THE UNIVERSITY OF TEXAS
M. D. ANDERSON CANCER CENTER

Tumor mutation status will predict metabolic response to metformin in NSCLC

ELIGIBILITY CHECK

_____(Y) 1. Does the patient have histologic proof of lung cancer documented by biopsy sufficient for mutational analysis?

_____(Y) 2. Does the patient have non-small cell lung cancer documented by FDG PET/CT and negative brain MRI?

_____(Y) 3. Is the patient medically inoperable or refuse thoracic surgery?

_____(N) 4. Does the patient have a random blood glucose >200 mg/dl or is the patient currently on metformin or other hypoglycemic agent?

_____(N) 5. Does the patient have a creatinine > normal institutional limits or a diagnosis of chronic kidney disease?

_____(N) 6. Has the patient ever had lactic acidosis?

_____(Y) 7. Does the patient plan to receive hypofractionated RT at MD Anderson?

_____(Y) 8. Has the patient signed a study-specific informed consent form?

_____________________________Patient's Name

_____________________________Verifying Physician

_____________________________Patient ID#

_____________________________Birth date

_____________________________Sex

_____________________________ Race

_____________________________Radiotherapy Completion Date

_____________________________PET/CT Schedule Date

Completed by ___________________________________ Date ___________________

1.0 Introduction

Stereotactic Body Radiotherapy (SBRT) for NSCLC

Lung cancer remains the leading cause of cancer death in the United States in 2010 (Jemal, Siegel et al. 2010). SBRT for stage I lung cancer in medically inoperable patients appears promising, the RTOG 0236 phase II multi-institutional study achieved 3-year survival and local control rates of 55.8% and 90.6% (Timmerman, Paulus et al. 2010). The goal of SBRT is the delivery of higher doses per treatment over fewer treatments, otherwise known as hypofractionated RT. In comparison, the local control rate for conventional RT was reported as 30 to 40% (Armstrong and Minsky 1989; Dosoretz, Katin et al. 1996). However, as tumor size increases, both local and distant failure rates increase, with local failure rates of close to 40% in some series (Chi, Liao et al. 2010). Thus, additional therapy for these patients is required.

^{18}F-FDG PET/CT after SBRT for NSCLC

One difficulty in utilizing SBRT or hypofractionated RT for therapy is determining response to treatment. CT scans following SBRT are difficult to interpret due to post-SBRT lung consolidation, making accurate estimations of local failure difficult (Bradley, Moughan et al. 2010). Retrospective review has suggested the utility of using post-treatment FDG-PET/CT scans for determining local failure following SBRT (Henderson, Hoopes et al. 2010; Chang, Liu et al. 2012), with a post-SBRT maximum SUV threshold of 5 associated with a negative predictive value of 100%. However, the utility of PET/CT in this context, as well as the optimum timing of imaging following SBRT has yet to be determined.

Metformin as an anti-cancer agent

Several epidemiologic studies have shown significantly decreased rates of non-small cell lung cancer (NSCLC) in diabetics taking metformin (Hsieh, Lee et al. 2012; Mazzone, Rai et al. 2012). Although this phenomenon is not universal (Smiechowski, Azoulay et al. 2012), a recent meta-analysis of over 210,000 total patients and 21,000 diabetics found that lung cancer incidence was significantly decreased in metformin users (RR 0.67, p=0.05)(Noto, Goto et al. 2012). Data from similar populations implicate this drug in improved survival following a cancer diagnosis. Specifically, in the meta-analysis described above, metformin was found to be associated with a decrease in cancer mortality in all sites (RR 0.66, p=0.005)(Noto, Goto et al. 2012). Additionally, several studies have linked improved survival following a lung cancer diagnosis and metformin use. Specifically, Tan and colleagues showed that patients with locally advanced NSCLC taking metformin had a median overall survival (OS) of 20 months (Tan, Yao et al. 2011). The remaining patients were then stratified by glucose control regimen (insulin vs. other); with median OS being approximately 13 months in both groups. This is in line with our own observations, with metformin use being independently associated with improved OS on multivariate analysis in both HNSCC and NSCLC (Skinner, Sandulache et al. 2012). There are significant pre-clinical data showing that metformin exerts an anti-neoplastic effect on a variety of lung cancer cell lines as well as in preclinical animal models (Algire, Zakikhani et al. 2008; Algire, Amrein et al. 2011; Zaugg, Yao et al. 2011; Ashinuma, Takiguchi et al. 2012; Chaudhary, Kurundkar et al. 2012; Javeshghani, Zakikhani et al. 2012; Kato, Gong et al. 2012; Luo, Hu et al. 2012; Menendez, Oliveras-Ferraros et al. 2012; Salani, Maffioli et al. 2012; Sikka, Kaur et al. 2012; Wu, Li et al. 2012).

Metformin is also a potent radiosensitizer. This phenomenon was first described by Sanli and colleagues, with metformin acting to radiosensitize NSCLC cells independent of LKB1 activation (Sanli, Rashid et al. 2010). We and other groups have found similar effects in multiple tumor types in the aero-digestive tract, including cancer of the head and neck (HNSCC), esophagus, and lung (Liu, Scholz et al. 2012; Song, Lee et al. 2012).

Biomarkers of metformin sensitivity

The mechanisms underlying the anti-neoplastic function of metformin are complex, although AMPK activation and mTOR inhibition are thought to play an important role. Metformin as well as another biguanide, phenformin, were found to have a much more striking effect in tumors with absent LKB1 (Algire, Amrein et al. 2011; Shackelford, Abt et al. 2013). However, this observation may not be universal, as a separate study showed the effectiveness of metformin in LKB1 intact cells (Xiao, He et al. 2012). The anti-neoplastic effects of metformin have also been found to be limited to tumor cells with non-functional p53 (Buzzai, Jones et al. 2007), a finding that is consistent with work in our laboratory (Sandulache, Skinner et al. 2012; Skinner, Sandulache et al. 2012). Tumor cells with dysfunctional p53 and/or LKB1 lack the capability of responding appropriately to metabolic stress, leading to cell death following treatment with a metabolically targeted agent like metformin, while sparing normal surrounding cells (Pollak 2012).

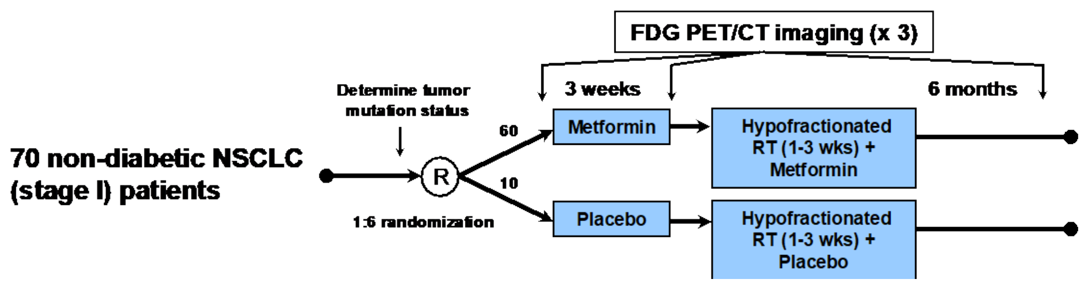

Figure 1. Protocol timeline. [70 non-diabetic study subjects will enroll in this early phase II study. Each will receive a ^{18}F-FDG PET/CT imaging of their lung tumor on entry. Biopsy material will tested for mutations. Patients will be stratified by tumor size (≥4 cm vs. <4 cm) and randomized at 1:6 weighted to metformin treatment. Subsequently, patients randomized to metformin treatment will receive 3 weeks of metformin followed by repeat ^{18}F-FDG PET imaging. All patients will receive their primary SBRT treatment per our standard practice. They will take metformin until completion of RT. They will have

Metformin and FDG-PET/CT

It is known that metformin alters the uptake of FDG in normal tissues. Specifically, metformin causes a selective increase in FDG-uptake in normal bowel (Gontier et al., 2008; Massollo et al., 2013; Ozülker et al., 2010), as well as in colon cancer cells *in vitro*(Habibollahi et al., 2013). However, metformin administration significantly decreased cell proliferation and FDG uptake in neoplastic tissue in mice fed a high fat diet (Mashhedi et al., 2011). On retrospective review we have observed that metformin use does not preclude the use of FDG-PET/CT as a surrogate response for response in esophageal cancer (Skinner et al., 2012b) following chemoradiation.

Hypothesis: Tumor mutation status (genotype) versus glucose uptake (phenotype) will more accurately predict metformin therapy response in lung cancer.

Significance and summary

This is a prospective trial evaluating the use of the imaging of glucose uptake via FDG-PET/CT scan as well as mutations associated with pre-clinical sensitivity to the drug metformin as possible clinical useful indicators of tumor sensitivity to this drug. We will perform a 1:6 randomization of patients stratified by tumor size (≥4 cm vs. <4cm), with a goal of 60 patients treated with open label metformin and hypofractionated RT as described below and 10 patients treated with hypofractionated RT alone. Subjects will have a core biopsy of the primary tumor and FDG-PET/CT scans at baseline and 6 months after hypofractionated RT as well as an additional FDG-PET/CT scan 3 week after starting metformin. Although multiple groups are investigating the use of metformin as an adjunct to cancer therapy, its effects as an cancer therapeutic remain to be tested. Additionally, it is unclear what tumor biomarkers will be predictive for tumor response to this agent. Further, metformin has an unclear effect on FDG uptake, with both decreases and increases reported in the literature (Gontier et al., 2008; Habibollahi et al., 2013; Mashhedi et al., 2011; Massollo et al., 2013; Ozülker et al., 2010). As FDG-PET/CT is commonly used to asses response to therapeutic agents in cancer, it is important to determine the effect of metformin on this imaging modality in the prospective setting. Combining genetic and imaging profiles may also be useful to enrich for patients likely to respond to this agent. (Gontier et al., 2008; Massollo et al., 2013; Ozülker et al., 2010)

2.0 Objectives

2.1 To determine the effect of metformin on response in NSCLC patients treated with hypofractionated RT

2.2 To characterize the effect of metformin on tumor FDG-avidity.

2.3 Secondary objectives:

2.3.1 To evaluate mutations that lead to increased sensitivity to metformin.

2.3.2 To compare the anatomic versus metabolic response to metformin and hypofractionated treatment.

3.0 RECRUITMENT AND SELECTION

Patients with a pathologic diagnosis of NSCLC who are scheduled to receive thoracic hypofractionated RT will be recruited for this study. 70 patients will be involved in this single-blinded randomized study. All patients will undergo a FDG-PET/CT scan at baseline, following 3 weeks of metformin and at 6 months post-SBRT. Subjects will not be excluded based on age, gender, economic status, or race.

3.1 Patients will sign consent for the imaging procedure.

3.1.1 Patient will be registered on protocol in CORe.

3.1.1.1 Inclusion Criteria

3.1.1.1.1 Patients with pathologic diagnosis of lung NSCLC or squamous cell carcinoma.

3.1.1.1.2 Patients are to be treated with hypofractionated RT.

3.1.1.1.3 Patient is not a surgical candidate due to medical comorbidities determined by a thoracic surgeon or patient refusal.

3.1.1.1.4 Patient plans to receive treatment at MD Anderson.

3.1.1.1.5 Patients must sign informed consent.

3.1.1.1.6 Patient must have adequate renal function within 30 days prior to registration, defined as serum creatinine within normal institutional limits or creatinine clearance at least 60 ml/min

3.1.1.2 Exclusion Criteria

3.1.1.2.1 Patient has: random glucose >200 mg/dl or is taking an oral hypoglycemic agent or insulin at the time of study entry.

3.1.1.2.2 Patient has a history of lactic acidosis, chronic kidney disease or a creatinine ≥ 1.2 mg/dl

3.1.1.2.3 Women who are pregnant or breast feeding, as treatment involves unforeseeable risks to the participant, embryo, fetus, or nursing infant.

3.1.1.2.4 Patients with history of allergic reaction to metformin

3.1.2 Patients will complete the study on the day of the 6 month follow-up RT visit. They will not require additional follow-up.

4.0 PRE- AND POST-TREATMENT EVALUATION

4.1 All patients will provide informed consent prior to study entry.

4.2 All patients should have sufficient tumor tissue for mutational studies.

4.2.1 Archival tumor tissue will be evaluated for TP53, KRAS and STK11 mutations using the CMS50 available under the existing IPCT Clearinghouse Protocol (PA11-0852)

4.3 All patients will receive FDG-PET/CT scan at baseline (prior to metformin start), prior to RT and at 6 months (+/- 30 days) following RT.

4.4 All patients will have baseline (prior to starting metformin or placebo) and weekly laboratory studies while taking metformin or placebo:

4.4.1 Blood glucose

4.4.2 Creatinine

4.4.3 BUN

4.5 A one-time optional blood sample (10 ml) for circulation tumor DNA analysis collected either at baseline or during radiation treatment

5.0 NEOADJUVANT METFORMIN

5.1 All study subjects randomized will receive metformin or placebo for 3 weeks (+/- seven days) prior to RT treatment and for up to 2 weeks during RT treatment.

5.2 Metformin will be administered at a dose of 2000 mg in divided dose daily (500 mg am, 1000 mg noon, 500 mg pm). To reduce GI toxicity, patients will start metformin at 1000 mg daily in a divided dose (500mg am, 500 mg pm) for 1 week. If patients are not able to tolerate advancing the dose to 2000mg daily after one week, patients may take between 1000mg to 1500mg daily for the duration of the trial. If this dose is not tolerable, the patient will stop study medication.

5.3 Study subjects will be instructed to stop taking metformin/placebo and contact the study PI if they develop any of the following symptoms of lactic acidosis: unusual tiredness, dizziness, severe drowsiness, chills, blue/cold skin, muscle pain, fast/difficult breathing, slow/irregular heartbeat, stomach pain with nausea, vomiting, or diarrhea.

6.0 ^{18}F-FDG PET/CT ACQUISITIONS

6.1 The study coordinator will identify patients and confirm informed consent. PET/CT imaging sessions will be performed at the CABI General Electric Discovery 690 FX PET/CT scanner. In the event PET/CT scans cannot be performed at CABI, electronic copies of PET scans will be allowed for image analysis.

6.2 The patients will be imaged lying supine and flat on the scanner bed in approximately the same position as their radiotherapy.

6.3 The patients will undergo PET/CT imaging using [^{18}F]-2-fluoro-2-deoxyglucose positron emission tomography (^{18}F-FDG), using a standard approved radiopharmaceutical dose and administration selected by the nuclear medicine physician (120 min).

Each study subject will receive ^{18}F-FDG PET/CT imaging 3 times: at baseline (prior to metformin start), prior to radiotherapy, and 6 months (+/- 30 days) following completion of radiotherapy. Patient preparation prior to imaging will follow the NCI consensus recommendations (Shankar, Hoffman et al. 2006). Each patient will receive between 5.18 and 7.77 MBq per kilogram of body weight of ^{18}F-FDG injected 65 (+/-5) minutes prior to initiation of imaging (Shankar, Hoffman et al. 2006). Patient will receive their PET/CT imaging on a General Electric Discovery 690 FX PET/CT scanner with the same arm positioning (up/down) as utilized in their CT radiotherapy planning. 4D acquisition techniques will be utilized. An average CT will be acquired and utilized for attenuation correction (Pan, Mawlawi et al. 2006).

6.4 The uptake time, time between injection of ^{18}F-FDG and initiation of the PET emission acquisition, will be between 60 to 70 minutes. An average CT will be acquired for attenuation correction purposes.

7.0 SBRT TREATMENT

7.1 At MD Anderson, we have treated > 600 lung SBRT cases since 2004 with a 3-year average of 130 cases per year.

7.2 All patients in this study are medically inoperable (or refuse thoracic surgery) and have non-small cell lung cancers in whom the standard of care at MD Anderson is SBRT to a total dose of 50-70 Gy in 4-15 daily treatment fractions. The radiation treatment planning, prescription radiation dose, and administration will be determined by their attending radiation oncology physician. It is expected each patient in this study will receive RT per our standard of care practice.

7.3 Tissue heterogeneity correction should be applied for planning. Daily on-board imaging using CT on-rail or cone beam CT is required before each fraction for stereotactic delivery. Orthogonal portal films should be taken to verify the treatment isocenter before each treatment.

8.0 REGISTRATION PROCEDURES

8.1 Informed consent is required prior to participation in this study. The study coordinator will inform potential subjects about the study opportunity and ask if they would like to participate. If the patient is interested, they will be given an opportunity to read the informed consent and authorization document specific to the study and ask questions of the study coordinator. The patient will be informed about: 1) the rationale for the study; 2) the logistics of the study; 3) the risks of the study; 4) how the data will be used. Consent will be obtained by study coordinator. The patient will be given a copy of the consent, and asked to sign another copy for our records.

8.2 Patients will be registered in CORe after pretreatment evaluation is completed and eligibility criteria are met.

8.3 The principal investigator and all key personnel have completed NIH approved institutional and HIPAA training in the conduct of medical research studies

9.0 STATISTICAL CONSIDERATIONS

9.1 Objectives

The primary objectives are to compare tumor response by PERCIST between metformin and placebo cohorts and between genotypes of three candidate genes for metformin patients. Secondary objectives will consider the correspondence of the RECIST and PERCIST tumor response methods and the predictive power of pre-treatment glucose utilization. In addition, we will evaluate the predictive power of pre-treatment glucose utilization with mutation status for resultant metformin disease control (DC) using RECIST and PERCIST criteria.

9.2 Study Design.

Patients will be randomized 6:1 to metformin versus placebo in the Clinical Oncology Research system (CORe). All patients will undergo a FDG-PET/CT scan at baseline (prior to metformin start), prior to RT and at 6 months (+/- 30 days) post-RT. Response will be determined at 6 months post-treatment via relative change from pre-treatment tumor SUV of [^{18}F]-FDG-PET after 3 weeks induction metformin or placebo to the tumor SUV at 6 months post-treatment. Subjects will not be excluded based on age, gender, economic status, or race.

9.3 Power and Sample Size Calculations.

9.3.1 Metformin versus Placebo

The metformin versus placebo (N=60 metformin vs N=10 placebo) comparison attains 80% power to detect a difference in magnitude of at least 1 standard deviation using a two-sided Mann-Whitney test at the 0.05 significance level.

9.3.2 Genotype Comparisons

Among the N=60 patients treated with metformin, we expect 40-50% patients to present disruptive TP53 mutations, 25-50% to present Kras activating mutations, and 15-30% to present inactivating STK11 mutations. A two-sided test at the 0.05 significance level attains at least 81-84% power to detect a difference in magnitude of at least 0.8 standard deviations between cohorts of patients with functional and disruptive TP53 mutations; at least 80-90% power to detect a difference in magnitude of at least 0.9 standard deviations between cohorts of patients with activated and inactivated Kras mutations; at least 80-96% power to detect a difference in magnitude of at least 1.1 standard deviations between cohorts of patients with functional and inactivated STK11 mutations. Among the cohort of patients that present disruptive

TP53 mutations, the test attains at least 80-96% power to detect a difference in magnitude of at least 1.5 standard deviations between cohorts of patients with activated and inactivated Kras mutations and at least 80-94% power to detect a difference in magnitude of at least 1.7 standard deviations between cohorts of patients with functional and inactivated STK11 mutations.

9.4 Analysis Plan.

9.4.1 Primary Objectives

Mann-Whitney tests will be used to test for genotype effects of within each of the three candidate genes and between study arms (control versus metformin treated). Among the N=70, we will compare tumor response at 6 months post-treatment between study arms (N=60 metformin vs N=10 placebo).

9.4.2 Secondary Aim 1

The first secondary objective will evaluate the correspondence of the RECIST and PERCIST tumor response methods. Each tumor's relative change in maximum transaxial diameter on CT after 3 weeks induction metformin (N=60) will be matched with the corresponding relative change at 3-weeks from pre-treatment tumor SUV of [^{18}F]-FDG-PET. The relationship among the pairs will be assessed for linear dependence using Pearson's product moment correlation coefficient. The sample size of N=60 patients provides 80% power to detect a positive correlation of at least 0.32 using a one-sided test of null hypothesis of independence.

9.4.3 Secondary Aim 2

The second secondary objective will consider the predictive power of pre-treatment glucose utilization with mutation status for resultant metformin disease control (DC) using RECIST and PERCIST criteria. DC for RECIST will require CR, PR, or SD after 3 weeks induction metformin. DC for PERCIST will require a reduction in tumor SUV of [^{18}F]-FDG-PET after 3 weeks induction metformin. The accuracy of pre-treatment SUV of [^{18}F]-FDG-PET in predicting DC will be evaluated using area under the receiver operator characteristic curve (AUROC) for each tumor genotype independently and combined. For a one-sided test of the null hypothesis of indiscriminate prediction (AUROC=0.5), the sample size of N=60 patients provides at least 80% power to detect an AUROC of at least 0.71 at the 0.05 significance level given that the resultant disease control rate is at least 25% in the combined analysis. In addition, inference with multivariate logistic regression will be used to assess the effect of pre-treatment SUV of [^{18}F]-FDG-PET in the presence of genotype status. Confounders of post-radiation chemotherapy and steroid use will be adjusted for using linear mixed regression modeling.

10.0 POTENTIAL RISKS

10.1 CT risks. The patients recruited to this study will receive three CT imaging sessions in combination with FDG-PET scan for this study. The major risk from multiple CT scans is from the radiation. Each CT scan will deliver approximately 0.05 Gy to the patient. However, each patient will undergo radiation therapy that will deliver at least 45 Gy to the tumor and scatter doses of at least 2.5 Gy (5% of the prescribed dose) to the entire chest.

10.2 FDG-PET risks. The patients recruited to this study will receive two PET/CT imaging sessions for this study. The radiation dose from the PET/CT scans described in sections 5.3 and 5.4, is still quite small relative to radiation dose from a standard CT scan.

10.3 Radiation dose. The total radiation dose from the procedures in this study will be predominately from the three 4D CT scans, which is about 0.15 Gy for the three scans. The additional radiation of patients undergoing several CT scans as defined in this protocol will result in negligible additional dose. The additional risk is thus likely to be immeasurable.

10.4 Metformin treatment. Metformin has a well-established safety profile and is used clinically, not only in diabetics, but also in non-diabetic patients with polycyctic ovarian syndrome(Tang et al., 2012). Metformin is not associated with hypoglycemia in non-diabetic patients(Harborne et al., 2003). Although a clinical concern during metformin treatment is lactic acidosis (which limits the use of this drug to patients without impaired renal function), a recent meta-analysis found the incidence of this being 4.4/100,000 patient years and less than that observed in patients using other hypoglycemic regimens(Salpeter et al., 2010). Metformin is associated with acute GI side effects (loose stools, nausea), however these effects are mitigated by gradual dose-escalation and usually resolve within the first few weeks of treatment.

11.0 LONG TERM GOALS

SBRT or hypofractionated RT is an effective treatment for many medically inoperable patients with stage I lung NSCLC. However, with larger (T2) tumors local and distant failure is as high as 40% in some series (Chi et al., 2010). For many of these patients medical comorbidities limit adjuvant or concurrent systemic therapy. The current study seeks to investigate the minimally toxic agent metformin as an adjunct to SBRT or hypofractionated RT. Our goal is to compare the response of lung NSCLC to metformin with RECIST versus PERCIST criteria. Secondarily, we will compare tumor mutation status versus tumor metabolic activity to predict tumor sensitivity to metformin. Secondarily, we hope to examine the genetic profile of these tumors to determine markers of sensitivity to metformin.

12.0 DATA MONITORING & ADVERSE EVENT REPORTING

12.1 Data Monitoring. The Data and Safety Monitoring Plan (DSMP) is established to ensure the safety of research participants and the integrity of the study data. This study is an intervention study with a known minimally toxic agent that is routinely used in the clinic. As such, this is a relatively low risk study and data monitoring will occur on an occurrence basis with regular review by the PI. The study staff (PI, Clinical research coordinator, research nursing, etc.) is responsible for collecting and recording all clinical data. As these results are collected, all ≥ 3 toxicities and adverse events will be identified, graded for severity and assigned causality, reported to the required entities, and compiled for periodic review. After assigning causality, the PI will decide the course of action for the study participant. The PI will evaluate all AE's and determine whether the adverse event affects the risk/benefit ratio of the study and whether modifications to the protocol or informed consent form are required. Throughout this process, the PI will inform and collaborate with the IRB and Clinical Research Compliance.

12.2 Toxicity Grading. The Common Terminology Criteria for Adverse Events version 4.0 will be used to grade all $\geq$ 3 (CTCAE v4.0; available at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm) treatment-related adverse events. All patient encounter or treatment areas should have access to a copy of these criteria.

12.3 Adverse Events. All $\geq$ 3 adverse events (AE) must be reported to the Protocol PI. All serious adverse events, defined as grade 4 or 5 toxicity, will be reported to the institutional review board (IRB) of The University of Texas M. D. Anderson Cancer Center according to institutional reporting guidelines and using institutional reporting forms (see Appendix C). Reports of serious adverse events will be delivered to Clinical Research Compliance and will be submitted to the U.S. Food and Drug Administration by the safety coordinator according to 21CFR 312.32.

12.4 AE Reporting. The following AEs experienced by patients accrued to this protocol and attributable to the protocol treatment (definitely, probably, or possibly related) should be reported (from the start of protocol treatment to 30 days after protocol treatment):

12.4.1 Death on study.
12.4.2 Hospitalization or prolongation of hospitalization on study
12.4.3 Life-threatening event
12.4.4 Persistent or significant disability or incapacity

13.0 DATA CONFIDENTIALITY PLAN

All patient-reported outcome, laboratory, radiographic, and clinical data gathered in this protocol will be stored in a password-protected database. All patient information will be handled using anonymous identifiers. Linkage to patient identity is only possible after accessing a password-protected database. Access to the database is only available to individuals directly involved in the study. Information gathered for this study will not be reused or disclosed to any other person or entity, or for other research. Once the research has been completed, identifiers will be retained for as long as is required by law and by institutional regulations, and at that point will be destroyed.

14.0 Study Calendar

Procedures	Baseline	Treatment Phase						Follow-up
		Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Month 6[2]

PET/CT (at CABI)	X[3]				X			X[3]
Metformin/Placebo[7]		X	X	X	X	X[5]	X	
SBRT (50 Gy in 4 fx)					X			
SBRT (70 Gy in 10 fx)					X	X		
Hypofractionated RT (45-60 Gy in 15 fx					X	X	X	
Blood chem (Glucose, Cr, BUN)	X[1]	X[4]	X[4]	X[4]	X[4]	X[4,5]	X[4,8]	
Optional blood for circulating DNA	X[6]							
Toxicity Evaluation		X	X	X	X	X[5,8]	X[8]	
Drug diary		X	X	X	X	X[5]	X[8]	

[1] Within 30 days prior to registration
[2] Within +/- 30 days
[3] As part of SOC prior to study entry
[4] While taking metformin/placebo
[5] For 70 Gy in 10 treatment
[6] One 10 ml purple top collected once either at BL or during treatment phase
[7] +/- 7 days for the first 3 week period
[8] For 45-60 Gy in 15 treatment

15.0 REFERENCES:

Algire, C., L. Amrein, M. Bazile, S. David, M. Zakikhani and M. Pollak (2011). "Diet and tumor lkb1 expression interact to determine sensitivity to anti-neoplastic effects of metformin in vivo." Oncogene **30**(10): 1174-1182.

Algire, C., M. Zakikhani, M.-J. Blouin, J. H. Shuai and M. Pollak (2008). "Metformin attenuates the stimulatory effect of a high-energy diet on in vivo llc1 carcinoma growth." Endocrine-related cancer **15**(3): 833-839.

Armstrong, J. G. and B. D. Minsky (1989). "Radiation therapy for medically inoperable stage i and ii non-small cell lung cancer." Cancer Treatment Reviews **16**(4): 247-255.

Ashinuma, H., Y. Takiguchi, S. Kitazono, M. Kitazono-Saitoh, A. Kitamura, T. Chiba, Y. Tada, K. Kurosu, E. Sakaida, I. Sekine, N. Tanabe, A. Iwama, O. Yokosuka and K. Tatsumi (2012). "Antiproliferative action of metformin in human lung cancer cell lines." Oncology reports **28**(1): 8-14.

Bradley, J. D., J. Moughan, M. V. Graham, R. Byhardt, R. Govindan, J. Fowler, J. A. Purdy, J. M. Michalski, E. Gore and H. Choy (2010). "A phase i/ii radiation dose escalation study with concurrent chemotherapy for patients with inoperable stages i to iii non-small-cell lung cancer: Phase i results of rtog 0117." International journal of radiation oncology, biology, physics **77**(2): 367-372.

Buzzai, M., R. G. Jones, R. K. Amaravadi, J. J. Lum, R. J. DeBerardinis, F. Zhao, B. Viollet and C. B. Thompson (2007). "Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth." Cancer Research **67**(14): 6745-6752.

Chang, J. Y., H. Liu, P. Balter, R. Komaki, Z. Liao, J. Welsh, R. J. Mehran, J. A. Roth and S. G. Swisher (2012). "Clinical outcome and predictors of survival and pneumonitis after stereotactic ablative radiotherapy for stage i non-small cell lung cancer." Radiation oncology (London, England) **7**: 152.

Chaudhary, S. C., D. Kurundkar, C. A. Elmets, L. Kopelovich and M. Athar (2012). "Metformin, an antidiabetic agent reduces growth of cutaneous squamous cell carcinoma by targeting mtor signaling pathway." Photochemistry and photobiology **88**(5): 1149-1156.

Chi, A., Z. Liao, N. P. Nguyen, J. Xu, B. Stea and R. Komaki (2010). "Systemic review of the patterns of failure following stereotactic body radiation therapy in early-stage non-small-cell lung cancer: Clinical implications." Radiotherapy and Oncology **94**(1): 1-11.

Dosoretz, D. E., M. J. Katin, P. H. Blitzer, J. H. Rubenstein, D. H. Galmarini, G. R. Garton and S. A. Salenius (1996). "Medically inoperable lung carcinoma: The role of radiation therapy." Seminars in Radiation Oncology **6**(2): 98-104.

Henderson, M. A., D. J. Hoopes, J. W. Fletcher, P.-F. Lin, M. Tann, C. T. Yiannoutsos, M. D. Williams, A. J. Fakiris, R. C. McGarry and R. D. Timmerman (2010). "A pilot trial of serial 18f-fluorodeoxyglucose positron emission tomography in patients with medically inoperable stage i non-small-cell lung cancer treated with hypofractionated stereotactic body radiotherapy." International Journal of Radiation Oncology*Biology*Physics **76**(3): 789-795.

Hsieh, M.-C., T.-C. Lee, S.-M. Cheng, S.-T. Tu, M.-H. Yen and C.-H. Tseng (2012). "The influence of type 2 diabetes and glucose-lowering therapies on cancer risk in the taiwanese." Experimental diabetes research **2012**.

Javeshghani, S., M. Zakikhani, S. Austin, M. Bazile, M.-J. Blouin, I. Topisirovic, J. St-Pierre and M. N. Pollak (2012). "Carbon source and myc expression influence the antiproliferative actions of metformin." Cancer Research.

Jemal, A., R. Siegel, J. Xu and E. Ward (2010). "Cancer statistics, 2010." CA: a cancer journal for clinicians **60**(5): 277-300.

Kato, K., J. Gong, H. Iwama, A. Kitanaka, J. Tani, H. Miyoshi, K. Nomura, S. Mimura, M. Kobayashi, Y. Aritomo, H. Kobara, H. Mori, T. Himoto, K. Okano, Y. Suzuki, K. Murao and T. Masaki (2012). "The antidiabetic drug metformin inhibits gastric cancer cell proliferation in vitro and in vivo." Molecular cancer therapeutics **11**(3): 549-560.

Liu, H., C. Scholz, C. Zang, J. H. Schefe, P. Habbel, A.-C. Regierer, C.-O. Schulz, K. Possinger and J. Eucker (2012). "Metformin and the mtor inhibitor everolimus (rad001) sensitize breast cancer cells to the cytotoxic effect of chemotherapeutic drugs in vitro." Anticancer research **32**(5): 1627-1637.

Luo, Q., D. Hu, S. Hu, M. Yan, Z. Sun and F. Chen (2012). "In vitro and in vivo anti-tumor effect of metformin as a novel therapeutic agent in human oral squamous cell carcinoma." BMC cancer **12**: 517.

Mazzone, P. J., H. Rai, M. Beukemann, M. Xu, A. Jain and M. Sasidhar (2012). "The effect of metformin and thiazolidinedione use on lung cancer in diabetics." BMC cancer **12**: 410.

Menendez, J. A., C. Oliveras-Ferraros, S. Cufí, B. Corominas-Faja, J. Joven, B. Martin-Castillo and A. Vazquez-Martin (2012). "Metformin is synthetically lethal with glucose withdrawal in cancer cells." Cell cycle **11**(15): 2782-2792.

Noto, H., A. Goto, T. Tsujimoto and M. Noda (2012). "Cancer risk in diabetic patients treated with metformin: A systematic review and meta-analysis." PLOS ONE **7**(3).

Pan, T., O. Mawlawi, D. Luo, H. H. Liu, P. C. Chi, M. V. Mar, G. Gladish, M. Truong, J. Erasmus, Jr., Z. Liao and H. A. Macapinlac (2006). "Attenuation correction of pet cardiac data with low-dose average ct in pet/ct." Med Phys **33**(10): 3931-8.

Pollak, M. N. (2012). "Investigating metformin for cancer prevention and treatment: The end of the beginning." Cancer Discovery **2**(9): 778-790.

Salani, B., S. Maffioli, M. Hamoudane, A. Parodi, S. Ravera, M. Passalacqua, A. Alama, M. Nhiri, R. Cordera and D. Maggi (2012). "Caveolin-1 is essential for metformin inhibitory effect on igf1 action in non-small-cell lung cancer cells." FASEB journal : official publication of the Federation of American Societies for Experimental Biology **26**(2): 788-798.

Sandulache, V. C., H. D. Skinner, T. J. Ow, A. Zhang, X. Xia, J. M. Luchak, L.-J. C. Wong, C. R. Pickering, G. Zhou and J. N. Myers (2012). "Individualizing antimetabolic treatment strategies for head and neck squamous cell carcinoma based on tp53 mutational status." Cancer **118**(3): 711-721.

Sanli, T., A. Rashid, C. Liu, S. Harding, R. G. Bristow, J.-C. Cutz, G. Singh, J. Wright and T. Tsakiridis (2010). "Ionizing radiation activates amp-activated kinase (ampk): A target for radiosensitization of human cancer cells." International journal of radiation oncology, biology, physics **78**(1): 221-229.

Shackelford, D. B., E. Abt, L. Gerken, D. S. Vasquez, A. Seki, M. Leblanc, L. Wei, M. C. Fishbein, J. Czernin, P. S. Mischel and R. J. Shaw (2013). "Lkb1 inactivation dictates therapeutic response of non-small cell lung cancer to the metabolism drug phenformin." Cancer cell **23**(2): 143-158.

Shankar, L. K., J. M. Hoffman, S. Bacharach, M. M. Graham, J. Karp, A. A. Lammertsma, S. Larson, D. A. Mankoff, B. A. Siegel, A. Van den Abbeele, J. Yap and D. Sullivan (2006). "Consensus recommendations for the use of ^{18}f-fdg pet as an indicator of therapeutic response in patients in national cancer institute trials." J Nucl Med **47**(6): 1059-1066.

Sikka, A., M. Kaur, C. Agarwal, G. Deep and R. Agarwal (2012). "Metformin suppresses growth of human head and neck squamous cell carcinoma via global inhibition of protein translation." Cell cycle (Georgetown, Tex.) **11**(7): 1374-1382.

Skinner, H. D., V. C. Sandulache, T. J. Ow, R. E. Meyn, J. S. Yordy, B. M. Beadle, A. L. Fitzgerald, U. Giri, K. K. Ang and J. N. Myers (2012). "Tp53 disruptive mutations lead to head and neck cancer treatment failure through inhibition of radiation-induced senescence." Clinical cancer research : an official journal of the American Association for Cancer Research **18**(1): 290-300.

Smiechowski, B. B., L. Azoulay, H. Yin, M. N. Pollak and S. Suissa (2012). "The use of metformin and the incidence of lung cancer in patients with type 2 diabetes." Diabetes Care **36**(1): 124-129.

Song, C. W., H. Lee, R. P. M. Dings, B. Williams, J. Powers, T. D. Santos, B.-H. Choi and H. J. Park (2012). "Metformin kills and radiosensitizes cancer cells and preferentially kills cancer stem cells." Scientific reports **2**.

Tan, B.-X., W.-X. Yao, J. Ge, X.-C. Peng, X.-B. Du, R. Zhang, B. Yao, K. Xie, L.-H. Li, H. Dong, F. Gao, F. Zhao, J.-M. Hou, J.-M. Su and J.-Y. Liu (2011). "Prognostic influence of metformin as first-line chemotherapy for advanced nonsmall cell lung cancer in patients with type 2 diabetes." Cancer **117**(22): 5103-5111.

Timmerman, R., R. Paulus, J. Galvin, J. Michalski, W. Straube, J. Bradley, A. Fakiris, A. Bezjak, G. Videtic, D. Johnstone, J. Fowler, E. Gore and H. Choy (2010). "Stereotactic body radiation therapy for inoperable early stage lung cancer." JAMA **303**(11): 1070-1076.

Wu, B., S. Li, L. Sheng, J. Zhu, L. Gu, H. Shen, D. La, B. D. Hambly, S. Bao and W. Di (2012). "Metformin inhibits the development and metastasis of ovarian cancer." Oncology reports **28**(3): 903-908.

Xiao, X., Q. He, C. Lu, K. D. Werle, R.-X. Zhao, J. Chen, B. C. Davis, R. Cui, J. Liang and Z.-X. Xu (2012). "Metformin impairs the growth of liver kinase b1-intact cervical cancer cells." Gynecologic Oncology **127**(1): 249-255.

Zaugg, K., Y. Yao, P. T. Reilly, K. Kannan, R. Kiarash, J. Mason, P. Huang, S. K. Sawyer, B. Fuerth, B. Faubert, T. Kalliomäki, A. Elia, X. Luo, V. Nadeem, D. Bungard, S. Yalavarthi, J. D. Growney, A. Wakeham, Y. Moolani, J. Silvester, A. Y. Ten, W. Bakker, K. Tsuchihara, S. L. Berger, R. P. Hill, R. G. Jones, M. Tsao, M. O. Robinson, C. B. Thompson, G. Pan and T. W. Mak (2011). "Carnitine palmitoyltransferase 1c promotes cell survival and tumor growth under conditions of metabolic stress." Genes & development **25**(10): 1041-1051.