will be built into the study schedule to promote compliance.

5. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. Reported adverse events (AEs) and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

- The study agent is extended-release metformin.

- ER metformin 500 mg per day (1 tablet QD) for 1 week, then 1,000mg per day (2 tablets QD) for the second week, then 2,000 mg (2 tablets BID) for the remaining treatment period.
- Duration of treatment is 12-14 weeks.

5.2 Study Agent Administration

- For the first week 1 tablet will be taken QD with evening meal, then for the second week 2 tablets will be taken QD with evening meal, and for the remaining weeks 2 tablets will be taken BID with morning and evening meals.
- Tablets should be swallowed whole and never crushed or chewed.

5.3 Run-in Procedures

Not applicable.

5.4 Contraindications

Metformin is contraindicated in patients with

- Renal disease or renal dysfunction. Metformin will be discontinued for $eGFR \leq 30$ mL/min.
- Known hypersensitivity to metformin hydrochloride
- Acute or chronic metabolic acidosis
- Iodinated contrast media, intravascular use in radiologic studies; possible acute alteration of renal function resulting in increased risk of lactic acidosis
- Hepatic impairment, as defined in the exclusion criteria

If a subject requires a radiologic exam involving intravascular administration of iodinated contrast materials while participating in the study, she/he must discontinue study agent for at least 48 hours prior to having the exam and will not be permitted to restart study medication until at least 48 hours following the procedure and only after renal function has been re-evaluated and found to be normal.

5.5 Concomitant Medications

Participants may not use non-study metformin or other biguanides while on study.

Cationic drugs (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, or vancomycin) that are eliminated by renal tubular secretion theoretically have the potential for interaction with metformin by competing for common renal tubular transport systems. Careful patient monitoring is recommended in patients who are taking cationic medications that are excreted via the proximal renal tubular secretory system.

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (e.g., biopsy) should also be included.

5.6 Dose Modification

The CTCAE version 4 will be used for toxicity reporting.

Dose reductions will be made only if the toxicities are possibly, probably, or definitely related to the study drug. For adverse events not related or unlikely to be related to study drug, the dose may be suspended at the discretion of the investigator.

Grade 1 toxicity or less, no dose modification will be made unless the adverse event is unacceptable to the physician or participant.

Grade 2 toxicity, dosage will be reduced by 500 mg (current dose level minus 500 mg). For second appearance of grade 2 toxicity, therapy will be discontinued until toxicity resolves to grade 1 or less not to exceed a 4 week period. At that time, the use of study drug will be resumed with the dosage further reduced by 500 mg. For third appearance of grade 2 toxicity, participants will be taken off study.

Grade 3 toxicity possibly related to drug, therapy will be discontinued until toxicity resolves to grade 1 or less not to exceed a 4 week period. At that time, the use of study drug will be resumed with the dosage reduced by 500 mg. For grade 3 toxicity definitely or probably related to drug, participants will be taken off study. For second appearance of grade 3 toxicity, participants will be taken off study.

Each toxicity will be evaluated according to the grade at presentation, even if the same toxicity presents with a different prior grade. If a participant experiences a grade 2 toxicity followed by a grade 3 toxicity, the dose will be discontinued until toxicity resolves to grade 1 or less not to exceed a 4 week period. For a third episode of either grade 2 or 3 toxicities the drug will be discontinued. Conversely, if a participant has a grade 3 toxicity followed by a grade 2 toxicity with the appropriate changes to drug dose, a third appearance of either a grade 2 or grade 3 toxicity will lead to discontinuation of the study drug. Grade 4 toxicity, participants will be taken off study.

The participant will come off study if the drug cannot be restarted in 4 weeks (cumulative throughout duration of study) for any of the toxicities.

The participant will come off study for $eGFR \leq 30$ mL/min.

The study site principal investigator will be notified immediately of any grade 3 or 4 adverse events. The study investigators will decide if the adverse event is related to the agent.

5.7 Adherence/Compliance

5.7.1 Participants will be considered compliant for secondary “per protocol” statistical analysis if they have taken $\geq 80\%$ of their assigned study doses based on capsule count.

5.7.2 The primary measure of compliance is pill count. The secondary measure of compliance will be the Intake Calendar. Serum levels of study agent will also be used to confirm compliance.

6. PHARMACEUTICAL INFORMATION

6.1 Metformin (IND exempt)

Metformin is an oral drug approved in the US for the management of hyperglycemia in type 2 diabetes mellitus (T2DM) in conjunction with diet and exercise [33, 34]. It is also commonly used off-label for polycystic ovary syndrome in women. Its major mechanism of action is the suppression of hepatic gluconeogenesis which reduces serum glucose and secondarily reduces insulin levels [35]. Compelling evidence demonstrates that in a variety of human cancer cell lines and animal models, metformin treatment

reduces tumor cell growth in part by reducing mTORC1 activity [12-15]. On the molecular level, metformin is believed to inhibit mitochondrial electron transfer from complex I to II, thereby resulting in lower cellular ratio of ATP/ADP levels which can secondarily activate adenosine monophosphate kinase (AMPK) via the tumor suppressor liver kinase B1[36]. Additional activities of metformin also include activation of muscular glucose uptake transporters [37, 38] and repression of glucagon receptor adenylate cyclase activation [39, 40].

The drug product, metformin ER tablet (NDC 62037-0571-01; Actavis [Parsippany, NJ], formerly Watson Laboratories [Corona, CA]) (<http://www.medicationdaily.com/metformin-hydrochloride/ndc/62037-0571-01>) contains 500 mg metformin HCl and the following inactive ingredients: hypromellose 2208, colloidal silicon dioxide, and magnesium stearate. The tablet comprises a monohydrophilic polymer matrix system in which metformin HCl is combined with a drug release controlling polymer to form the matrix. After administration, fluid from the gastrointestinal (GI) tract enters the tablet, causing the polymers to hydrate and swell. Drug is released slowly from the dosage form by a process of diffusion through the gel matrix that is essentially independent of pH. The hydrated polymer system is not rigid and is expected to be broken up by normal peristalsis in the GI tract. The biologically inert components of the tablet may occasionally remain intact during GI transit and will be eliminated in the feces as a soft, hydrated mass.

6.2 Reported Adverse Events and Potential Risks

Metformin is considered to have a benign safety profile [41, 42]. According to the US prescribing information (PI) for metformin HCl ER (NDC 62037-0571-01), common adverse events (AEs) for metformin ER *vs.* placebo were diarrhea (9.6% *vs.* 2.6%) and nausea/vomiting (6.5% *vs.* 1.5%) (<http://www.medicationdaily.com/metformin-hydrochloride/ndc/62037-0571-01>). In a clinical study of immediate-release (IR) metformin *vs.* placebo in patients with T2DM, GI symptoms such as diarrhea (53.2% *vs.* 11.7% placebo), nausea and vomiting (25.5% *vs.* 8.3% placebo), abdominal discomfort (6.4% *vs.* 4.8% placebo), flatulence (12.1% *vs.* 5.5% placebo), indigestion (7.1% *vs.* 4.1% placebo), and asthenia (9.2% *vs.* 5.5% placebo) were the most common reactions to the drug. These symptoms were dose-dependent, transient, and resolved spontaneously with continued use. Diarrhea led to discontinuation of medication in 6% of subjects treated with metformin. Additional adverse reactions reported in 1 to 5% of subjects receiving active drug, and more commonly reported than in the placebo group, include abnormal stools, hypoglycemia, myalgia, lightheaded, dyspnea, nail disorder, rash, increased sweating, taste disorder, chest discomfort, chills, flu syndrome, flushing and palpitation.

A recently published multicenter, randomized, placebo-controlled trial in patients with type 2 diabetes reported on vitamin B₁₂ deficiency with long-term metformin treatment. Compared with placebo, metformin treatment was associated with a mean decrease in vitamin B₁₂ concentration of ~19% (95% confidence interval (CI) -24% to -14%; *p*<0.001). The absolute risk of vitamin B₁₂ deficiency (<150 pM) at study end was 7.2 percentage points higher in the metformin group than in the placebo group (95% CI 2.3 to 12.1; *p*=0.004) [43].

According to the metformin HCl ER PI, lactic acidosis is a rare, but serious, metabolic complication that can occur due to metformin accumulation during treatment with metformin. When it occurs, it is fatal in approximately 50% of the cases. Lactic acidosis may also occur in association with a number of pathophysiologic conditions, including diabetes mellitus, and whenever there is significant tissue hypoperfusion and hypoxemia. Lactic acidosis is characterized by elevated blood lactate levels (>5 mM), decreased blood pH, electrolyte disturbances with an increased anion gap, and an increased lactate/pyruvate ratio. Metformin plasma levels >5 µg/mL are generally found when the drug is implicated as the cause of lactic acidosis.

The incidence of lactic acidosis in patients receiving metformin is very low (~0.03 cases/1000 patient-years, with ~0.015 fatal cases/1000 patient-years). In more than 20,000 patient-years of exposure to metformin in

clinical trials, there were no reports of lactic acidosis. Reported cases have occurred primarily in diabetic patients with significant renal insufficiency, including both intrinsic renal disease and renal hypoperfusion, often in those with multiple concomitant medical/surgical problems and who take multiple concomitant medications. Patients with congestive heart failure require pharmacologic management, in particular those with unstable or acute congestive heart failure who are at risk of hypoperfusion and hypoxemia, are at increased risk of lactic acidosis. The risk of lactic acidosis increases with the degree of renal dysfunction and with patient age. Drug should be withheld in the presence of any condition associated with hypoxemia, dehydration, or sepsis. Because impaired hepatic function can significantly impair the ability to clear lactate, metformin should generally be avoided in patients with clinical or laboratory evidence of hepatic disease. Alcohol can potentiate the effects of metformin on lactate metabolism. Drug treatment should be temporarily discontinued prior to any intravascular radiocontrast study and for any surgical procedure [44, 45].

In support of the previously reported low risk of lactic acidosis, a recently published systematic review assessed the risk of lactic acidosis with metformin use in type 2 diabetes mellitus. Pooled data from 347 comparative trials and cohort studies revealed no cases of lactic acidosis in 70,490 patient-years of metformin use. The authors concluded that there was no evidence from prospective comparative trials or from observational cohort studies that metformin is associated with an increased risk of lactic acidosis compared to other antihyperglycemic treatments [46, 47].

Unlike sulfonylureas, metformin does not produce hypoglycemia in either T2DM patients or normal subjects, and does not cause hyperinsulinemia. Non-diabetic patients with polycystic ovary syndrome have been safely treated with a hypocaloric diet in combination with oral metformin 850 mg twice daily for a duration of 12 months [48, 49].

6.3 Availability

Metformin HCl ER 500 mg tablet (NDC 62037-0571-01; Actavis, Parsippany, NJ, formerly Watson Laboratories, Corona, CA) will be supplied to investigators by the NCI, DCP.

6.4 Agent Distribution

Agents will only be released by NCI, DCP after documentation of IRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

NCI, DCP-supplied agents may be requested by the Investigator (or their authorized designees) at each Organization. DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request. Agents are requested by completing the DCP Clinical Drug Request form (NIH-986) (to include complete shipping contact information) and faxing or mailing the form to the DCP agent repository contractor:

John Cookinham
MRIGlobal
DCP Repository
1222 Ozark Street
North Kansas City, MO 64116
Phone: (816) 360-3805
FAX: (816) 753-5359
Emergency Telephone: (816) 360-3800

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF). The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to the research/investigational pharmacy staff at each site. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant.

6.6 Packaging and Labeling

DCP will package, label and distribute agent for all DCP-supplied agents.

6.7 Storage

Metformin HCl ER tablets should be stored at 20–25°C (68–77 °F) in a secure, locked area of the research facility, with excursions permitted to 15–30 °C (59–86°F).

6.8 Registration/Randomization

Participants will be considered registered on the date they sign the approved informed consent document with a member of the study staff. The study coordinator will contact the UAZ Consortium Office for a participant identification number (PID) when the subject has been consented. This study does not involve randomization.

6.9 Blinding and Unblinding Methods

Not applicable.

6.10 Agent Destruction/Disposal

DCP-supplied agents: at the completion of investigation, all unused study agent will be returned to NCI, DCP Repository according to the DCP “Guidelines for AGENT RETURNS” and using the DCP form “Return Drug List”.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Evaluation/ Procedure ¹	Baseline	Agent Start Day 1 of Week 1	Weeks 1-5	Week 6 (± 1 week)	Weeks 7-11	Week 12-14 or Early Termination	Follow-Up (2-4 weeks post- intervention)
Informed Consent	X						
Assess Eligibility	X						
Medical History	X			X		X	
Vital Signs ² / Height ³ and Weight	X			X		X	
Physical Exam ⁸	X			X		X	
Performance status	X						
Concomitant medications	X		X	X	X	X	X
Assessment of tobacco and alcohol use	X					X	
Fasting blood for CBC/diff, CMP ⁷ , C- peptide, HbA1c	X					X	
Urine pregnancy test if applicable	X	X ⁴		X		X	
Examination of oral cavity	X			X		X	
Lesion measurement	X			X		X	
Photography	X			X		X	
Biopsies ⁵	X					X	
Saliva collection for research biomarkers ¹⁰	X					X	
Fasting blood collection for research biomarkers	X					X	
Dispense Study Agent ⁴		X		X			
Collect Study Agent				X		X	
Review Agent Diary/Record				X		X	
Adverse Events			X	X	X	X	X
Telephone/Email Contact			X ⁶		X ⁹		X

¹ Initiation of study agent should occur within 9 weeks of baseline biopsy. On-study evaluations should be conducted with the following timeframes: week 6 interim visit can be scheduled +/- 1 week; total agent intervention duration is 12 – 14 weeks; post-intervention visit can be scheduled between weeks 12-14; the agent should be taken until the evening before the post-intervention visit.

² Vital signs include temperature, blood pressure, and pulse.

³ Only at baseline.

⁴ Eligible participants may have agent shipped to them. Urine pregnancy test for women of childbearing potential does not need to be repeated at agent start if the baseline test was done within 4 weeks prior to agent start.

⁵ At baseline, a biopsy will be performed on the lesion that meets the size criteria or on the largest lesion, if multiple lesions that meet the size criteria. If smaller lesions meet the size criteria and are of clinical concern to treating physician, they may be biopsied. The lesion with the worst histology following the central pathology review will be the target lesion and re-biopsied at post-intervention. If the target lesion is not visible post-intervention, a biopsy will be obtained at the site of the previous lesion biopsy. Biopsy of visually normal cheek mucosa will be obtained at baseline and post-intervention.

⁶ Telephone (or email) contact within 3 days of starting the agent and before and after each dose escalation. Participants are eligible for dose escalation only in the absence of grade 2 or higher toxicities.

⁷ CMP includes serum glucose, urea nitrogen, creatinine, sodium, potassium, chloride, bicarbonate, total protein, albumin, calcium, alkaline phosphatase, ALT, AST, total bilirubin. Calculations included with the CMP are: BUN/creatinine ratio, anion, gap, globulin, alb/glob ratio, eGFR.

⁸ Physical exam includes assessment of chest, heart, abdomen, and lower extremities for pedal edema.

⁹ Telephone (or email) contact at Week 9 (± 3 days).

¹⁰ Saliva should be collected between 8 am and 12 noon, if feasible.

7.2 Baseline Testing/Prestudy Evaluation

Participants will undergo a Baseline Testing/Pre-Study Evaluation in which the informed consent form and medical records release and tissue release form will be signed. Participants will be assessed for study eligibility. This clinic visit should be scheduled between 8 am and 12 noon, when feasible.

Participants will undergo a brief physical exam including assessment of chest, heart, abdomen, and lower extremities for pedal edema. Weight, height, vital signs (temperature, blood pressure, pulse) and performance status will be collected.

Participants will also be evaluated for concomitant medications, medical history, baseline symptoms and signs, and assessment of tobacco and alcohol use. The tobacco and alcohol history will be recorded directly onto the CRF which will be considered the source document for these data.

Participants will undergo an oral exam for lesion measurement and photography. Bi-dimensional measurements for all lesions will be recorded. All lesions that meet the size criteria ($\geq 8 \times 3 \text{mm}$) will be considered target lesions for clinical response assessment. Lesions that do not meet the size criteria ($< 8 \times 3 \text{mm}$) will be measured but recorded as non-measurable. Color photographs of all lesions will be taken. A biopsy will be performed on the lesion that meets the size criteria or on the largest lesion, if multiple lesions meet the size criteria. If smaller lesions meet the size criteria and are of clinical concern to treating physician, they may be biopsied. All biopsies will be sent for local pathology evaluation, followed by central pathology review. The lesion with the worst histology following the central pathology review will be selected as the target lesion for histologic response assessment and for tissue biomarker evaluation.

If participant underwent a pre-enrollment biopsy within 6 weeks prior to the Baseline visit and the pre-enrollment biopsy has been histologically confirmed with adequate archival tissue for biomarker analysis, a biopsy of the lesion will not be obtained at the Baseline clinic visit. Rather, archival tissue will be collected. In these participants, the same size criteria for eligibility apply.

The final histologic eligibility will be determined by a centralized pathology review (see section 10.2).

Biopsy of visually normal cheek mucosa will be performed on all participants to evaluate tissue markers, including those who had a prior lesion biopsy that is qualifying.

Saliva will be collected for metformin levels and cytokine biomarkers. Saliva should be collected between 8 am and 12 noon, if feasible.

Fasting blood will be collected for complete blood count with differential (CBC-diff), comprehensive metabolic panel (CMP), C-peptide, and HbA1c. Fasting blood will also be collected for serum metformin levels, serum cytokine biomarkers, and genomic analysis of blood DNA.

Urine pregnancy test will be performed for potentially fertile women.

Participants may return within 7 days of the Baseline Testing/Pre-Study Visit for procedures not performed during the Baseline Testing/Pre-Study Visit. Participants will be provided with an AE Diary and instructions on how to complete the AE Diary.

7.3 Evaluation During Study Intervention

After eligibility is confirmed, participants will be provided with an initial supply of metformin ER 500 mg tablets and an Intake Calendar (agent/medication log). The agent supply and Intake Calendar may be provided to the participants during a clinic visit or may be mailed to the participants, although potentially

fertile women will need to have a negative urine pregnancy test within 4 weeks before provision of agent supply. Initiation of study agent should occur within 9 weeks of baseline biopsy.

Participants will be instructed on how to complete the Intake Calendar. The Calendar will be marked with agent start date and dosage increase dates. Participants will be instructed to take Metformin ER 500 mg per day for one week, followed by dose escalation to 1,000 mg per day for one week, followed by dose escalation to 2,000 mg/day for the remainder of the 3 month period. Suitability for dose escalation will be assessed via telephone (or email) contact prior to starting the intervention and each escalation as participants are eligible for dose escalation only in the absence of grade 2 or higher toxicities. Additional telephone (or email) contact will also be made following dose escalation. There will be an additional interim telephone (or email) contact at week 9 (\pm 3 days) to assess adverse events and encourage compliance.

An interim clinic visit will occur after 6 (\pm 1) weeks of treatment. During this visit, participants will undergo a brief physical assessment of the chest, heart, abdomen, and lower extremities for pedal edema, be assessed for changes in medical history, and be evaluated for weight and vital signs. They will return unused pills. Unused pills will be counted and may be re-issued along with a new supply of study pills. AEs, concomitant medications, and compliance will be reviewed. A urine pregnancy test will be repeated, if applicable.

Participants will undergo an oral exam for lesion measurement and photography. Bi-dimensional measurements for all lesions will be recorded. Lesions that do not meet the size criteria ($<8 \times 3$ mm) will be measured but recorded as non-measurable. Color photographs of all lesions will be taken.

7.4 Evaluation at Completion of Study Intervention

Participants will return after 12-14 weeks of agent intervention for post-intervention evaluation. This visit should be scheduled between 8 am and 12 noon because of the saliva collection. During this visit, participants will return unused pills and be evaluated for weight, vital signs, AEs, concomitant medications, and tobacco and alcohol assessment. They will undergo a brief physical assessment of the chest, heart, abdomen, and lower extremities for pedal edema and be assessed for changes in medical history. The tobacco and alcohol history will be recorded directly onto the CRF which will be considered the source document for these data.

Participants will undergo an oral exam for lesion measurement and photography. Bi-dimensional measurements for all lesions will be recorded. Lesions that do not meet the size criteria ($<8 \times 3$ mm) will be measured but recorded as non-measurable. Color photographs of all lesions will be taken.

A biopsy will be performed on the selected target lesion for histopathology and tissue markers. If the target lesion is not visible post-intervention, a biopsy will be obtained at the site of the previous lesion biopsy. A biopsy of visually normal cheek mucosa will be performed for tissue markers.

Saliva will be collected for metformin levels and cytokine biomarkers.

Fasting blood will be collected for complete blood count with differential (CBC-diff), comprehensive metabolic panel (CMP), C-peptide, and HbA1c. Fasting blood will also be collected for serum trough metformin levels and serum cytokine biomarkers. Urine pregnancy test will be repeated, if indicated.

7.5 Post-intervention Follow-up Period

Participants will be contacted by telephone (or email) 2-4 weeks later for a final AE assessment of drug, and to be provided with final biopsy report. Participants will be reminded to return the AE Diary to the study office in a pre-stamped envelope.

7.6 Methods for Clinical Procedures

7.6.1 Measurement of lesions

All lesions will be measured in a bi-directional plane. The lesion length is the longest side and the width is perpendicular to the longest length. Lesions that do not meet the size criteria (<8x3mm) will be measured but recorded as non-measurable. The bi-directional measurements will be recorded on standard head and neck cancer staging maps. For example:

Lesion #1 8 mm length x 3 mm width = 24 mm² (product)

Lesion #2 9 mm length x 3 mm width = 27 mm² (product)

7.6.2 Lesion photographs

Color photographs of all lesions will be obtained prior to biopsy and digitally stored for the documentation of clinical response, however, the investigators' lesion measurements will be the official measurements for baseline and post-treatment assessments. For monitoring purposes, the participant's initials and PID will be on each photograph.

7.6.3 Standard Biopsy Procedures

7.6.3.1 Local anesthetic: topical anesthesia consisting of viscous lidocaine, or topical application of 20% benzocaine, will be initially applied, and then the biopsy site will be injected with 1 or 2% lidocaine with or without 1:1000,000 epinephrine.

7.6.3.2 Incisional biopsy:

Lesion Biopsy

- For the baseline biopsy, using a Baker 4 mm punch, the center or clinically most suspicious area of the lesion is biopsied with a twisting motion. The circular punched out tissue is grasped gently with fine toothed forceps and excised just deep to the submucosa with the #15 scalpel. Care is taken to avoid collecting significant amounts of fat or muscle and to avoid crushing the specimen.
- Biopsy sample is placed mucosa side up on a clean hard surface such as a piece of wax paper, Whatman filter paper, or equivalent.
- The specimen will be oriented on the Whatman paper to further reduce artifact and preserve orientation. The specimen will be sent to the institutional pathology lab in 10% formalin (see section 10.2) for paraffin embedding, routine histopathology and stored for biomarker analyses.
- The above collection procedure will be utilized in an identical fashion for the post-treatment biopsy. The biopsy will be obtained from the most suspicious part of the lesion adjacent to the baseline biopsy. If no lesion persists post-treatment, then the biopsy will be taken from the site adjacent to the original biopsy.

Biopsy of visually normal cheek mucosa

A 3 mm punch biopsy will be collected from visually normal cheek mucosa in the similar fashion as the lesion biopsy.

7.6.3.3 Hemostasis is achieved by either direct pressure or suture, or silver nitrate stick.

7.6.3.4 Participants are provided with oral wound care instructions: 4-6 times daily local rinsing with saline and standard post surgical instructions including notification of consulting professional for heavy bleeding, severe pain, or fever.

7.6.3.5 For analgesia, participants will be told to take acetaminophen 325-650 mg po qid PRN. However, it is anticipated that the participants will not require significant pain control after the above procedure. Care should be taken to avoid Stenson's duct by greater than 1 cm after it is identified.

7.6.4 Saliva Collection

The collection of unstimulated whole saliva will be conducted as described below:

The patient should not have eaten for 90 minutes prior to the collection procedure. Saliva should be collected between 8 am and 12 noon, if feasible. During the collection period, the subject shall be

seated straight up with eyes open and head tilted slightly forward. The subject will be instructed to minimize oro-facial movements to minimize influence on salivary flow (the subject should not swallow and should not speak during the collection process). Immediately before the collection begins, the subject is instructed to swallow. The patient allows the saliva to accumulate in the floor of the mouth for 60 seconds without swallowing. The patient empties the entire accumulated saliva into the pre-weighed container, a 50 ml Falcon tube. The procedure is then repeated 4 more times for a total collection time of 5 minutes. Subjects are instructed not to swallow during the entire 5-minute collection period.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

The primary endpoint is to determine whether 12-14 weeks of metformin intervention would improve the clinical response of oral premalignant lesions. Subjects who show complete or partial clinical response following the metformin intervention will be considered clinical responders.

Clinical response will be evaluated by the following criteria [50]:

Complete Response (CR): Disappearance of all evidence of lesion(s).

Partial Response (PR): Greater than or equal to 50% reduction in the sum of the products of diameters of lesion(s) measurable at baseline. Non-measurable lesion(s) may not increase greater than or equal to 25% in size and no new lesion may appear.

No Change (NC): No change in the size of the lesion(s) identified at baseline and no new lesions appearing, i.e., anything that is not CR, PR, or PD.

Progressive Disease (PD): Any increase greater than or equal to 25% in the product of the diameters of any lesion(s) measurable at baseline or in the estimated size of lesion(s) non-measurable at baseline or the appearance of an unequivocal new lesion.

8.2 Secondary Endpoints (in order of priority)

8.2.1 Histologic response to metformin intervention in the target lesion

Histologic response will be evaluated by the following criteria:

Complete Response (CR): Complete reversal of dysplasia or hyperplasia to normal epithelium in the target lesion.

Partial Response (PR): Improvement of the degree of dysplasia or hyperplasia in the target lesion.

No Change (NC): No change in the degree of dysplasia or hyperplasia in the target lesion, anything that is not CR, PR or PD.

Progressive Disease (PD): Increase in the severity of grade of histology in the target lesion.

8.2.2 Tissue-based biomarkers

8.2.2.1 Metformin effect on cell proliferation and its molecular targets in the target lesion and in the normal tissue. The expression of the following markers will be assessed in the baseline and post-intervention biopsies.

8.2.2.1.1 Marker of cell proliferation, Ki67

8.2.2.1.2 Molecular targets of metformin, including, in order of priority, pS6, pAKT^{S473}, p4EBP, pACC

8.2.2.2 Expression of frequent dysregulated molecular mechanisms and organic cation transporter 3 (OCT 3) in the target lesion and in the normal tissue, including, in order of priority, EGFR, pEGFR, p53, PTEN, pERK, p16, and OCT3. The expression of these biomarkers will be assessed in the baseline

and post-intervention biopsies to determine the biochemical consequences of the metformin treatment, and their potential relationship with the clinical response to metformin.

- 8.2.2.3 Targeted analysis of cancer-associated genes in the target lesion and blood DNA collected at baseline to determine the impact of genomic alterations on the biological and biochemical consequences and clinical response to metformin.
- 8.2.3 Serum and saliva based biomarkers
 - 8.2.3.1 Metformin effect on serum metabolic markers (C-peptide, glucose and HbA1c)
 - 8.2.3.2 Metformin concentrations in serum and saliva at baseline and post-intervention
 - 8.2.3.3 Metformin effect on serum and saliva inflammatory and angiogenic cytokines, including interleukin (IL)-6, IL-8, growth-related oncogene-1 (GRO-1), and vascular endothelial growth factor (VEGF). These cytokines will be measured in samples collected at baseline and post-intervention.
- 8.2.4 Exploratory Endpoints
 - 8.2.4.1 To characterize changes in the saliva microbiome before and after metformin intervention, including both the absolute microbial load and taxonomic composition.
 - 8.2.4.2 To evaluate the potential microbiome signatures that are correlated with treatment response.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

8.4 Off-Study Criteria

Participants may go ‘off-study’ for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/serious adverse event, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death, determination of ineligibility (including screen failure).

8.5 Study Termination

NCI, DCP as the study sponsor, and the FDA have the right to discontinue the study at any time.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

Tissue biomarkers, including Ki67, pS6, pAKT^{S473}, p4EBP, pACC, EGFR, pEGFR, p53, PTEN, pERK, p16, and OCT3 will be assessed by immunohistochemistry (IHC). IHC is the best and most validated

technique for measuring protein levels of the biomarkers of interest in paraffin-embedded tissue. It allows analyses of relative expression levels of each biomarker and determination of localization of each marker.

Serum and saliva metformin concentrations will be determined by chromatography-tandem mass spectrometry method, which provides high sensitivity and specificity for metformin analysis.

Serum and saliva inflammatory and angiogenic cytokines, including IL-6, IL-8, GRO-1, and VEGF, will be measured by Luminex Multianalyte System. Luminex™ technology allows for simultaneous quantification of multiple proteins in a single sample.

Genomic analyses will be conducted in blood DNA and DNA extracted from the target lesion by targeted sequencing using the current Expanded Solid Tumor Panel at the Center for Advanced Laboratory Medicine, University of California San Diego. This panel covers all exons for 399 genes, which is designed to detect hotspot mutations and key fusion of approximately 400 genes often associated with cancer progression, including oral cancer. Importantly, targeted sequencing has been applied successfully to DNA extracted from formalin-fixed paraffin-embedding tissue sections.

The microbial load in the saliva samples will be determined using the flow cytometry method described by Vandeputte et al. [51]. Samples are first diluted ten-fold in sterile saline solution, then filtered across a 5 µm filter to dissociate aggregates and remove human epithelial cells. The sample is then stained with SYBR green, a cell membrane permeable fluorescent DNA stain. Populations with increased intensity specific to SYBR green fluorescence above a stained vehicle control are enumerated with counting beads to extrapolate the number of microbial cells per mL of original sample. By using serially diluted saliva samples, we have shown that we can reproducibly detect the microbial load across a 1,000-fold range of dilutions. The total number of microbial cells per mL of human saliva can then be used to normalize the sequencing results to represent the number of bacterial taxa per mL instead of compositional data that only identifies the ratio of microbes. In preliminary experiments with healthy individuals, we found a 100-fold difference in microbial load per mL of saliva. This dramatic difference would have a large impact on interpretation of microbial sequencing results from saliva, and accounting for this variability in our clinical cohort will improve our ability to detect meaningful changes.

For shotgun metagenomic sequencing, DNA will be extracted in triplicate from 200 µl aliquots of frozen, unstimulated saliva using our high-throughput, automated DNA extraction pipeline [52]. Next, 5 ng of each aliquot of extracted DNA will be used to create shotgun metagenomic libraries with the Kapa HyperPlus kit. We have established an automated, miniaturized shotgun metagenomics library preparation pipeline, where we can readily process 384 samples at one-tenth the volume and a fraction of the cost within a single day. Importantly, we have also recently optimized a method to reduce the amount of human DNA from saliva samples for improved microbial assessment [53]. Sequencing data will be processed as previously described [53]. Briefly, demultiplexed samples will be trimmed and quality filtered using Atropos v 1.1.5, a fork of Cutadapt. Reads aligning to the host genome will be identified using Bowtie 2 v2.3.0 and filtered from downstream analysis. The host-filtered microbial reads will be profiled using MetaPhlAn v2.0 and the taxonomic output matrix will be filtered to represent only the relative abundance of the most specific taxonomic level. We will normalize the resulting matrix to account for differences in microbial load as described by Vandeputte et al. Bray-Curtis and Binary Jaccard beta diversity analysis will be performed using QIIME2 to visualize the impact of metformin on the salivary microbiome.

9.2 Comparable Methods

Proposed methods are standard methodologies used in other research studies. The resulting data will be able to be compared to existing data.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

Clinical chemistry, hematology, C-peptide, and HbA1c will be performed at the institutional or commercial diagnostic laboratories.

Immunohistochemistry will be performed in the laboratory of Dr. Alfredo Molinolo at UCSD.

Serum and saliva cytokine markers will be performed at the University of Maryland Cytokine Core Laboratory.

Genomic analysis will be performed at the UCSD Center for Advanced Laboratory Medicine.

Serum and saliva metformin concentrations will be determined in the University of Arizona Cancer Center Analytical Chemistry Shared Resource led by Dr. Sherry Chow.

10.2 Collection and Handling Procedures

Baseline

Ten milliliters of fasting blood (1 x 7 ml SST tube; 1 x 3 ml Lavender-EDTA tube) will be collected at Baseline for CBC with diff, CMP, HbA1c, and C-peptide. SST tubes will be held at room temperature for 30 min followed by centrifugation. Lavender-EDTA tube will be inverted gently 5 times. Both will be prepped, labeled, and packaged according to the recommendation from the diagnostic lab. These samples will be sent immediately for analysis.

The lesion biopsy will be placed in a standard pathology container for tissue fixation in 10% formalin and labeled according to the requirements from the pathology lab. Biopsy of normal mucosa will be placed in a standard pathology container for tissue fixation in 10% formalin and labelled according to the requirements from the pathology lab.

Ten milliliters of fasting blood will be collected at Baseline for research biomarkers (1 x 7 ml SST tube; 1 x 3 ml Lavender-EDTA tube for serum markers and genome analysis, respectively). SST tubes will be held at room temperature for 30 min followed by centrifugation. Serum will be aliquoted evenly into 10 x 1 ml cryovials. Lavender-EDTA tube will be inverted gently 5 times. Cryovials and Lavender-EDTA tubes will be labeled with the study ID, participant ID, and visit type (baseline). Samples will be stored at -80°C prior to analysis.

Saliva will be collected into a 50 ml conical tube for cytokine markers. Saliva will be aliquoted evenly into 10 x 1 ml cryovials. Cryovials will be labeled with the study ID, participant ID, and visit type (baseline). Samples will be stored at -80°C prior to analysis.

Post-Intervention

Ten milliliters of fasting blood (1 x 7 ml SST tube; 1 x 3 ml Lavender-EDTA tube) will be collected at post-intervention for CBC with diff, CMP, HbA1c, and C-peptide. SST tubes will be held at room temperature for 30 min followed by centrifugation. Lavender-EDTA tube will be inverted gently 5 times. Both will be prepped, labeled, and packaged according to the recommendation from the diagnostic lab. These samples will be sent immediately for analysis.

The lesion biopsy will be placed in a standard pathology container for tissue fixation in 10% formalin and labelled according to the requirements from the pathology lab. Biopsy of normal mucosa will be placed in a

standard pathology container for tissue fixation in 10% formalin and labelled according to the requirements from the pathology lab.

Seven milliliters of fasting blood will be collected at post-intervention for research biomarkers (1 x 7 ml SST tube for serum markers). SST tubes will be held at room temperature for 30 min followed by centrifugation. Serum will be aliquoted evenly into 10 x 1 ml cryovials. Cryovials will be labeled with the study ID, participant ID, and visit type (post). Samples will be stored at -80°C prior to analysis.

Saliva will be collected into a 50 ml conical tube for cytokine markers. Saliva will be aliquoted evenly into 10 x 1 ml cryovials. Cryovials will be labeled with the study ID, participant ID, and visit type (post). Samples will be stored at -80°C prior to analysis.

Twelve to fifteen formalin-fixed paraffin-embedded unstained tissue sections (4 µm) from biopsy collected at baseline and post-intervention will be requested from each institution's pathology department for measurement of tissue biomarkers.

Centralized pathology review

Centralized pathology review will occur for eligibility assessment and after study completion. The H&E stained section of the target lesion biopsy will be scanned by study personnel at each site using Aperio or equivalent scanner. Dr. Alfredo Molinolo at UCSD will perform an independent centralized pathology review of the scanned slides.

In case of disagreement between the local site and central path review conducted by Dr. Molinolo, the following actions will take place:

1. For minor discrepancy (one level change) in diagnoses, Dr. Molinolo's evaluation will be considered final, for consistency.
2. For major discrepancy (two levels or more difference), the case will be referred to Dr. Stephen Hewitt at the NCI for independent review. The consensus evaluation of two pathologists in agreement will be used as the final diagnosis.
3. For disagreement among all three pathologists (local site, Dr. Molinolo, and Dr. Hewitt), Dr. Molinolo and Dr. Hewitt will discuss the case and come to a consensus.

A designated UCSD site coordinator will be trained to compare the review from the local pathologist with the central pathology review and ensure timely notification to the site that the participant is eligible.

10.3 Shipping Instructions

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations. Biologic specimens (Category B, UN3373) will be in leak-proof primary and secondary receptacles with puncture resistant packaging and absorbent material. Shipments are to be preceded with phone/email contact to the receiving lab to assure the shipment will be met and processed promptly.

Tissue sections will be shipped from the study sites (or delivered at UCSD) at room temperature to the laboratory of Dr. Alfredo Molinolo at UCSD:

Alfredo A. Molinolo, M.D., Ph.D.
Moores Cancer Center, Room 3332
3855 Health Sciences Drive
La Jolla, CA 92093
(858) 246-0179

amolinolo@ucsd.edu

Frozen serum and saliva samples and frozen blood samples will be shipped overnight in batches on dry ice from the study sites (or delivered on dry ice at UCSD) to the laboratory of Dr. J. Silvio Gutkind at UCSD:
J. Silvio Gutkind, Ph.D.
Moore's Cancer Center, Room 2344
3855 Health Sciences Drive
La Jolla, CA 92093
(858) 534-5980
sgutkind@ucsd.edu

At the end of the study, frozen serum and saliva samples for metformin levels will be shipped overnight from UCSD on dry ice to:
Catherine Cordova c/o Chow Laboratory
University of Arizona Cancer Center, Room 4971
1515 N. Campbell Ave.
Tucson, AZ 85724
(520) 626-5433
ccordova@uacc.arizona.edu

10.4 Tissue Banking

Biologic specimens collected during the conduct of each clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense. The specimens will be made available to the research community. Specimens sent to researchers will be coded such that participant identity will be protected.

In concordance with the NCI Genomic Data Sharing Policy, genomic data generated in this study will be deposited in dbGaP and will be available in a controlled-access manner.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

A list of AEs that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in §6.2, Pharmaceutical Information, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are completed (including run-in) must be recorded on the AE CRC (paper and/or electronic) whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for adverse event reporting.

- AE verbatim term
- System Organ Class (SOC)
- Common Terminology Criteria for Adverse Events v4.0 (CTCAE) AE term
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a serious adverse event (SAE)
- Whether or not the subject dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using 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/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

ADL

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc.*

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.1.4 Assessment of relationship of AE to treatment

The possibility that the adverse event is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

11.2 Serious Adverse Events

11.2.1 DEFINITION: Fed. Reg. 75, Sept. 29, 2010 defines SAEs as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*).
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality/birth defect
- Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed.

11.2.2 Reporting SAEs to DCP

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE form found at http://prevention.cancer.gov/sites/default/files/uploads/clinical_trial/SAE-Form.pdf.

11.2.2.2 Contact the DCP Medical Monitor by phone within 24 hours of knowledge of the event.

DCP Medical Monitor:
Eva Szabo, MD
Chief
Lung & Upper Aerodigestive Cancer Research Group
Division of Cancer Prevention, NCI, NIH
9609 Medical Center Drive, Room 5E-102, MSC 9781
Bethesda, MD 20892-9781 (For FedEx, use Rockville, MD 20850)
Phone: (240) 276-7011
FAX: (240) 276-7848
email: szabo@mail.nih.gov

Include the following information when calling the Medical Monitor:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug and expectedness

11.2.2.3 The Lead Organization and all Participating Organizations will email (preferred) or FAX written SAE reports to the DCP Medical Monitor within 48 hours of learning of the event using the paper SAE form. The written SAE reports will also be FAX'ed (650-691-4410) or emailed (safety@ccsainc.com) to DCP's Regulatory Contractor, CCS Associates (phone: 650-691-4400).

11.2.2.4 The DCP Medical Monitor and regulatory staff will determine which SAEs require FDA submission.

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.3 Follow-up of SAE

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE form in the appropriate format. Follow-up information should be sent to DCP as soon as available. SAE related to the study agent will be followed until resolved, or deemed unlikely to further resolved by the Protocol Chair, or until the subject withdraws consent for further follow-up. SAE unrelated or unlikely to be related to study agent will be followed for at least 30 days after the last dose of study agent.

12. STUDY MONITORING

12.1 Data Management

This study will report clinical data using the OnCore application from Forte Research Systems, Inc., as stated in the Master Data Management Plan. All users of the database will have appropriate education, training and experience to perform assigned tasks. The data collection and management will be done according to the Consortia 2012 DMP.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRF) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDE). The approved CRFs will be used to create the electronic CRF (e-CRF) screens in the OnCore application. Consortia site staff will enter data into the e-CRF for transmission to DCP according to pre-established DCP standards and procedures. Amended CRF will be submitted to the DCP Protocol Information Office for review and approval. Approved changes will be programmed into the OnCore database by the Consortium Data Management staff.

CRF Submission Information:

University of Arizona Early Phase Chemoprevention Consortium Office
Attn: Bonita Weible
1430 E. Fort Lowell, Suite 304
Tucson, AZ 85719
Phone: (520) 318-7178
Fax: (520) 514-6015
Email: UACC-CPRE@UACC.arizona.edu

12.3 Source Documents

Source documentation for this trial will consist of protocol-specific source documents as well as clinical and research laboratory reports. In the event of a Serious Adverse Event, medical records related to the event will be sought for source documentation of the event and its treatment, if any.

12.4 Data and Safety Monitoring Plan

The University of Arizona Cancer Center (UACC) Data and Safety Monitoring Board (DSMB) will provide oversight for subject safety for all UA Consortium clinical trials consistent with the National Institutes of Health Policy for Data and Safety Monitoring dated June 10, 1998; further guidance statement issued by the NIH on June 5, 2000, and the policy for Data and Safety Monitoring by Data and Safety Monitoring Boards. The UACC DSMB meets quarterly.

Regular monthly meetings of the UA Consortium, are used as a forum to review accrual rates, problematic issues relating to accrual and protocol implementation, adverse events occurrence, follow-up, and reporting; submission of all required study reports; and progress and outcomes of laboratory analyses.

12.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc.*), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

Not applicable.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This study is a phase IIa single-arm, open-label trial in individuals with oral leukoplakia or erythroplakia to explore the potential of metformin for oral cancer prevention. The primary endpoint analysis is to determine whether 12-14 weeks of treatment with therapeutic doses of metformin would improve the clinical response of oral premalignant lesions. The clinical response of each participant will be evaluated. A response rate of

30% is considered as poor treatment and a response rate of 60% is considered as a good treatment. No early stopping rules due to futility or efficacy will be implemented.

13.2 Randomization/Stratification

This is a single-arm, open-label trial so no randomization, stratification or blocking will be performed. Also, no formal interim analysis is planned.

13.3 Accrual and Feasibility

The study plans to accrue 26 eligible participants to initiate agent intervention. With a projected attrition rate of 20%, we anticipate to have at least 20 participants completing the agent intervention to provide study endpoint data. The planned duration of accrual is 24 months with the expected accrual rate of 1 per month among three study sites.

13.4 Primary Objective, Endpoint(s), Analysis Plan

The primary objective of this study is to determine whether 12-14 weeks of treatment with therapeutic doses of metformin would improve the clinical response of oral premalignant lesions. Each participant's clinical response will be categorized into Complete Response (CR), Partial Response (PR), No Change (NC) or Progressive Disease (PD), evaluated by the criteria described in § 8.1. A participant with CR or PR is considered as a respondent. The preclinical data demonstrate that metformin defers carcinogen-induced HNSCC development and progression (see §2.3). Based on the positive preclinical data, the main interest of this study is in measuring and evaluating the improvement not in evaluating the unlikely negative effect. Therefore, a one-sided one-sample binomial exact test at a significance level of 5% will be performed to see if the clinical response rate is greater than 30% (poor treatment). A sample size of 20 achieves 87% power to detect a response rate 0.30 higher than a poor treatment response.

13.5 Secondary Objectives, Endpoints, Analysis Plans

The secondary objectives of this study are to evaluate the histologic response to metformin intervention, to determine the metformin effect on cell proliferation and its molecular targets, to determine the metformin effect on frequent dysregulated molecular mechanisms and OCT expression, and to determine the impact of genomic alterations on the biological and biochemical consequences and clinical response to metformin.

Additional secondary objectives include evaluation of the metformin effect on serum metabolic markers, measurements of metformin concentrations in serum and saliva, and evaluation of metformin effect on serum and saliva inflammatory and angiogenic cytokines.

Similar to the primary endpoint, the histologic response rate will be calculated and a one-sided 95% confidence interval based on the exact method will be derived. Based on a small sample size of 20, nonparametric methods, e.g. signed rank test, will be performed to evaluate each of the changes in tissue, serum, and saliva markers. In addition, a univariate logistic regression model with the clinical response as the outcome variable will be fitted to explore if any of the expression of frequent dysregulated mechanisms, OCT3 levels, and any genomic alterations is associated with the clinical response to metformin.

To identify statistically significant changes in the saliva microbiome before and after metformin treatment, we will first evaluate changes in alpha diversity among matched pairs using non-parametric analogous Wilcoxon rank-sum test (Mann-Whitney test). To test for significant differences in beta diversity (e.g. if pre-treatment and post-treatment samples cluster in principle coordinates analysis space), permutational multivariate analysis of variance (PERMANOVA) will be used. Importantly, we are currently performing experiments in healthy volunteers to define the stability of the saliva microbiome over the course of a day

and in response to routine perturbations (e.g. brushing teeth, eating a meal). The results from these experiments will allow us to determine the appropriate effect size and assess potential correlations between the saliva microbiota and therapeutic outcome.

With an anticipated attrition rate of 20%, we will try to reduce the fraction of participants with missing outcomes as much as possible. Also, multiple imputation techniques will be used to handle missing data.

13.6 Reporting and Exclusions

Participants will be considered compliant for statistical analysis if they have taken $\geq 80\%$ of their assigned study doses based on pill count. As mentioned earlier, multiple imputation techniques will be used to handle missing data.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of drug. Descriptive statistics of the type and frequency of all adverse events will be generated, including 95% confidence intervals.

13.8 Evaluation of Response

All subjects with endpoint data will be assessed for response to intervention, based on the endpoints described above in Sections 13.4 and 13.5.

Sub-analyses may be performed on the subsets of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of intervention, major protocol violations, etc.). However, sub-analyses may not serve as the basis for drawing conclusions concerning efficacy, and the reasons for excluding participants from the analysis should be clearly reported.

13.9 Interim Analysis

No formal interim statistical analyses are planned for this Phase IIA trial. Accrual, data collection, and any adverse events will be monitored on a regular basis.

13.10 Ancillary Studies

None.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Signed and dated current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 Lab certification (*e.g.*, CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.2.4 Documentation of training in “Protection of Human Research Subjects” for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.5 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 Signed Investigator’s Brochure/Package Insert acknowledgement form

14.2.7 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator for each site and initialed by all study personnel listed on the form

14.2.8 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent. A Model Consent Form for Use of Tissue for Research is available through a link in the DCP website.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the IRB at each Organization at which the protocol will be implemented.

Any subsequent changes to the informed consent must be approved by NCI, DCP, the Consortium Lead Organization's IRB, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to the DCP Regulatory Contractor:

Paper Document/CD-ROM Submissions:

Regulatory Affairs Department
CCS Associates
2001 Gateway Please
Suite 350 West
San Jose, CA 95110
Phone: 650-691-4400
Fax: 650-691-4410

E-mail Submissions:

regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review, which will then be electronically forwarded to the DCP Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

Study procedures performed during study visits will be covered by the study budget. Research tests will not be billed to the subject. Subjects may incur minimal out-of-pocket expenses for transportation but will not be charged for study agent or any study-related activities. Subjects will receive monetary compensation which they may use at their discretion for out of pocket cost such as transportation. If injury occurs, medical care will be provided and charged to the subject's insurer.

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CONSENT FORM

Study Title for Study Participants:

Testing metformin to prevent oral cancer in people with oral leukoplakia or erythroplakia.

Official Study Title for Internet Search on <http://www.ClinicalTrials.gov>:

M4OC-Prevent: Metformin for Oral Cancer Prevention

What is the usual approach to my oral leukoplakia?

You are being asked to take part in this study because you have an oral premalignant lesion (OPL). OPL looks like red or whitish plaques or lesions in the mouth that do not rub off. These areas can be associated with a higher risk of cancer. People who are at increased risk and choose not to participate in a study are usually followed closely by their doctor to watch for the development of cancer.

What are my other choices if I do not take part in this study?

Currently there are no approved medicines to treat white (leukoplakia) and/or red (erythroplakia) patches in the mouth. Alternatives to participation in this study include having no treatment at all, using other investigational agents for this condition, observation or surgery. Surgery sometimes works well, but surgery can affect the function of your tongue or mouth and the problem comes back at least one third of the time. Observation means coming to the doctor frequently to be examined for signs of cancer. It is recommended that you have regular follow-up and monitoring of any worrisome white (leukoplakia) and/or red (erythroplakia) patches in the mouth. Please talk to your doctor about specific follow-up recommendations for you.

Why is this study being done?

The purpose of this study is to test metformin to find out what effects, if any, it has on you and your risk of developing oral cancer. Metformin is a drug approved in the US for the treatment of type 2 diabetes and is a pill. The study will evaluate whether the drug can stop changes in the mouth that are related to oral pre-cancer growth (oral leukoplakia or erythroplakia) or oral cancer. There will be about 26 people taking part in this study.

What are the study groups?

This study has one study group. All participants will receive the study drug, metformin.

How long will I be in this study?

You will be in the study for up to 22 weeks. You will receive the study drug for 12-14 weeks. Even if you do not finish the study, your doctor will continue to watch you for side effects and follow your condition for 2-4 weeks.

What extra tests and procedures will I have if I take part in this study?

You will need to have the following exams, tests or procedures to find out if you can be in the study. These exams, tests or procedures may be part of regular care for someone with oral

premalignant lesions. However, they may be done more often because you are in this study. All exams, tests and biopsies will be performed in the clinic.

Before you begin the study:

You will need to have the following extra tests, and/or procedures in a Baseline Visit to find out if you can be in the study.

- The study staff will discuss this consent form with you and answer any questions you may have. Once you have signed it, the following procedures will be done.
- You will be fasting (no food or drinks, except water) for 8 hours before your appointment for the pre-study evaluation.
- A brief physical exam of your chest, heart, abdomen, and lower legs and feet as well as height, weight, and vital signs (blood pressure, pulse, and temperature).
- A review of your medical history and current medications, including any symptoms you may be currently experiencing.
- A review of your tobacco and alcohol use.
- Collection of 2 teaspoons of blood for routine blood tests for clinical care (a complete blood count and a group of blood tests of your body's chemical balance and metabolism), C-peptide (blood test that indicates if your body is producing insulin) and Hemoglobin A1c (blood test that measures the average blood sugar in your blood over the last 2-3 months).
- Collection of 2 teaspoons of blood for research samples.
- A sample of your saliva will be collected between 8 am and 12 noon, if feasible
- Urine test for pregnancy, if you are a woman who could become pregnant.
- Your mouth will be examined. Lesions (white and/or red patches) in your mouth may be measured and photographed as determined by the study physician.
- A biopsy of your mouth lesion will be obtained unless you had a mouth lesion biopsy performed within 6 weeks prior to the Baseline visit and the tissue from that biopsy is appropriate for the study and available to the researchers. A biopsy of your inside cheek will also be obtained.
- You will be given a symptom diary to record any illness or injury (adverse events) during the study.

Once you are determined to be eligible, you will return to the clinic for the following procedures:

- Urine test for pregnancy, if you are a woman who could become pregnant.
- You will be given study medication and instructions for taking it. The study medication will be taken by mouth. For your safety, as long as you have no alarming side effects, the dose of medication will be increased after the first week and increased again after the second week.
- A review of your symptom diary and current medications.
- You will be given a study calendar to mark each day you take the study medication. Alternatively, the study medication and study calendar may be mailed to you. The study staff may conduct this visit by phone.

Week 1-5

- You will be contacted by telephone or email to review any changes in your current medications, any symptoms you may be experiencing and to answer questions you may have about the study medication before and after you increase the dose of medication to the next level.

Week 6 - You will return to the clinic for the following procedures:

- A brief physical exam of your chest, heart, abdomen, and lower legs and feet as well as weight, and vital signs.
- Urine test for pregnancy, if you are a woman who could become pregnant.
- Examination of your mouth.
- A review of your study calendar, symptom diary, current medications, and change in medical history.
- Return unused medication and receive a new supply of study medication.

Week 7-11

- You will be contacted by telephone or email to review any changes in your current medications, any symptoms you may be experiencing and to answer questions you may have about the study medication.

Week 12 - You will return to the clinic for the following procedures:

- You will need to be fasting (no food or drinks, expect water) for 8 hours before your appointment.
- A brief physical exam of your chest, heart, abdomen, and lower legs and feet as well as weight and vital signs.
- A review of your tobacco and alcohol use.
- Return unused medication.
- Urine test for pregnancy, if you are a woman who could become pregnant.
- Your mouth will be examined. Lesions in your mouth may be measured and photographed as determined by the study physician.
- A biopsy of your mouth lesion and of your inside cheek will be obtained.
- A review of your study calendar, symptom diary, current medications, and change in medical history.
- Collection of 2 teaspoons of blood for routine blood tests, C-peptide and Hemoglobin A1c.
- Collection of 2 teaspoons of blood for research samples.
- A sample of your saliva will be collected between 8 am and 12 noon, if feasible.
- A review of your study calendar and symptom diary.

You will continue to keep a diary of any illness or injury for at least 2 weeks following the last dose of study agent. You will then be contacted by the study staff to go over your current medications any symptoms you may have. You will also be given the results of your final biopsy.

What possible risks can I expect from taking part in this study?

If you choose to take part in this study, there is a risk that you may:

- Lose time at work or home and spend more time in the hospital or doctor's office than usual.
- There is a risk someone could get access to the personal information in your medical records or other information researchers have kept about you. Someone might be able to trace this information back to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.

- You will be asked to complete questionnaires during the study. Some of the questions could make you feel uncomfortable. You have the right to refuse to answer any question for any reason.

The metformin used in this study may affect how different parts of your body work, such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health.

There is also a risk that you could have side effects.

Here are important points about side effects:

- The study doctors do not know who will or will not have side effects.
- Some side effects may go away soon, some may last a long time, or some may never go away.
- Some side effects may interfere with your ability to have children.
- Some side effects may be serious and may even result in death.

Here are important points about how you and the study doctor can make side effects less of a problem:

- Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.
- The study doctor may be able to treat some side effects.
- The study doctor may adjust the study drugs to try to reduce side effects.

The most common side effects that we know about metformin are shown below. Some may be serious. There might be other side effects that we do not yet know about. If important new side effects are found, the study doctor will discuss these with you.

OCCASIONAL, SOME MAY BE SERIOUS

In 100 people receiving metformin, from 4 to 20 may have:

- Diarrhea
- Nausea, vomiting
- Infection
- Pain in belly
- Heartburn
- Bloating, passing gas
- Dizziness
- Headache
- Changes in taste
- Low blood sugar
- Low blood level of vitamin B₁₂, rarely associated with anemia
- Accidental injury
- Lack of energy and strength/weakness
- Runny nose

OCCASIONAL, SOME MAY BE SERIOUS (continued)

In 100 people receiving metformin, from 4 to 20 may have:

- Abnormal stools
- Muscle pain
- Shortness of breath
- Nail changes
- Rash
- Increased sweating
- Chest discomfort
- Chills
- Flu-type symptoms
- Sudden reddening of the face and/or neck
- Abnormal heartbeat

RARE, SOME MAY BE SERIOUS

In 100 people receiving metformin, 3 or fewer may have:

- Lactic acidosis* (signs and symptoms: feeling very weak, tired, or uncomfortable; increasing sleepiness; unusual muscle pain; trouble breathing; slow or irregular heart beat; unusual or unexpected stomach pain; feeling cold; low blood pressure associated with feeling lightheaded and dizzy)

***Lactic acidosis is a rare (0.03 per 1000 patient-years) but serious toxicity associated with metformin use.** When it occurs, it is fatal in approximately 50% of the cases. Risk of lactic acidosis is highest in patients with preexisting kidney or liver failure, severe infections, congestive heart failure, or poor oxygenation. Additional risk factors are excessive alcohol consumption, major surgeries, and treatment with intravascular iodine-based contrast agents used in CT (x-ray) scans. Such scans and surgeries are not planned for this study; however, please notify your study doctor if you are planning to undergo CT scans or elective surgical procedure while on study.

POSSIBLE, SOME MAY BE SERIOUS

The frequency of some individual side effects has not yet been determined:

- Constipation
- Leg pain

Reproductive risks: You should not get pregnant, breastfeed, or father a baby while in this study. The metformin used in this study could be very damaging to an unborn baby. Check with the study doctor about what types of birth control, or pregnancy prevention, to use while in this study. If you are pregnant, you will not be enrolled on this study. If you become pregnant or suspect that you are pregnant, you must tell your study doctor right away. Getting pregnant will result in your removal from this study.

Risks related to biopsy and blood tests: Biopsies and blood tests can cause mild discomfort and/or bleeding at biopsy site and minimal pain, bruising and/or bleeding at the blood draw site. Less likely is the possibility of significant bleeding at the biopsy site requiring a visit to doctor. There will be some pain and discomfort with the **biopsy procedures** in spite of the use of the anesthetic. Risk of severe bleeding or infection from the biopsy procedure is very low. In the event that you have some oozing from your biopsy site, a single stitch will be used to stop the bleeding.

Some of the research tests may be about genes. Genes carry information about features that are found in you and in people who are related to you. Researchers are interested in the way that genes affect how your body responds to treatment.

- **What are the possible risks?**

There is a risk that someone could trace the information in a central database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.

There are laws against the misuse of genetic information, but they may not give full protection. New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

- **How will information about me be kept private?**

Your samples will be identified by a unique study code only. Information that identifies you will not be given to anyone, unless required by law. If research results are published, your name and other personal information will not be used.

What possible benefits can I expect from taking part in this study?

This study may or may not help you because we do not know how the study drugs will compare to the usual approach for your condition. This study may help us learn things that could help people in the future.

Can I stop taking part in this study?

Yes. You can decide to stop at any time. If you decide to stop for any reason, it is important to let the study doctor know as soon as possible so you can stop safely. If you stop, you can decide whether or not to let the study doctor continue to provide your medical information to the organization running the study. The study doctor will tell you about any new information or changes in the study that could affect your health or your willingness to continue in the study.

The study doctor may take you out of the study:

- If your health changes.
- If the study is no longer in your best interest.
- If new information becomes available.
- If you do not follow the study rules.
- If the study is stopped early for any reason by the sponsor, IRB or FDA.

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights.

What are the costs of taking part in this study?

The metformin will be supplied at no charge while you take part in this study. The cost of study-specific biopsies and exams, tests, and any other procedures will be paid for by the study.

The baseline oral exam, lesion photography, lesion biopsy and pathology may be considered standard of care. When appropriate, these costs will be billed to you or your insurance company. You will have to pay for any costs (including deductibles and co-payments) not covered by your health insurer.

Before you decide to be in the study, you should check with your health plan or insurance company to find out exactly what they will pay for.

If you complete all of the required visits and tests, you will receive \$100 each for the baseline visit, week 6 visit and week 12 visit at the end of the study. This money is to reimburse you for your time and help cover any cost you may have in being on the study. If for any reason you are unable to complete the entire study, the amount of compensation will be less. It will be based on how long you are in the study.

What happens if I am injured or hurt because I took part in this study?

If you feel you have been injured or hurt as a result of taking part in the study, it is important that you tell the study doctor immediately. You will get medical treatment if you are injured or hurt as a result of taking part in this study.

The study sponsors will not pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance coverage, you would be responsible for any costs. Even though you are in a study, you keep all of your legal rights to receive payment for injury caused by medical errors.

Who will see my medical information?

Your privacy is very important to us and we will make every effort to protect it. Your information may be given out if required by law. For example, certain states require doctors to report to health boards if they find a disease like tuberculosis. However, we will do our best to make sure that any information that is released will not be able to identify you. Some of your health information, and/or information about your specimen, from this study will be kept in a central database for research. Your name or contact information will not be put in the database.

There are organizations that may inspect your records. These organizations are required to make sure your information is kept private. Some of these organizations are:

- The study sponsor, the National Cancer Institute (NCI) and NCI agents and partners, and the study Coordinating Center, the University of Arizona Cancer Center.
- The Institutional Review Board, IRB, is a group of people who review the research with the goal of protecting the people who take part in the study.
- The Food and Drug Administration and the National Cancer Institute in the US, and similar organizations if other countries are involved in the study.
- The National Cancer Institute will obtain information for this clinical trial under data collection authority Title 42 U.S.C. 285.
- Every health care personnel who provides services to you in connection with this study
- Any laboratories, other individuals/organizations that analyze your health information in connection with this study as defined by protocol

Where can I get more information?

The National Cancer Institute will obtain information from this clinical trial under data collection authority Title 42 U.S.C. 285.

You may visit the NCI website at <http://cancer.gov> for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at: 1-800-4-CANCER (1-800-422-6237).

A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Who can answer my questions about this study?

You can talk to the study doctor about any questions or concerns you have about this study or to report side effects or injuries. Contact the study doctor _____ (*insert name of study doctor[s]*) at _____ (*insert telephone number*).

This section is about optional studies you can choose to take part in.

There may be some specimens (biopsy tissue, and blood serum) remaining once the study is complete. These specimens will become the property of the National Cancer Institute if you give

your permission for them to store and use your remaining samples and health information for future medical research. These samples may be stored indefinitely. You can take part in the main research study described above without giving your consent for your samples to be stored. Some of these studies may be about genes. Genes carry information about features that are found in you and in people who are related to you. Researchers are interested in the way that genes affect how your body responds to treatment.

In addition, the study team is required to put your individual genetic data generated from the main study and health information in a controlled-access database hosted by the National Institutes of Health. Only researchers who apply for and get permission to use the information for a specific research project will be able to access the information. Your genetic data and health information will not be labeled with your name or other information that could be used to identify you. Researchers approved to access information in the database will agree not to attempt to identify you.

- **What are the possible risks?**

The greatest risk to you is the unintentional release of your personal information. People may develop ways in the future that would allow someone to link your genetic or medical information back to you. It is also possible that there could be violations to the security of the computer systems used to share the codes linking your genetic and medical information to you. We will do our best to make sure that your personal information will be kept private. The chance that this information will be given to someone else is very small.

- **How will information about me be kept private?**

When your sample(s) is sent to the researchers, no information identifying you (such as your name or social security number) will be sent. Samples will be identified by a unique study code only. Researchers receiving your sample and information will not know who you are. They must also sign an agreement that they will not try to find out who you are. Information that identifies you will not be given to anyone, unless required by law. If research results are published, your name and other personal information will not be used.

- **What are the possible benefits?**

You will not benefit from taking part. The researchers, using the samples from you and others, might make discoveries that could help people in the future.

- **Are there any costs or payments?**

There are no costs to you or your insurance for storing or using your remaining samples. If any of the research leads to new tests, drugs, or other commercial products, you will not share in any profits.

- **What if I change my mind?**

If you decide you no longer want your samples to be used, you can call the study doctor, _____, (*insert name of study doctor for main trial*) at _____ (*insert telephone number of study doctor for main trial*) who will let the researchers know. Then, any sample that remains in the bank will no

longer be used. Samples or related information that have already been given to or used by researchers will not be returned.

Consent for optional studies

If you allow your remaining specimens, genetic data, and health information to be used for future medical research, you can specify your consent below. Consent for optional studies is entirely voluntary and may be withdrawn at any time. If you have any questions, please talk to the study staff or the investigator.

My biopsy tissue may be kept for use in future medical research. Yes No
(circle one)

My saliva may be kept for use in future medical research. Yes No
(circle one)

My blood serum may be kept for use in future medical research. Yes No
(circle one)

My blood may be kept for use in future medical research. Yes No
(circle one)

I agree that my study doctor, or their representative, may contact me or my physician to see if I wish to participate in other research in the future. Yes No
(circle one)

My genetic data and health information can be released, with no direct identifiers, into scientific databases. Yes No
(circle one)

This is the end of the section about optional studies.

My Signature Agreeing to Take Part in the Main Study

I have read this consent form or had it read to me. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the main study and any additional studies where I circled 'yes'.

Participant's Signature

Date of signature

Signature of person(s) conducting the informed consent discussion

Date of signature

APPENDIX A

Karnofsky Performance Status Criteria

Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.