PHASE II TRIAL OF FLAXSEED TO PREVENT PNEUMONOPATHY AFTER CHEMORADIATION FOR LUNG CANCER

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List of Abbreviations

CRF: case report form

CTCAE: Common Terminology Criteria for Adverse Events

ED: enterodiol

EL: enterolactone

FS: flaxseed

GCP: Good Clinical Practice

GRAS: Generally Recognized as Safe

NCI: National Cancer Institute

NSCLC: non-small cell lung cancer

PI: principal investigator

RILI: radiation-induced lung injury

RNS: reactive nitrogen species

ROS: reactive oxygen species

RP: radiation pneumonitis

SAE: serious adverse event

SDG: secoisolariciresinol diglucoside

WBC: white blood cells

XRT: radiation therapy

Study Summary

Title	**PHASE II TRIAL OF FLAXSEED TO PREVENT PNEUMONOPATHY AFTER CHEMORADIATION FOR LUNG CANCER**
Short Title	Dietary Flaxseed in NSCLC
Protocol Number	
Phase	Phase II
Methodology	Single-arm study, 40g flaxseed ingested per day
Study Duration	Two years
Study Center(s)	Single-center
Objectives	To establish the utility of FS supplementation in patients receiving chemoradiation therapy for lung cancer; To validate the ability to measure biomarkers of oxidative stress and active FS metabolites in these patients; to quantify oxidative stress.
Number of Subjects	48 subjects
Diagnosis and Main Inclusion Criteria	Patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) receiving chemoradiotherapy with definitive intent
Study Product, Dose, Route, Regimen	Whole grain flaxseed diet consisting of 40g flaxseed ingested per day
Duration of administration	Approximately eight to nine weeks
Reference therapy	none
Statistical Methodology	This is a single-arm phase II trial. The primary endpoint will be absence of radiation pneumonitis (RP) of grade 3 or above within 6 months from the end of XRT, according to the CTCAE v.4.0 scale. The most recent pooled Radiation Therapy Oncology Group analysis showed that the rate of $\geq$ grade 3 RP was 18%[51], so the null hypothesis "clinical response" rate for this study is 82%. A sample size of n=39 achieves 80% power to detect a response rate of 94.1% in a one-sided binomial exact test with target significance level no larger than α=0.1. The actual significance level achieved by this test is 0.062. We will reject the null hypothesis that FS treatment has no effect on the RP rate if there are 3 or fewer cases of RP in the 39 evaluable subjects. Assuming a death rate of 10% prior to 6 months and a 10% inevaluable rate, **we will seek to enroll 48 patients**.

1 Introduction

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

1.1 Background

The effectiveness of radiotherapy for thoracic malignancies is limited by the high radiosensitivity of normal lung parenchyma [1, 2]. Clinically significant radiation lung injury occurs in up to 30% of patients irradiated for lung cancer [3] and 10-15% of other thoracic oncology patients [4]. A greater proportion of patients have subclinical adverse effects of radiation on the lung, identifiable by imaging and/or physiologic testing [5]. Highly reactive compounds known as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are induced in large quantities by radiation therapy (XRT) and have been implicated in this form of lung injury [6].

Two phases of radiation-induced lung injury have been described in humans [1]. Acute radiation pneumonitis, thought to be largely inflammatory and exudative in nature, can occur several weeks to six months post-irradiation, and can be life threatening if a large volume of lung has been affected [7]. In the second phase of radiation-induced lung injury that can occur months to years after irradiation, the lung tissue enters a fibrotic state in which the numbers of inflammatory cells decrease and a marked thickening of alveolar walls occurs due to collagen deposition.

There is currently no known effective pharmacologic therapy for the prevention of acute or chronic radiation pneumonopathy. Systemic oral and intravenous corticosteroids are routinely used to treat clinically significant acute radiation pneumonitis, but the administration of these drugs does not alter the risk of developing late radiation fibrosis. The exact mechanism by which the acute radiation pneumonitis phase transitions to the late fibrotic phase is unclear. However, as recently reviewed by Robbins and Zhao [9], studies implicate a chronic state of oxidative stress in radiation-induced late fibrosis; it is widely recognized that irradiation is associated with both a rapid and a chronic increase in ROS/RNS production. This persistent state of oxidative stress may therefore be a target for inhibition of radiation injury and radioprotection of normal tissues [10] to allow for more effective radiotherapy and improved clinical outcomes.

Currently the only means to avoid life-threatening or fatal radiation pneumonopathy is to minimize the amount of lung exposed to dangerous radiation levels. A safe and effective biologic radioprotector would thus be extremely useful. Preclinical data suggest that antioxidant molecules and/or enzymes might offer protection of the lung [11-13].

FS has been piloted in multiple diseases at this institution[39]. We previously conducted a pilot trial of FS in NSCLC patients treated with chemoradiation by our team at the Pulmonary Division of the University of Pennsylvania, led by Dr. Christofidou-Solomidou PhD, and Dr. Berman and Dr. Rengan from the Department of Radiation Oncology as follows: 14 subjects were randomized to one FS or control muffin daily from start to 2 weeks after RT. Fourteen patients (control,7; FS,7) were enrolled. The tolerability rates were 42.9 versus 71.4% (p=0.59) for FS

and control, respectively. Mean percentages of intended number of muffins consumed were 37% versus 73% (p=0.12). ED and EL increased at onset of FS and decreased with discontinuation, confirming bioavailability. Isoprostane and 8-oxo-dGuo were detectable. There was a trend towards decreased rates of pneumonitis in FS. While FS in the administered muffin formulation did not meet tolerability criteria due to taste (not toxicity), we were able to show FS bioavailability and quantify oxidative stress markers.[40]

1.2 Investigational Agent

Flaxseed (FS) is a non-toxic, whole grain commonly used as a nutritional supplement that has gained popularity because it is a rich source of natural antioxidants. FS is composed of high concentrations of omega-3 fatty acids and lignans. Omega-3 fatty acids reduce inflammation and may be helpful in treating a variety of autoimmune diseases [15-17]. Lignans, widely occurring plant compounds closely related to lignin (which forms the woody component of trees and other plants), possess antioxidant properties [18]. The FS lignan precursor secoisolariciresinol diglucoside (SDG), isolated from FS, is metabolized in the mammalian intestine to the lignans enterodiol (ED) and enterolactone (EL). The oxygen radical scavenging properties of the FS lignans have been shown in vitro to act either by direct hydroxyl radical scavenging activity [19, 20] or by inhibition of lipid peroxidation [21]. In addition, the FS lignan SDG may exert antioxidant activity by inhibiting ROS produced by white blood cells (WBC) by acting as a platelet-activating-factor antagonist [22]. The antioxidant properties of FS lignans have been verified in animal models of endotoxic shock [22], diabetes [23] and in carbon tetrachloride-induced oxidative stress [24]. Although ROS production and oxidant stress have been implicated in the etiology of a variety of acute and chronic lung injury forms, the therapeutic or preventive use of dietary FS or FS-derived lignans has not been extensively evaluated in pulmonary disease.

1.3 Preclinical Data

Our group was the first to publish the use of FS in murine models of acute lung injury including hyperoxia and acid aspiration, and our data suggest the importance of its antioxidant properties [25]. Additionally, we have shown that FS can ameliorate ischemia/reperfusion lung injury in a murine model (manuscript under review). In a murine model of thoracic radiation induced lung fibrosis, dietary FS supplementation improves survival and overall health, and it reduces oxidative lung damage in mice receiving a single dose of thoracic radiation (manuscript under review).

1.4 Clinical Data to Date

There is no available clinical research data on the use of dietary FS in the setting of thoracic radiation therapy in humans.

1.5 Dose Rationale and Risk/Benefits

Dose Rationale: Previous studies have investigated dose of FS and have shown tolerability, increase in urinary lignan levels[41-43], and decrease in diabetes and atherosclerotic disease for doses 15-50 g[38,44,45]. In this study, subjects will ingest 40g of FS per day in the form of ground, wholegrain FS, incorporated in the manner of their choice. The intolerability of the formulation in the NSCLC population[39] prompted the switch to packets of ground FS, which has been shown to have excellent tolerability and is currently being utilized in pulmonary studies of cystic fibrosis and lung transplant patients[40].

Potential Risks: Our original phase I studies shows that there is no significant safety issues with flaxseed ingestion during the prescribed time, although there is little data beyond six weeks of FS ingestion. There is no data on safety in children, nor in pregnancy and lactation. There are isolated reports of FS induced contact allergy [27, 28]. FS has not yet attained GRAS (Generally Recognized as Safe) status from the FDA. While 50g of ingested FS daily for one month has been well tolerated, subjects experienced up to a 30% increase in bowel movements [29]. An upper total dose of 40g of FS daily was chosen to minimize its laxative effects.

Potential Benefits: Subjects receiving study diet will receive close monitoring for toxicity. Subjects will be encouraged to continue a healthy diet in the setting of possible XRT induced esophagitis. In addition, flaxseed may reduce the risk of RILI; however, we do not know if this is the case and is the subject of research in this study.

Risk Benefit Ratio: The potential benefits of this study outweigh the potential risks. Data from this study will provide evidence of efficacy of administering dietary FS in a phase II setting which will allow us to perform a larger, phase III clinical trial. It will be the first time markers of oxidative stress and FS metabolites will be systematically measured in this population.

2 Study Objectives

Primary Objective:
Determine in a phase II trial whether FS supplementation decreases the risk of RILI in patients receiving definitive chemoradiotherapy for locally advanced or metastatic NSCLC, and to collect additional toxicity and tolerability data from FS administration in this population.
Secondary Objective:
Determine how FS modulates the markers of inflammation and oxidative damage.

1. Quantify the gene and protein expression levels of representative nrf2- regulated antioxidant enzymes HO-1 and NQO-1 from buccal swabs of NSCLC patients.
2. Quantifying urinary markers of systemic oxidative stress including 8,12-iso-iPF2α-VI isoprostane (F2α-IsoPs) and 8-oxo-7,8-dihydro-2'deoxyguanosine (8-oxo-dGuo) and quantifying serum levels of FS lignans ED and EL.

3 Study Design

3.1 General Design

See Table 1 (Study Flow Chart)

This single arm Phase II trial will investigate the feasibility of dietary FS supplementation in a population of patients receiving definitive chemoradiotherapy for lung cancer. Subjects will be male or female with locally advanced or metastatic non-small cell lung cancer (NSCLC) requiring irradiation of the mediastinum.

We propose to have 48 subjects ingest FS starting approximately 1 week before their definitive XRT begins, and for the duration of their therapy (approximately 7-8 weeks). The subjects will be asked to stop eating the study FS after completion of their radiotherapy for a total of approximately 8-9 weeks of FS administration.

Enrolled subjects will be seen at a pre-irradiation visit approximately one week prior to the start of XRT and weekly during the course of radiotherapy (approximately seven to eight weeks of therapy) and diet administration. Subjects will be assessed at each weekly visit for toxicity including measures of dermatitis, nausea, vomiting, diarrhea, esophagitis, cough, and dyspnea. In addition, at the visits as indicated in the study flow-sheet below, patients will complete a FACT-L questionnaire, on specimen days, which has been shown to be correlative of lung-cancer specific patient-reported outcomes (Ring et al. 2008). This, along with the flaxseed logs, will be filled out by the patients in RedCap, which is a web-based secure portal used at the University of Pennsylvania for research and patient surveys and has been well-described in its use in the literature (Paul A. Harris, Robert Taylor, Robert Thielke, Jonathon Payne, Nathaniel Gonzalez, Jose G. Conde, Research electronic data capture (REDCap) - A metadata-driven methodology and workflow process for providing translational research informatics support, J Biomed Inform. 2009 Apr;42(2):377-81.). If a patient does not have computer access, the study coordinator will sit with the patient to enter the information into RedCap. Toxicity will be scored according to the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0 for adverse event reporting. Research visits will be scheduled to coincide with subjects' standard of care clinic visits whenever possible.

Following completion of radiotherapy, subjects will be assessed at approximately one month and approximately six months. At approximately six months post XRT, subject involvement and study monitoring will be completed. Due to unanticipated side-effects of treatment, scheduling issues or logistical difficulties, the specimen collection and/or visit schedule will be modified at the discretion of the principal investigator.

Specimen collection will include obtaining blood, urine and buccal swabs at a total of five time points:

1) At a pre-irradiation visit prior to starting the FS diet
2) Approximately one week after starting the FS diet at the start of XRT
3) At approximately 4 weeks following the start of XRT
4) At the conclusion of XRT therapy
5) At approximately one month post XRT completion

On the days of specimen sampling, subjects will be asked not to eat their daily FS until after sample collection, or at least 4 hours before their visit. Collection of urine specimens and buccal swabs will be obtained by appropriately trained research study staff. Collection of blood samples will be obtained by research staff appropriately trained in venipuncture techniques.

The Investigational Drug Service (IDS) at the University of Pennsylvania will be responsible for flaxseed receipt, storage and dispensing to research staff. Subjects receiving radiotherapy will have daily visits to the radiation oncology department as standard of care for fractionated therapy and will be given FS as necessary by research staff after it is dispensed by IDS.

3.2 Primary Study Endpoints

The primary endpoints to be measured in this study include:

- Rate of RILI after FS administration in a population of patients receiving definitive chemoradiotherapy for lung cancer.
- Toxicity and tolerability data of dietary FS administration during chemoradiotherapy.

3.3 Secondary Study Endpoints

The secondary endpoints to be measured in this study include:

- Measures of biomarkers of oxidative stress in this population.
- Measures of serum levels of FS metabolites.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Male or female, ages 18 and older
2. Current diagnosis of non-small cell lung cancer (NSCLC) including patients who have metastatic disease requiring definitive thoracic and mediastinal radiotherapy with concurrent chemotherapy.
3. Able to provide written informed consent and comply with all study procedures
4. Total planned radiation dose to gross disease **60-70** Gy.

4.2 Exclusion Criteria

1. Current diagnosis of disease of the gastrointestinal system, liver, or kidneys which could result in altered metabolism or excretion of the study medication (history of major gastrointestinal tract surgery, gastrectomy, gastrostomy, bowel resection, etc.) or history of chronic gastrointestinal disorders (ulcerative colitis, regional enteritis, or gastrointestinal bleeding)
2. Known hypersensitivity to flaxseed or any of its metabolites, or wheat products
3. Taking or has taken an investigational drug within **14** days
4. Taking or has taken Amifostine or Mucomyst (N-acetylcysteine) within **14** days
5. Current or prior use of flaxseed, flax-containing products, soybeans, or soy-containing products
6. Current or prior use (limited to prior **14** days) of dietary supplements such as herbals or botanicals
7. Prior thoracic and/or mediastinal radiation therapy

4.3 Subject Recruitment and Screening

Subjects will be recruited from investigator clinical practices and include men and women who will be receiving chemoradiotherapy with definitive intent for non-small cell lung cancer (NSCLC) including patients with locally advanced or metastatic disease. Subjects will undergo an informed consent process in accordance with GCP (see section 11 Ethical Considerations). Informed consent will be obtained prior to the performance of any study procedures. Subjects must meet all of the inclusion and none of the exclusion criteria...

Documentation of Eligibility: Screening assessments will be performed to determine subject eligibility criteria, which will be documented on a case report form. The PI will confirm and sign off on the inclusion and exclusion criteria on a case report form (CRF) prior to the patient being

formally enrolled in the study. (See sections 4.1 and 4.2 for complete listing of eligibility criteria).

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Any subjects experiencing a serious adverse event (SAE) felt to be related to study drug will be withdrawn from the study. Subjects will be withdrawn if discontinuation from the study is deemed by the principal investigator (PI) to be in their best interest, namely if an abnormal response of the subject's malignancy to radiotherapy outside of normal limits is observed. Subjects may be withdrawn at any time at the discretion of the PI. Any subject withdrawing their consent to participate in the study or their authorization to use their protected health information will be withdrawn from the study. Subjects withdrawn may be replaced at the discretion of the principal investigator.

Subjects may be withdrawn due to any of the following:

1. Intolerable side effects
2. Clinical deterioration
3. Failure to attend outpatient and treatment visits
4. Failure to provide lab specimens
5. Failure to cooperate and provide toxicity and tolerability measures

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Even though subjects may be withdrawn prematurely from the study, toxicity data will continue to be assessed as part of the normal radiotherapy protocol as determined by the investigator. Routine tests will be performed as standard of care as part of the ongoing radiotherapy.

5 Study Drug

5.1 Description

(Also see section 1.2 Investigational Agent)
Flaxseed is a non-toxic whole grain nutritional supplement with high content of natural antioxidants.

5.2 Treatment Regimen

Subjects will be asked to eat FS daily starting approximately 1 week before of the initiation of radiotherapy. The subjects will be asked to continue eating FS for the duration of their therapy – approximately 7 to 8 weeks – for a total flaxseed administration of approximately 8 to 9 weeks.

5.3 Method for Assigning Subjects to Treatment Groups

This is a single arm trial and all subjects will be assigned the same treatment regimen.

5.4 Preparation and Administration of Study Drug

FS will be stored by the Investigational Drug Service at the University of Pennsylvania. FS will be dispensed to subjects on a weekly basis during radiation therapy. Dispensing will occur

during subjects' daily visits for radiotherapy as part of standard of care on an as needed basis to allow for ingestion during the study treatment period.

5.5 Subject Compliance Monitoring

Study subjects will be asked to complete a "FS log" during the study treatment period, and a research technician will assess treatment regimen compliance by determining quantities of FS eaten. This will occur on a weekly basis during subjects' daily visits for radiotherapy. If a subject is deemed to be significantly non-compliant with the FS diet, the reason will be documented. This will be entered in RedCap by the Clinical Research Coordinator. Patients will also get text message or phone call reminders to take their flaxseed.

5.6 Prior and Concomitant Therapy

A comprehensive medical history including medication history will be obtained prior to enrollment to determine if subjects meet eligibility criteria. (See sections 4.1 and 4.2 for complete listing of eligibility criteria).

Prior therapy not permitted:

1. Any dietary supplements such as herbals or botanicals within the prior **14** days.
2. Prior flaxseed or flax-containing products, soybeans or soy-containing products.
3. Prior thoracic radiotherapy.
4. Any investigational agent within the prior **14** days.
5. Amifostine or Mucomyst within the prior **14** days.

Prior therapy permitted:

1. Vitamins or multivitamins including calcium and vitamin D.

Concomitant therapy not permitted:

1. Flaxseed, flax-containing products, soybeans, or soy-containing products.
2. Any herbals or botanicals.

5.7 Packaging

FS grain will be will be packaged by the supplier in individual packets for daily consumption. The study subjects will be provided with instructions to ingest 40g of daily FS.

5.8 Blinding of Study Drug

This is a single arm study and as such there will be no blinding of treatment assignment.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Ground whole flaxseed will be manufactured, packaged and provided to the University of Pennsylvania by Golden Valley Flax by Hylden Farms of North Dakota, Park River, North Dakota. It will be received by the Investigational Drug Service (IDS) at the University of Pennsylvania.

5.9.2 Storage

Ground whole grain flaxseed will be stored by the Investigational Drug Service (IDS) at the University of Pennsylvania in a cool dry place per product instructions protected from direct light.

5.9.3 Dispensing of Study Drug

All FS will be dispensed in subject-specific packages in sufficient quantities to allow for the ingestion during the study treatment period. No extra FS will be provided. The subject will be provided with a one week supply of individually packaged FS with clear instructions given at the start of the treatment period to consume. Subjects will be given logs to track FS consumption. The Investigational Drug Service (IDS) at the University of Pennsylvania will dispense flaxseed to research staff that will provide it to the study participants.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of study materials shipped, consumed, and remaining. This reconciliation will be logged on the reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study materials. Materials destroyed on site will be documented in the study files.

6 Study Procedures

See Table 1 (Study Flow Chart).

6.1 Visit 1: Pre-irradiation (Week -1)

At Visit 1 (Pre-irradiation), subjects will receive information regarding the study and informed consent will be obtained. A baseline CTCAE toxicity evaluation will be performed. Flaxseed and FS log will be distributed to subjects. Subjects will be asked to start daily FS ingestion. A blood sample and urine sample will be obtained for baseline urinary isoprostanes (a marker of oxidative stress) and plasma lignan levels (flaxseed metabolites). The blood sample obtained will be up to 15mL in volume. A buccal swab will also be obtained.

Please note that radiation is not an experimental question.

Standard pre-irradiation procedures will be performed as follows:

The radiation dose will be prescribed at mid-separation on the central ray for two equally weighted beams. For all other beam arrangements, the dose will be prescribed at the center of the target area or at the intersection of central rays of the beams.

Simulation for all fields is required. CT-based treatment planning is required for all subjects and will be used to define the target volume. The gross tumor volume (GTV) will consist of all known sites of disease including the primary tumor and all pathologically enlarged ($\geq$ 1 cm in short axis) or FDG-PET positive lymph nodes. Discontinuous volumes are allowed. Elective nodal irradiation is not allowed and therefore the GTV will be equal to the clinical target volume (CTV) for this study. The planning target volume (PTV) will be constructed from the GTV by an automatic margining tool supervised and edited by the treating radiation oncologist. The

irradiation target volume will be defined by shaped ports with custom-made blocks or multileaf collimation. Portal verification shall be done for all treated fields. Megavoltage equipment is required with minimum peak photon energies of 6 MV. SAD techniques should be used. Each field will be treated every session. Interruptions in therapy should be discussed with the principal investigator but will be instituted at the discretion of the attending radiation oncologist.

Dose calculation should be performed using inhomogeniety corrections to account for differences in tissue density across the thorax. The total dose to gross disease will be **60-70** Gy. The prescribed dose will be constrained to keep the volume of lung receiving 20 Gy (V20) to be less than 35%. This will be administered in 2.0 Gy daily fractions without a planned treatment break. Treatment will be continuous, 5 days per week for 7-8 consecutive weeks.

Normal tissue doses: Dose-volume histograms should be performed for spinal cord, lungs, and heart. The maximal spinal cord dose should not exceed 45 Gy. No more than 35% of the total lung volume should receive greater than 20 Gy. No more than 50% of the total cardiac volume should receive greater than 40 Gy. **The esophagus will be contoured on all patients and the dose volume parameters will be obtained. This is not a dose-limiting structure, and therefore no specific constraints will be mandated.**

6.2 Visit 2: Start of Irradiation (Week 0)

At Visit 2 (Start of Irradiation), toxicity evaluation will be performed and CTCAE scoring determined. Blood, urine, and buccal samples will be obtained. The blood sample obtained will be up to 15mL in volume. Definitive radiation therapy will commence.

6.3 Visits 3 to 9(10): Radiation Weeks 1 through 7(8)

Toxicity evaluation and CTCAE scoring will be obtained. FS compliance will be assessed once each week and FS log will be reviewed.

At approximately 4 weeks following the start of XRT, blood urine, and buccal samples will be obtained. Specimens will be obtained only once at this time point during the course of XRT. The blood sample obtained will be up to 15mL in volume

Subjects will be instructed not to eat their FS within 4 hours before radiation, and preferably after radiation treatment on those days. Subjects will be instructed to stop eating FS at the completion of their prescribed radiotherapy.

6.4 Post Radiation 1 Month Follow-up

Toxicity evaluation and CTCAE scoring will be performed, with bloodwork being performed as clinically indicated as part of standard of care. Urine and blood sampling for urinary isoprostanes and plasma FS lignan levels will be assessed. A buccal swab will also be obtained.

6.5 Post Radiation 6 Month Follow-up

Toxicity evaluation and CTCAE scoring will be performed, with blood work being performed as clinically indicated as part of standard of care. Study cessation will be at the 6-month post XRT visit.

Table 1. Study Flow Chart

Event	Pre-study Eligibility	1 week prior to XRT	Start of XRT	Weekly during XRT	End of XRT	1 month post XRT	6 months post XRT
Visit Week		Visit 1 Week -1	Visit 2 Week 0	Visits 3 to 8(9) Week 1 to 7(8)			
Medical History	X						
Informed Consent	X						
FS Consumption		X*	X*	X*	X*		
FS Log Review		X	X	X	X		
Toxicity Evaluation		X	X	X	X	X	X
FACT-L Questionnaire		X	X	X***	X	X	X
Specimen Collection							
Urine specimen		X**	X	X***	X	X	
Blood specimen		X**	X	X***	X	X	
Buccal Swab		X**	X	X***	X	X	

*FS consumption to begin after first specimens are obtained approximately 1 week prior to XRT and to continue every day during XRT and end at the end of XRT.
** The first specimen collection can be done any time after consent but prior to first FS consumption.
***Specimen collection and FACT-L questionnaires during XRT will occur at one time point only (approximately 4 weeks following the start of XRT)

7 Statistical Plan

7.1 Study Design

This is a single arm phase II flaxseed diet supplement for subjects with locally advanced or metastatic NSCLC who are scheduled to begin conventional chemoradiation therapy with definitive intent. Flaxseed is thought to be a protectant for radiation pneumonitis. Subjects will be told to ingest 40g of flaxseed daily.

FS will be ingested daily for 8-9 weeks, including 7-8 weeks during radiation and for 1 week prior to radiation. Subjects will be queried weekly about their FS consumption while on the diet. Serial measurements of plasma levels of flaxseed metabolites, urine levels of oxidative stress markers, and buccal swabs will be obtained at 5 time points: baseline (prior to start of radiation), at week 0 of radiation, 4 weeks into radiation, at the end of radiation and at one month post-radiation. This assessment is important because NSCLC subjects receiving radiation therapy often develop esophagitis and swallowing difficulties.

7.2 Statistical Analyses and Decision-making

Primary Objective: This is a single-arm phase II trial. The primary endpoint will be absence of radiation pneumonitis (RP) of grade 3 or above within 6 months from the end of RT, according to the CTCAE v.4.0 scale. The most recent pooled Radiation Therapy Oncology Group analysis showed that the rate of ≥ grade 3 RP was 18%[51], so the null hypothesis "clinical response" rate for this study is 82%. A sample size of n=39 achieves 80% power to detect a response rate of 94.1% in a one-sided binomial exact test with target significance level no larger than α=0.1. The actual significance level achieved by this test is 0.062. We will reject the null hypothesis that FS treatment has no effect on the RP rate if there are 3 or fewer cases of RP in the 39 evaluable subjects. Assuming a death rate of 10% prior to 6 months and a 10% inevaluable rate, **we will seek to enroll 48 patients**. As the Department of Radiation Oncology at Penn is one of the busiest centers in the country and currently treats approximately 235 patients daily, and the thoracic oncology service is the busiest clinic service within the Department and has exceptional rates of enrollment of patients onto investigator-initiated clinical trials, we expect to have no difficulty enrolling 48 patients in two years.

Secondary Objective: We will analyze the continuous secondary endpoints (Aim 2) using mixed linear models that include random effects for subject. We will inspect the data before analysis to identify a transformation to normality, if needed. A simpler form of the analysis would be to conduct a paired *t* test comparing baseline to post-treatment values. With n=39 evaluable subjects, we will be able to detect a change in the mean of 0.533 standard deviations with 90% power in a two-tailed test with type I error rate α=0.05. We will collect blood and urine for markers of antioxidant activity and systemic inflammation, and buccal swabs for antioxidant enzyme epithelial gene expression at the specified time points. We will analyze the data using mixed linear models that include random effects for subject. This will effectively be the same as a paired t-test on differences across the treatment periods.

Multiplicity: We will take an intermediate approach to avoid potential spurious claims of statistical significance due to the multiplicity of outcomes. We will consider the sets of outcomes — antioxidant activity, epithelial gene expression, and clinical safety and efficacy — separately, adjusting for multiplicity as needed within each by the Simes procedure, which is more powerful than Bonferroni but provides the same level of control for the family-wise error rate.

7.3 Sample Size and Study Duration

A maximum of 48 subjects with locally advanced or metastatic NSCLC who undergo definitive chemoradiation will be accrued over approximately two years. The study will close when the final subject has undergone the approximately 6-month post-radiation follow-up visit.

7.4 Subject Population(s) for Analysis

The all-treated population will be used for all study analyses. For purposes of this study, the all-treated population is defined as the group of subjects ingesting at least 1 week of FS.

8 Safety and Adverse Events

8.1 Adverse Event Reporting

Serious adverse events will be reported to the IRB and DSMC per institutional policy.

All events meeting the DSMC reporting requirements will be entered into the mandatory Velos AE/SAE form. Once an event is entered, an e-mail alert to Doris Shank (doris.shank@uphs.upenn.edu) will be sent. A study CRF will not replace the ACC central reporting form. Information in Velos will be accurately maintained and updated in a timely manner. If new/updated information is learned about the event, the event will be amended or corrected promptly. An investigator will determine the grade, attribution and expectedness of all events.

The following timeline will be adhered to for reporting events to the DSMC:

1. All grade 3 or higher events (AE or SAE) within five business days of knowledge
2. All unexpected deaths within 24 hours of knowledge.
3. All other deaths within 30 days of knowledge. Deaths of subject's off-study for greater than 30 days from the last study treatment/intervention are not reportable.

8.2 Stopping Rules

If the subject develops adverse gastrointestinal complaints (Grade 3 or higher GI toxicity), or if the subject develops other significant side effects otherwise deemed unrelated to current radiotherapy, or if the subject displays unexpected lack of tumor responsiveness to radiotherapy, then the subject will be removed from the study.

9 Protocol Exceptions and Deviations

9.1 Protocol Exceptions

A one time, **intentional** action or process that departs from the IRB and CTSRMC approved study protocol, intended for **one** occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, **advance** documented IRB and DSMC approval is required.

9.2 Protocol Deviations

A one time, **unintentional** action or process that departs from the IRB and DSMC approved study protocol, involving one incident and **identified retrospectively**, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation must be reported to the DSMC within 5 business days and the IRB within 10 business days.

9.3 Reporting of Protocol Exceptions and Deviations

Protocol Exceptions and Protocol Deviations will be approved or reported to the IRB and DSMC per institutional requirements.

10 Data Handling and Record Keeping

10.1 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 subject(s) in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

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

10.1.1 Unintentional Disclosure

Upon discovering that PHI may have been or has been disclosed to anyone not specified in the HIPAA disclosure consent, the investigator will report the disclosure to the Institutional Officer in the Office of Research Compliance and Integrity. The report should contain details about the type of data disclosed and the extent of the disclosure (number of subjects, who received it etc.).

10.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a research study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents and may be paper, electronic or a combination of both. 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 CONFIDENTIAL. This material is the property of the University of Pennsylvania. Do not disclose or use except as authorized in writing by the study sponsor.

10.3 Case Report Forms

We will not use specific case report forms.

10.4 HIPAA Retention Period (45 CFR164.530(j))

Protected Health Information (PHI) Research Requests (HIPAA1-008):
Records documenting research requests, privacy board review or privacy officer expedited review, background material, and acceptance or denial of request. Retain 6 years after research completed.

Protected Health Information Disclosure Records (HIPAA1-009):
Documenting the release of PHI, including **both authorized and unauthorized** releases. Should include the date of release, to whom the information was released, and the circumstances of the release. Retain 6 years after research completed.

Maintenance of HIPAA records is independent of the regulations for clinical study records. All records of PHI research requests and any type of release will maintained for 6 years after the research is fully terminated.

11 Study Monitoring, Auditing, and Inspecting

11.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan in Attachment 1. The investigator will allocate adequate time for such monitoring activities.

11.2 Auditing and Inspecting

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

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

12 Ethical Considerations

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

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.

13 Study Finances

13.1 Funding Source

This study will be financed through a R21 grant through the National Institutes of Health (NIH).

13.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) 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 prior to participation in this study. All University of Pennsylvania investigators will follow the University conflict of interest policy.

13.3 Subject Stipends or Payments

FS will be provided free of charge. Subjects will not be paid for their participation in this study.

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