A pilot study of metformin as a chemoprevention agent in non-small cell lung cancer

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List of Abbreviations

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IND	Investigational New Drug Application
iPSC	Induced Pluripotent Stem Cells
IRB	Institutional Review Board
NSCLC	Non-Small Cell Lung Cancer
PHI	Protected Health Information
PI	Principal Investigator
RFS	Recurrence Free Survival
SAE	Serious Adverse Event/Serious Adverse Experience
SCC	Squamous Cell Carcinoma
SOP	Standard Operating Procedure

Study Summary

Title	A pilot study of metformin as a chemoprevention agent in non-small cell lung cancer
Running Title	A pilot study of metformin as a chemoprevention agent in non-small cell lung cancer
Protocol Number	12-006865
Phase	Phase II-Pilot study
Methodology	Patients will be identified and enrolled from Thoracic Surgical Clinics. Patients will undergo surgical resection with the collection of tumor tissue, normal lung, blood, and skin biopsies. For patients enrolled in the study that were found to have stage IA-IIIA SCC, starting less than 90 days from the time of surgery, patients will be randomized to receive either 850 mg po BID of metformin or observation. Metformin dosing will include a 4 week ramp up of 850 mg po daily prior to starting the BID dose, for a total of 6 months of Metformin. Patients will be followed for 2 years from the time of surgery to evaluate 2-year Recurrence Free Survival.
Overall Study Duration	08/01/2012 to 07/30/2017
Subject Participation Duration	2 Years
Single or Multi-Site	Single Site
Objectives	1. To evaluate the feasibility of patient randomization, accrual, and tissue collection in a pilot study of metformin versus observation following resection of stage IA-IIIA lung squamous cell cancer for patients. 2. To compare the 2-year recurrence free survival rate between metformin and observation.
Number of Subjects	100
Diagnosis and Main Inclusion Criteria	Suspected or biopsy proven Stage IA-IIIA NSCLC (must be proven squamous cell carcinoma at the time of surgery)medically fit for surgical resection (based on surgeon assessment), current or prior smoker, age >18, no existing untreated or prior cancer <5 years from diagnosis, willing and able to consent to study, undergo study interventions, and take study drug; not diabetic or currently taking metformin or other diabetic drug, ECOG performance status 0,1, or 2.
Study Product, Dose, Route, Regimen	Metformin dosing will include a 4 week ramp up of 850 mg po daily prior to starting the BID dose.
Duration of Administration	Six Months

Reference therapy	Observation

Introduction

Insufficient data for the feasibility of enrolling lung cancer patients to a trial protocol of metformin versus placebo with concurrent collection of biospecimens raises questions regarding the potential of performing a large, randomized, phase III study. The proposed pilot study will collect data to demonstrate the potential for enrolling patients to such a protocol, assess the feasibility of performing the study using the CCOP mechanism, collecting biospecimens from patients on study, and generate preliminary data to assess if a large, randomized, phase III study is indeed feasible and justified.

Metformin is a widely prescribed oral medication used as front-line therapy for type 2 diabetes. It has been shown to inhibit the growth of cancer cell lines, including breast cancer, in vitro and in vivo tumor models. Population and retrospective studies showed that metformin decreases the incidence of cancer and cancer-related mortality, and increases the response to neoadjuvant chemotherapy in diabetic patients. As a result, metformin is being investigated as a therapeutic agent in different clinical settings for many cancer subtypes. Evidence for an anti-cancer effect in NSCLC would justify its use as a preventative agent in the patient with treated disease. Successful execution of the correlative studies described will increase understanding of genomic changes in the precursor lesion bronchial dysplasia, and reveal potential therapeutic targets to prevent disease progression.

Phase I/II studies directed toward effects on existing tumors or pre-invasive lesions with surrogate endpoints have shown promise, but may not translate to real effects on the histologically normal tissue and cell types that are the true targets of chemoprevention. Our recent in vitro data indicating heterogeneity in metformin response suggest that all patients may not respond equally to its potential chemoprevention effects. The preliminary data to be gathered in the proposed pilot study will be critical to evaluate the feasibility of performing a randomized, phase III trial addressing this question as an Alliance study through the cooperative group infrastructure. The pilot data will also serve as important preliminary data for a correlative studies R01 grant in conjunction with a larger trial, with aims of evaluating the effects of metformin on patients with resected Stage IB-IIIA NSCLC, creating cohorts of metformin responders and non-responders based on disease recurrence, and finally generating iPS cells for in vitro studies and development of a metformin response index. This pilot trial will also address an under-represented area (lung cancer) in the Alliance Prevention Committee portfolio.

1.1 Background

Non-small cell lung cancer (NSCLC)

Worldwide, lung cancer remains the leading cause of cancer-related death in men and the second leading cause of cancer-related deaths in women (Jemal et al., 2011). The American Cancer Society estimates an incidence of 221,130 new cases of lung cancer for 2011 with 156,940 deaths (Siegel et al., 2011). Non-small cell lung cancer (NSCLC) accounts for more than 80% of all pulmonary neoplasms with greater than 60% of patients presenting with advanced or metastatic disease at the time of diagnosis [SEER; Little 2007]. Despite having a better chance for cure following surgical resection, even patients with early stage disease have a relatively high

risk of recurrence, with reported local recurrence rates ranging from 13% to 45% for Stage I disease and 17% to 55% for Stage II disease [Kelsey 2009, Lardinois 2005, Goodgame 2009]. Despite advances in screening, targeted therapies, adjuvant chemotherapy, minimally invasive surgery, and stereotactic radiation, the overall approach to lung cancer care has changed little in over 25 years, with relatively minimal improvements in survival.

Metformin as a chemoprevention agent

Metformin is an insulin sensitizer commonly used to treat diabetes. It lowers serum insulin levels and also directly inhibits cell growth. In addition to inhibiting gluconeogenesis, it also activates AMP kinase in epithelial cells by an LKB dependent mechanism. AMP kinase activation suppresses cell proliferation in malignant and nonmalignant cells by two different pathways -- inhibition of mTOR as well as through upregulation of p53/p21. Metformin may exert anti-tumour effects through both insulin dependent and insulin-independent mechanisms. Metformin also has indirect anti-proliferative effects related to lower systemic levels of insulin. The drug is well tolerated even by individuals without diabetes and does not cause hypoglycemia. Its main limited side effects are transient nausea and diarrhea. Lactic acidosis, a major adverse effect, is rare and seen only in those with concomitant renal failure or liver disease. Recent studies have shown its potential as a cancer prevention drug in cancers such as breast, prostate, pancreas, and colon.

In observational epidemiologic work, Evans et al. reported the risk of subsequent cancer diagnosis (all cancer types) was reduced in type 2 diabetics who received metformin (OR 0.85 for any vs. no metformin exposure). The protective effect increased with greater metformin exposure (measured as total dose prescribed or total duration of use). Additionally, Bowker et al. reported cancer mortality was lower in diabetics receiving metformin (vs. sulfonylureas or insulin, HR 0.50-0.77), but they did not study diabetics not receiving any drug therapy. Furthermore, while confirming lower cancer risk in diabetics receiving metformin (versus those not receiving metformin). Finally, Libby et al. have reported a significantly reduced risk of incident cancers (HR 0.63, 95% CI 0.53-0.75) in diabetics receiving metformin (versus those not receiving metformin); they recommended a randomized trial be conducted to “assess whether metformin is protective in a population at high risk for cancer”. Although many preventative agents have been or are being explored in NSCLC, such as sulindac, celecoxib, selenium, and others, none to date have shown proven benefit.

Metformin for non-small cell lung cancer

Large, phase III randomized studies for evaluating the effects of metformin as a chemoprevention agent are already underway in breast cancer. Although preliminary data suggests a potential effect in non-small cell lung cancer (Memmott RM et al., 2010), no trials addressing the question are yet open and enrolling. The recurrence rate for treated NSCLC ranges from 30-70% based on clinical and pathologic stage. For patients in the Stage IB-IIIA stage categories, adjuvant platinum-based chemotherapy has shown proven benefit with an increase in 5 year survival of 5-13%. Despite this improvement, recurrence rates remain high. Preliminary data has suggested activity of metformin in reducing cancer risk. Because metformin is a generic, inexpensive, well known and generally well tolerated oral agent that is commonly used to treat diabetes (including lung cancer patients who have diabetes), its evaluation as a potential adjuvant treatment for NSCLC can take place in an accelerated fashion. As lung

squamous cell carcinoma commonly affects the proximal airways, in contrast to lung adenocarcinoma, which typically affects more distal airway-alveolar units, the potential to bronchoscopically survey for precursor lesions such as bronchial dysplasia or carcinoma in-situ is feasible. Given this, we will institute the described pilot study protocol to evaluate the feasibility of metformin administration and tissue collection and to characterize the genomic alterations associated with these disease phenotypes under the influence of metformin administration. Interestingly, metformin has been linked to a number of the newly-identified genes identified with significant mutations in lung squamous cell carcinoma, including PTEN, PIK3CA, TP53, TSC1, BRAF, Notch1 and CREBBP. Whether metformin modifies the functional outcome for any of the novel mutations identified in invasive lung squamous cell carcinoma will be addressed in this pilot study.

Genomic alterations in the development and progression of NSCLC

A convergence of the ongoing rapid decrease in cost combined with increases in throughput for massively parallel sequencing technologies have set the stage for an exponential increase in the amount of detailed tumor sequencing data available. Pending results from the NCI sponsored TCGA projects in addition to other large-scale sequencing efforts for NSCLC will no doubt shed further light on recurring mutations in NSCLC and relevant molecular subtypes. DNA sequencing of NSCLC has been incredibly productive to date. The potential for EGFR-directed therapy in lung adenocarcinoma was originally described by Paez et al. (2004), in a project sequencing the activation domain from numerous tyrosine kinase receptors in the human genome from NSCLC tumor specimens. Through this work and other studies (Lynch et al., 2004), mutations in EGFR were discovered that correlated with response to gefitinib, and the era of targeted therapy for lung adenocarcinoma was born. EGFR-directed tyrosine kinase inhibitors such as erlotinib and gefitinib have now been studied extensively in a number of large international clinical trials, demonstrating equivalence with platinum-based chemotherapy for patients harboring sensitizing EGFR mutations (Mok et al., 2009). In a similar manner, EML4/ALK translocations were discovered by sequencing an expression library from a surgically resected lung adenocarcinoma specimen. Soda and colleagues (2007) found an inversion within chromosome 2p that leads to the formation of a fusion gene comprising portions of the echinoderm microtubule-associated protein-like 4 (EML4) gene and the anaplastic lymphoma kinase (ALK) gene. Functional studies in mouse NIH3T3 cells demonstrated that forced expression of this human fusion tyrosine kinase generated transformed foci in culture and subcutaneous tumours in nude mice. Data suggest that patients who harbor this mutation do not benefit from treatment with EGFR-kinase inhibitors, but do show sensitivity to ALK inhibition. Recent clinical studies have shown that patients with EML4-ALK fusion NSCLC have a better overall survival with crizotinib treatment, a novel ALK inhibitor, than with standard platinum-based chemotherapy (Shaw et al., 2011).

Despite these dramatic advances, little is known about precursor lesions for NSCLC. Adenocarcinoma in situ (AIS) remains a poorly understood entity from the standpoint of genomic alterations. The availability of well-characterized biospecimens for genome sequencing studies has hampered efforts in understanding this presumed precursor lesion for lung adenocarcinoma. Lung squamous cell carcinoma, in contrast, has a readily accessible precursor lesion termed bronchial dysplasia. Bronchoscopic studies of patients with NSCLC show a high frequency of bronchial dysplasia and carcinoma in situ. No detailed genomic characterization of

such lesions has yet been performed. Targeted agents which have been developed for the treatment of lung adenocarcinoma are largely ineffective for lung squamous cell carcinoma. The underlying genomic alterations in lung SCC are poorly described, and no targeted agents are currently approved for the treatment of lung SCC.

Metformin pharmacogenomics using patient-derived iPS cells

A number of recent pilot chemoprevention trials in multiple tumor types have attempted to show signals for specific agents by applying them first against established, invasive tumors. The validity of this strategy in identifying chemoprevention agents remains to be determined. A contrarian view holds that such a strategy will never accurately identify true chemoprevention agents. Histologically normal bronchial airway epithelia represent the field within which pre-invasive and then invasive malignancy develops in lung SqCC, and these cells represent the true target tissue for chemoprevention strategies in this disease. Assessing "response" however in the absence of a target lesion remains elusive. A further potential flaw in standard chemoprevention approaches is the unlikely possibility that all patients will show equivalent responsiveness to a specific strategy. Correctly identifying "responders" and "non-responders" to most chemoprevention agents has not been feasible, but may represent a crucial link to finding successful approaches. For example, recent data have suggested that LKB1 (STK11) mutations may influence responsiveness to either metformin or phenformin in vitro (Shackelford et al., 2013). Such genotypes in developing tumors might account for some of the heterogeneity in metformin responsiveness that has been observed in epidemiological studies across multiple tumor types.

Since the discovery of induced pluripotent stem cells (iPSCs) in 2007, this technology has been used to model diseases, interrogate drug response and toxicity, and create multiple differentiated cell types in unlimited quantity to evaluate potential for therapeutic transplantation (Yamanaka, 2012). Through ectopic expression of a series of pluripotency transgenes in somatic cells, specifically Oct4, Sox2, Nanog, and c-Myc, these cells develop properties similar to embryonic stem cells, with the ability to self-renew and differentiate into any cell population from all 3 primary germ layers (Takahashi et al., 2007). Because this technology does not involve embryo destruction, it bypasses the ethical controversies associated with derivation of traditional embryonic stem cells. In addition to the compelling possibility of using iPSCs for therapeutic transplantation, there is a growing realization that in vitro pharmacogenomic studies may be an important application. Patient-derived iPS cells have been utilized to evaluate the pharmacogenomics of drug response in a growing number of disease and organ models. This principle has been applied to study cardiotoxicity, hepatotoxicity, and other pharmacogenomic paradigms in a patient, cell type and disease-specific context not feasible using other systems (Liang et al., 2013 and Medine et al,. 2013). Patient-specific iPS cells and their differentiated progeny may represent a new model for personalized medicine, with the ability to exhibit "patient in a dish" disease phenotypes or drug responses (Mordwinkin, et al., 2013). This model system represents unexplored potential to evaluate patient-specific responsiveness in chemoprevention.

1.2 Dose Rationale and Risk/Benefits

Metformin is mainly associated with gastro-intestinal side effects. Previous trials have been done comparing metformin to a placebo that provided information about the frequency and magnitude of the main side effects and the contribution of GI symptoms to dropout rates. It was found that gastrointestinal toxicity occurred more frequently in patients receiving metformin than placebo. However lower doses of metformin (our dose is 1700mg/day) lead to less drop out than doses ≤ 2000mg/day. All patients are unlikely to respond equally to the chemoprevention effects of metformin. Evaluation of metformin response in vitro using lung epithelial cells derived from iPSCs offers a model to develop strategies for individualized chemoprevention.

2 Study Objectives

Primary objective for pilot study:

1. To evaluate the feasibility of patient randomization, accrual, and tissue collection in a pilot study of metformin versus observation following resection of stage IA-IIIA lung squamous cell cancer for patients.
2. To compare the 2-year recurrence free survival (RFS) rate between metformin and observation.

Secondary objective(s) for pilot study:

1. To develop the metformin sensitivity index based on iPS cell lines from the first 20 patients randomized to the metformin arm.

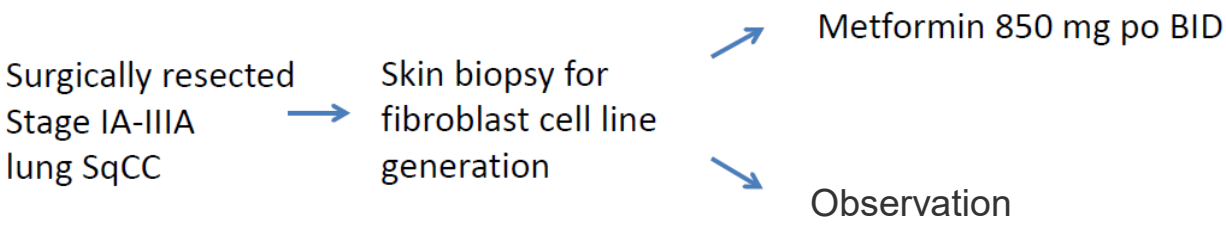
2. To apply the metformin sensitivity index on the remaining 25 patients randomized to metformin and the 45 observation patients (70patients total) to compare the 2-year RFS rate between metformin-sensitive and metformin-not-sensitive patients

3. To compare adverse events between metformin and observation arms using CTCAE version 4.

3 Study Design

3.1 General Design

Patients will be identified and enrolled from Thoracic Surgical Clinics. Patients will undergo surgical resection with the collection of tumor tissue, normal lung tissue, blood, and skin biopsies. For patients enrolled in the study that were found to have stage IA-IIIA SCC, starting less than 90 days from the time of surgery, patients will be randomized to receive either 850 mg po BID of metformin or observation. Metformin dosing will include a 4 week ramp up of 850 mg po daily prior to starting the BID dose, for a total of 6 months of Metformin. Patients will be followed for 2 years from the time of surgery to evaluate 2-year RFS. We plan to randomize a total of 100 patients (50 per arm), such that 90 patients are evaluable for the primary endpoint.



3.2 Embedded Correlative Studies

Patients will be enrolled in the correlative studies as the volume of tissue permits.

1. Derivation of fibroblast cultures from skin biopsy samples.
   Skin biopsies will be taken at the time of surgical resection. Biopsy samples taken in surgery will be taken as a 2mm x 2cm excision of a wedge of skin at the edge of the incision. Such excisions are frequently done as part of routine skin closure for optimal skin cosmesis following surgery. Samples are transported to the laboratory, and the epidermis and hypodermis removed. The dermis is cut into small pieces (<2 mm^2) before plating in each well of a 6-well plate along with Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 1% L-glutamine and 1% penicillin/streptomycin. Within an average of nine days, dermal fibroblasts are visible by light microscopy.

2. iPS cell reprogramming from skin fibroblasts.
   For defined metformin "responders" and "non-responders", iPSCs will be generated. We plan to create a total of 50 iPS cell lines for subsequent in vitro differentiation and testing of metformin response. Fibroblast cell lines are plated at 10^5 per 24 wells then transduced with infectious supernatants bearing the stemness factors OCT3/4, SOX2, KLF4 and c-MYC25 . Transduced fibroblasts are re-plated at confluence and iPS cells isolated after two weeks of clonal expansion. Cells are assessed by phase contrast microscopy for morphologic characteristics of iPS cells. iPS cell reprogramming will be performed in conjunction through the Regenerative Medicine Biotrust with our commercial partner ReGen Theranostics.

3. Evaluation of genomic alterations in histologically normal airway epithelium, pre-invasive lesions of lung SqCC, and invasive SqCC.
   DNA from biopsy specimens will be evaluated for DNA quantity and quality and subjected to mutation analysis using our panel of known genes. DNA will be prepared using standard protocols. A number of samples will also be used for exome sequencing as DNA quantities and funding permit. Data will be analyzed for the presence of mutations not present in germline DNA obtained via a blood sample. Mutation profiles will be compared between all patients receiving metformin versus placebo and correlated with clinical outcome. The subject will give a 4ml blood sample (collected in EDTA tube) to be used as normal comparative sample of DNA.

4. Biospecimen banking of blood, normal lung, lung tumor, and skin biopsy specimens and derivitives.
   All patients participating in the trial will be provided an opportunity to participate in Biospecimen banking for the correlative studies described above and future studies.

Samples will be stored in the Mayo Clinic Lung Specimen Registry, Regenerative Medicine Biotrust repository, or in Dr. Wigle's Thoracic Oncology Laboratory.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

- Suspected or biopsy proven Stage IA-IIIA lung squamous cell carcinoma (must be proven SCC at the time of surgery)
- Medically fit for surgical resection (based on surgeon assessment)
- Current or prior smoker
- Age > 18 years old
- Both Male and Female
- Willing and able to consent to study, undergo study interventions, and take study drug
- ECOG performance status 0, 1, 2
- Subject must start Metformin within 90 days of surgery.

4.2 Exclusion Criteria

- Currently taking metformin or other diabetic drugs
- Current or previous congestive heart failure, renal failure or liver failure
- Creatinine in Women of 1.4 or greater and Creatinine in Men of 1.5 or greater
- Existing untreated or prior cancer <5 years from diagnosis
- Received neo-adjuvant platinum-based chemotherapy or targeted therapy
- Receiving adjuvant platinum-based chemotherapy or targeted therapy after surgical resection

4.3 Subject Recruitment, Enrollment and Screening

- From the Principal Investigator or Co-Investigator clinical practices
- Evaluation of inclusion/exclusion criteria
- Informed consent

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

- Subject develops a diagnosis of diabetes
- Failure of subject to adhere to protocol requirements
- Subject decision to withdraw from the study (withdrawal of consent)

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Even though a subject has withdrawn from the study, it may be important to collect some follow-up or survival data on such subjects throughout the protocol defined follow-up period. Such data is important to the integrity of the final study analysis since early withdrawal could be related to the safety profile of the study drug. If a subject withdraws consent to participate in the study, for subject safety reasons, attempts should be made to obtain permission to collect follow up information whenever possible.

5 Study Drug

5.1 Description

The metformin will be compounded and supplied in 850 mg capsules form by the Mayo Clinic Pharmacy, Research Support.

5.2 Treatment Regimen

Metformin dosing will include a 4 week ramp up of 850 mg po daily prior to starting the BID dose.

5.3 Method for Assigning Subjects to Treatment Groups

The randomization will occur using an envelope system that has been prepared by the statistical department. A series of numbered envelopes will hold the designation of treatment arm. After the subject is consented the next envelope in numerical order will be opened, randomly assigning the treatment arm, by the study team.

5.4 Preparation and Administration of Study Drug

The metformin will be prepared by the Mayo Clinic Pharmacy, Research Support. At the time of the visit it will be distributed by the study team. Subsequent refills will be mailed to the subject by the pharmacy. The patient will receive dosing instruction by the study team.

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5.5 Subject Compliance Monitoring

Subjects will be provided a medication diary in which they should track their administration of the metformin. The study coordinator will make a monthly telephone call to assess the subject's compliance. Subjects who are found to be less than 90% compliant will be counseled in the importance of taking the treatment daily and the PI will be notified.

5.6 Prior and Concomitant Therapy

The subject's medications will be collected and reviewed prior to initiating the metformin or observation therapy to check for prior diabetes treatment. Subjects will be excluded if the are receiving treatment with diabetic agents. During the monthly telephone call the study coordinator will assess for any concomitant treatments with diabetic agents.

5.7 Packaging

The metformin will be packaged by the Mayo Clinic Pharmacy, Research Support. It will be distributed in 3 month increments of drug. The initial doses will be distributed to the subject by the study team. Refills will be sent to the subject by the Mayo Clinic Pharmacy, Research Support via mail.

5.8 Receiving, Storage, Dispensing and Return

5.8.1 Storage

The study agents will be stored by the Mayo Clinic Pharmcy, Research Support under their policies and procedures

5.8.2 Dispensing of Study Drug

The pharmacy will be notified by the study team of subject enrollment and which arm the subject has been randomized to. They will then dispense the appropriate agent to the subject.

5.8.3 Return or Destruction of Study Drug

The remaining study agent will be returned to the study team following 6 months of metformin administration. At the completion of the study, there will be a final reconciliation of drug shipped, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Visit 1

The subject will be identified by the Thoracic Surgical Clinics. The study coordinator will consent and enroll the subject. The subject will have a blood sample taken for research.

6.2 Visit 2

The subject will undergo surgical resection as part of their standard of care treatment. Stage IA-IIIA squamous cell carcinoma of the lung will be confirmed. During the surgical resection the patient will undergo collection of tumor tissue, normal lung tissue, and skin biopsies for research.

6.3 Visit 3

At the time of the subjects follow up (less than 90 days from the time of surgery) the subject will be seen be the study team. Eligibility criteria will be assessed and if the subject is still eligible they will be randomized and receive their study treatment (metformin or observation).

6.4 Monthly Telephone Calls

The study team will contact the subjects in the group taking Metformin monthly via telephone to assess drug compliance, adverse events, and any new diagnosis.

6.5 Half Yearly Follow Up

The subjects will be followed every 6 months following surgery for a total of 2 years. At the time of the first 6 month follow up the subjects receiving metformin will return any unused study drug and drug reconciliation will occur.

7 Statistical Plan

7.1 Statistical Methods

All patients meeting the eligibility criteria, and who started treatment, will be considered evaluable for the 2 year RFS rate endpoint. The proportion of 2 year RFS rates (along with the 95% confidence interval) will be estimated within each arm by the number of patients who are recurrence-free and alive at 2 years divided by the total number of evaluable patients in each arm, and compared using a chi-squared test.

Clinical data regarding recurrence-free survival (RFS) will be utilized for development of the metformin sensitivity index. Data collected will be summarized using descriptive statistics, including mean and standard deviation and median and inter quartile range for continuous variables, and using frequencies and percentages for categorical variables. Logistic regression models will be used to assess the impact of metformin sensitivity index metrics, treatment, and the interaction between the metformin sensitivity index metrics and treatment on the 2 year RFS status as the outcome of interest.

Adverse events (AEs) will be compared between metformin and placebo using CTCAE version 4. To evaluate the AE profiles associated with each intervention arm, the maximum grade for each type of AE will be recorded for each participant and frequency tables will be reviewed to determine the overall patterns. The number and severity of AEs (overall and by intervention group) will be tabulated and summarized across all grades. Chi-square tests will be used to compare the AE rates between metformin and placebo.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

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

Adverse Event Reporting Period
Adverse events will be reported after the subject begins taking the metformin or starts the observation period. The bronchoscopy and surgery are considered standard of care.

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

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

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in

this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

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

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also on the appropriate adverse event worksheet. All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

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

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

8.4 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 “Study Monitoring, Auditing, and Inspecting”). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9 Data Handling and Record Keeping

9.1 Confidentiality

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

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

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

9.2 Source Documents

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

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. Do not erase or use "white-out" for errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. If the reason for the correction is not clear or needs additional explanation, neatly include the details to justify the correction.

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents as outlined in the Mayo Clinic Research Policy Manual –"Access to and Retention of Research Data Policy."

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

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

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

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

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

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

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This study is financed through a grant from the Alliance for Clinical Trials in Oncology.

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor-investigator prior to participation in this study.

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