NIH
Clinical Research Protocol

Fibroblast Specific Inhibition of LOXL2 and TGFbeta1 Signaling in Patients With Pulmonary Fibrosis.

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Investigational Product:	Epigallocatechin-3-gallate (EGCG)
IND Number:	144120
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Development Phase:	Pilot Study
Sponsor:	Harold A. Chapman 513 Parnassus Ave, HSE 201 San Francisco, CA 94143
Funding Organization:	NIH and private foundation
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Coordinating Center:	UCSF

Approval:

PI or Sponsor Signature (Name and Title)

03/20/20
Date

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LIST OF ABBREVIATIONS

Add all other abbreviations referenced in the protocol and delete any not referenced in the protocol.

AE	adverse event
CFR	Code of Federal Regulations
CRF	case report form
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
$FEF_{25\%-75\%}$	forced expiratory flow
FEV_1	forced expiratory volume over one second
FVC	forced vital capacity
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
PI	Principal Investigator
PK	pharmacokinetic
SAE	serious adverse experience

PROTOCOL SYNOPSIS

TITLE	Fibroblast specific inhibition of LOXL2 and TGFbeta1 signaling in patients with pulmonary fibrosis.
SPONSOR	Harold A. Chapman
FUNDING ORGANIZATION	NIH
NUMBER OF SITES	2
RATIONALE	The marked anti-fibrotic effects in mice of combined LOXL2 and TGFbeta receptor kinase inhibition specifically in fibroblasts with an agent such as EGCG that is low cost and already experienced by many people compels an attempt at translation to humans with progressive fibrotic disease. We have recently verified that EGCG (100 mg/kg) given orally to mice completely blocks fibrosis and lung LOXL2 activity induced by bleomycin. In this aim we want to determine if we can block LOXL2, and TGFbeta1 signaling, in lungs of humans with fibrosis given oral EGCG.
STUDY DESIGN	This is an interventional study with two parts intended to test inhibition of a signaling pathway in vivo in ILD patients. Part 1: We will initially establish doses of oral EGCG that achieve plasma levels known to be safe in non-diseased human volunteers and likely to target fibroblast TGFbetaRI kinase by measuring EGCG levels at baseline, 0.5, 2, and 4 hours after administration. Part 2: Patients presenting to the UCSF ILD clinic and scheduled for lung biopsy because of uncertain imaging will be approached and consented patients will be randomized to EGCG-treated or untreated control groups. EGCG-treated group will take EGCG daily for a minimum of 2 week prior to surgery. Disposable fragments of biopsies will be either powdered for biochemical assays including pSmad3 and Snail 1or immunoprecipitated to correlate LOXL2 protein and LOXL2 enzyme activity, or dissociated for single cell analysis. Urine collected before and after EGCG exposure will be used to determine whether terminal collagen cross-link breakdown products, termed pyridinoline/deoxypyridinoline (PYD/DPD), are a reliable biomarker of drug response.
PRIMARY OBJECTIVE	Execute a proof-of-principle pilot study testing the hypothesis that oral EGCG will block LOXL2/TGFbeta receptor kinase activities and pro-fibrotic signaling in fibrotic lungs.
SECONDARY OBJECTIVES	If it becomes apparent that the pilot study will validate our central hypothesis then initiating the administrative procedure and incurring the administrative cost of preparing an IND application for a phase II trial with the FDA could proceed in parallel.
NUMBER OF SUBJECTS	35 (Part 1: 15 non-diseased patients; Part 2: 20 interstitial lung disease patients)
SUBJECT	Inclusion Criteria:

SELECTION CRITERIA	Part 1: Age 40-70 yrs old; no history of chronic lung disease Part 2: Diagnosis of interstitial lung disease (ILD); high-resolution CT imaging indicative of lung fibrosis of uncertain classification; referral for diagnostic surgical biopsy at UCSF. Exclusion Criteria: Both Part 1 and 2: any co-morbidity affecting hepatic function, such as HCV infection, cirrhosis, or using drugs with significant hepatic toxicities (total bilirubin >1.5× the upper limit of normal (ULN); aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3× ULN; alkaline phosphatase >2.5× ULN).
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Epigallocatechin-3-gallate (EGCG) Part 1: 450 mg, 600 mg, or 750 mg once orally Part 2: 600 mg once daily orally for at least 2 weeks (dose to be determined in Part 1).
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	N/A
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Part 1: up to 8 hours Part 2: up to 4 weeks The total duration of the study is expected to be 2 years.
CONCOMMITANT MEDICATIONS	Allowed: Standard therapy for ILD Prohibited: Drugs with significant hepatic toxicities
EFFICACY EVALUATIONS	N/A
PRIMARY ENDPOINT	Part 1: EGCG blood levels Part 2: LOXL2 activity and TGFbeta1 signaling markers Snail1, pSmad3, and single cell analysis in lung biopsy tissue; urinary collagen crosslinks (PYD/DPD)
SECONDARY ENDPOINTS	Tolerability
OTHER EVALUATIONS	N/A
SAFETY EVALUATIONS	Standard adverse event reporting.
PLANNED INTERIM ANALYSES	N/A

STATISTICS **Primary Analysis Plan**	Part 1: Part 2: LOXL2 protein levels and activity in lung tissue specimens will be compared to levels and activity in control lung tissue available from the UCSF ILD tissue bank using non-parametric analytical methods as appropriate. Post-treatment reductions in urinary PYD/DPD levels will be compared using parametric methods as appropriate.
Rationale for Number of Subjects	Part 1: Based on variability in prior dosing studies of EGCG in human volunteers, 5 volunteers per dose is at the minimum end to assess reliability of sufficient blood levels of EGCG, detected by LC/MS, in doses of 450 to 750 mg once orally. Part 2: For urine biomarker analyses, we are not sure of the needed population size but because each patient serves as their own control we believe 20 subjects should reveal whether there is any change in urinary PYD/DPD levels after at least 2 weeks of EGCG.

1 BACKGROUND

TGFβ1 is strongly implicated in the relentless accumulation of cross-linked, fibrillar collagens that characterize fibrotic diseases, especially IPF. Although attractive as a target, the critical roles of TGFβ1 in suppressing inflammation and epithelial proliferation give pause to the idea of global inhibition of TGFβ1 signaling. Indeed systemic inhibition of TGFβ1 can lead to the development of squamous skin tumors and autoreactive immunity. In addition, chronic administration of small molecule inhibitors of TGFβ1 receptor kinases have led to enhanced skin and colonic inflammation and abnormalities in cardiac valves. In an attempt to develop a more circumscribed inhibitor of TGFβ1 signaling centered on suppression of collagen accumulation we undertook a high-throughput, image-based phenotypic screen (HTS) of small molecules that block TGFβ1-induced epithelial-mesenchymal transition (EMT) of A549 cells in vitro, and suppress fibrosis in vivo, but not immediately inhibit TβRI kinase itself. We identified trihydroxyphenolic compounds such as epigallocatechin-3-gallate (EGCG) as potent (IC50 ~ 50 nM) blockers of TGFβ1 responses, Snail1 expression (a critical driver of mesenchymal expansion), and collagen deposition in vivo in the single dose bleomycin model of pulmonary fibrosis. Remarkably, the functional effects of trihydroxyphenolics required the presence of active lysyl oxidase-like 2 (LOXL2) thereby limiting effects to fibroblasts, the major LOXL2 producers (apart from tumor cells). Mechanistic studies revealed that the trihydroxyphenolics induce auto-oxidation of a LOXL2/3-specific lysine (K731) in a time-dependent reaction that irreversibly inhibits LOXL2 and converts the trihydroxyphenolic to a novel metabolite directly inhibiting TGFβ1 receptor kinase (TβRI). Combined inhibition of LOXL2 and TβRI activities by trihydroxyphenolics results in potent blockade of pathological collagen accumulation in vivo. Positive data generated from this human pilot study would compell a phase 2 clinical trial of a non-patentable, inexpensive agent such as EGCG as a therapeutic for IPF.

1.1 Overview of Non-Clinical Studies

The marked anti-fibrotic effects in mice of combined LOXL2 and TβR kinase inhibition specifically in fibroblasts with an agent such as EGCG that is low cost and already experienced by many people compels an attempt at translation to humans with progressive fibrotic disease. We have recently verified that EGCG (100 mg/kg) given orally to mice completely blocks lung LOXL2 induced by bleomycin. We want to determine if we can block LOXL2, and TGFβ1 signaling, in lungs of humans with fibrosis given oral EGCG.

We have obtained 10 samples of explanted IPF lung tissue banked in the UCSF Biorepository and 10 samples from IPF patients with FVC < 50% obtained through the NIH-funded LTRC, along with tissue from 9 mostly normal lungs not used for transplantation. Extracts of these samples were processed and analyzed for LOXL2 protein and enzyme activity. Overall, LOXL2 activity was significantly increased in the IPF population compared with normal, consistent with several prior reports that LOXL2 mRNA and protein are increased in IPF. These findings imply that inhibition of LOXL2 in vivo ini ILD subjects is feasible and can be measured.

Urine collected from two cohorts of IPF patients and controls at two sites: UT San Antonio Medical Center and UCSF were tested in the PYD/DPD EIA assay along with PYD/DPD standards and levels normalized to creatinine according to manufacturer's instructions. There are significant (Mann-Whitney) increases in urinary PYD/DPD in both IPF patient cohorts. In mice, the increase in PYD/DPD after bleomycin is blocked by a trihydroxyphenolic (corilagin).

1.2 Overview of Clinical Studies

N/A

2 STUDY RATIONALE

The marked anti-fibrotic effects in mice of combined LOXL2 and TbetaR kinase inhibition specifically in fibroblasts with an agent such as EGCG that is low cost and already experienced by many people compels an attempt at translation to humans with progressive fibrotic disease. We have recently verified that EGCG (100 mg/kg) given orally to mice completely blocks fibrosis and lung LOXL2 activity induced by bleomycin. In this aim we want to determine if we can block LOXL2, and TGFbeta1 signaling, in lungs of humans with fibrosis given oral EGCG.

2.1 Risk / Benefit Assessment

Potential Risks
a. By participating in the study patients are at risk for loss of confidentiality.
b. The risk of donation of 60 ml blood is minimal though transient pain, bleeding, and bruising at the site are possible. There is no risk of collection of voided urine samples.
c. There is potential risk for adverse effects from oral ingestion of EGCG. EGCG is currently listed as the main active compound in over 90 trials registered on clinicaltrials.gov. None of these relate to fibrosis. In at least two completed trials EGCG 800 mg per day was given between recruitment and surgery for both breast and prostate cancer and no serious adverse events were noted. In a meta analysis of green tea extract containing ~800-1200 mg EGCG per day in 34 trials over 10 yrs mild liver transaminitis was noted in 4 trials and overall the incidence of liver toxicity was estimated to be rare. No serious adverse events were noted. Several trials for "chemoprevention" employing 800 EGCG per day and lasting 6 months to a year in patients at risk for cardiovascular disease or cancer noted no adverse effects. Overall, it appears mild liver transaminitis may occur but this is rare.

There is no direct benefit at present for any of the subjects individually. There is the potential for improved therapy of pulmonary fibrosis progression if EGCG has the predicted biological impact and for a new noninvasive biomarker to track drug responses that may benefit future subjects with IPF.

IPF is the leading cause of death from pulmonary fibrosis in the US and the cause of death is largely due to the untreatable and progressive nature of the illness. Experiments proposed in this application are intended to support the development of a new therapeutic approach to IPF based on inhibition of LOXL2 and TGFβ1 signaling specifically in fibroblasts. This project is particularly important because the knowledge gained could

support a phase II clinical trial in IPF, raising the possibility of improved management of this relentless disease.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to execute a proof-of-principle pilot study in ILD patients with fibrosis testing the hypothesis that oral EGCG will block LOXL2/TbetaR kinase activities and pro-fibrotic signaling in fibrotic lungs.

3.2 Secondary Objectives

If it becomes apparent that the pilot study will validate our central hypothesis then initiating the administrative procedure and incurring the administrative cost of preparing an IND application for a phase II trial with the FDA could proceed in parallel.

4 STUDY DESIGN

4.1 Study Overview

This is an interventional, multi-site study with two parts intended to test inhibition of a signaling pathway in vivo in ILD patients.

Part 1: We will initially establish doses of oral EGCG that achieve plasma levels known to be safe in non-diseased human volunteers and likely to target fibroblast TGFbetaRI kinase by measuring EGCG levels as baseline, 0.5, 2, and 4 hours after administration.

Part 2: Patients presenting to the UCSF ILD clinic and scheduled for lung biopsy because of uncertain imaging will be approached and consented patients will be randomized to EGCG-treated or untreated control groups. EGCG-treated group will take EGCG daily for a minimum of 2 week prior to surgery. Disposable fragments of biopsies will be either powdered for biochemical assays including pSmad3 and Snail 1 or immunoprecipitated to correlate LOXL2 protein and LOXL2 enzyme activity, or dissociated for single cell analysis. Urine collected before and after egcg exposure will be used to determine whether terminal collagen cross-link breakdown products, termed pyridinoline/deoxypyridinoline (PYD/DPD), are a reliable biomarker of drug response.

4.2 Primary Efficacy Endpoint

Part 1: EGCG blood levels
Part 2: LOXL2 activity and TGFbeta1 signaling markers Snail1, pSmad3, and single cell analysis in lung biopsy tissue; urinary collagen crosslinks (PYD/DPD).

4.3 Secondary Efficacy Endpoints

N/A

4.4 Safety Evaluations

Standard adverse event reporting.

4.5 Other Evaluations (include only if applicable)

5 SUBJECT SELECTION

5.1 Study Population

Two study populations will be recruited in this study:

- Non-diseased volunteers. Healthy adults ages 40 to 70 will be recruited.
- Subjects presenting to the UCSF ILD clinic and then scheduled for a diagnostic VATs biopsy. These subjects will have mild to moderate reductions in FVC and DLCO and high resolution chest CT imaging indicative of fibrosis but fibrosis that is judged unclassifiable, meaning possible IPF, hypersensitivity pneumonitis, CVD-associated ILD, or other non-IPF fibrotic disorders. Approximately 10-20 diagnostic VATs biopsies are done each year at UCSF by one of our co-investigators, Jasleen Kukreja. Subjects scheduled for biopsy will be asked their willingness to voluntarily participate in this study.

5.2 Inclusion Criteria

Part 1: Age 40-70 yrs old; no history of chronic lung disease
Part 2: Diagnosis of interstitial lung disease (ILD); high-resolution CT imaging indicative of lung fibrosis of uncertain classification; referral for diagnostic surgical biopsy at UCSF.

5.3 Exclusion Criteria

Both Part 1 and 2: any co-morbidity affecting hepatic function, such as HCV infection, cirrhosis, or using drugs with significant hepatic toxicities (total bilirubin >1.5× the upper limit of normal (ULN); aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3× ULN; alkaline phosphatase >2.5× ULN).

6 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

6.1 Allowed Medications and Treatments

Standard therapy for ILD is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below.

7 STUDY TREATMENTS

7.1 Method of Assigning Subjects to Treatment Groups

There is no randomization for this study.

7.2 Blinding

There is no blinding for this study.

7.3 Formulation of Test and Control Products

We plan to use >95% EGCG capsules purchased from Teavigo as a single lot. No control product.

7.3.1 Formulation of Test Product

We plan to use >95% EGCG purchased from Teavigo. A composition of matter analysis by Covance of the lot used for encapsulation into 150 mg capsules is listed below. A single lot will be used for all dosing in this pilot study.

Analysis of Teavigo EGCG capsule 150 mg by Convance

	Test Product
Active Ingredient EGCG, mg/Serving Size	160
Other Ingredient, mg/ Serving Size	52
Total Sample Weight, mg/ Serving Size	212

7.3.2 Formulation of Control Product

N/A

7.3.3 Packaging and Labeling

EGCG is supplied in cartons containing 70 capsules.

Each carton of EGCG will be labeled with “Dietary Supplement”, the protocol number, a treatment number, the name of the sponsors, and directions for patient use and storage.

7.4 Supply of Study Drug at the Site

The Sponsor (or designee) will purchase EGCG capsules from Teavigo. The initial shipment will be shipped after site activation (i.e., all required regulatory documentation has been received by the Sponsor and a contract has been executed). Subsequent shipments will be made after site request for resupply.

7.4.1 Dosage/Dosage Regimen

Part 1: 450 mg, 600 mg, or 750 mg once orally
Part 2: 600 mg once daily orally for at least 2 weeks (dose determined in Part 1)..

7.4.2 Dispensing

Drs. Harold Chapman, Harold Collard, Jeffrey Golden have authority to dispense EGCG.

7.4.3 Administration Instructions

Part 1: 450 mg, 600 mg, or 750 mg once orally
Part 2: 600 mg once daily orally for at least 2 weeks (dose determined in Part 1)..

7.5 Supply of Study Drug at the Site

The Sponsor (or designee) will purchase EGCG capsules from Teavigo. The initial shipment will be shipped after site activation (i.e., all required regulatory documentation has been received by the Sponsor and a contract has been executed). Subsequent shipments will be made after site request for resupply.

7.5.1 Storage

Dietary Supplement EGCG should be stored by the study site at controlled room temperature, 15 to 30ºC (59 to 86ºF). If the temperature of storage in the clinic/pharmacy exceeds or falls below this range, this should be reported to the Sponsor or designee and captured as a deviation. Subjects will be instructed to store the medication in original packaging (foil pouch and protected from light) at room temperature according to the instructions outlined on the Drug Administration Instructions.

7.6 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The number of study drug dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

7.7 Measures of Treatment Compliance

Subjects will be asked to keep a patient diary noting the day and date they take their EGCG supplement and any adverse events. They will be asked to bring their patient diary to 2 week visit along with all used and unused EGCG containers.

8 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or subject's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

8.1 Clinical Assessments

8.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Baseline/Screening and at Study Days or Visits 2, and at early termination when

applicable. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

8.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

8.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at Screening.

8.1.4 Vital Signs

Body temperature, blood pressure, pulse, height and weight, and respirations will be performed after resting for 5 minutes on Study Day 1 and Day 14.

8.1.5 Other Clinical Procedures

N/A

8.1.6 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

The study will be stopped if it meets the criteria below:

- ≥ 2 participants experiencing a severe adverse event (SAE)
- Any arrhythmias, abnormal liver function, or abnormality in physical (including neurologic examination) that in the opinion of the Principal Investigator or Medical Monitor constitutes an SAE or an AE and that warrants discontinuation in ≥ 2 participants in the active group

8.2 Clinical Laboratory Measurements (include sections as appropriate)

8.2.1 Pregnancy Test

A urine pregnancy test will be performed for subjects of childbearing potential prior to administration of the EGCG treatment. All potential subjects of childbearing potential will be also counselled to use appropriate contraception. We will exclude pregnant subjects from participation in the trial.

8.2.2 Liver Function Test

Liver function testing will be obtained on Visit1 and Visit2 for all participating subjects. Any subjects have liver function test criteria above the limit will be excluded: total bilirubin >1.5× the upper limit of normal (ULN); aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3× ULN; alkaline phosphatase >2.5× ULN.

8.3 Pharmacokinetic Measurements

Part 1; EGCG levels as baseline, 0.5, 2, and 4 hours after administration

8.4 Research Laboratory Measurements (include sections as appropriate)

8.4.1 Cell Count and Differential

N/A

8.4.2 Sputum Cytokine Measurements

N/A.

8.4.3 Pharmacokinetic Measurements

Part 1: Blood for determination of plasma concentrations of EGCG will be collected from healthy volunteers 0, 0.5, 2, and 4 hours after the start of a single dosing. EGCG concentrations will be determined at Quintara Discovery using LC/MS method.

8.4.4 Fibrotic Marker Measurements

Part 2: Disposable fragments of biopsies will be collected from IPF patients treated (at least 2 weeks) with EGCG and analyzed in Chapman Lab. Tissues will be powdered for biochemical assays including pSmad3 and Snail 1 (Western blot using specific antibodies) or immunoprecipitated to correlate LOXL2 protein and LOXL2 enzyme activity (LOX enzyme activity kit from Abcam). Or fresh tissues will be dissociated for single cell analysis.

8.4.5 Urinary Collagen Crosslinks PYD/DPD Measurements

Urine specimens will be collected from ILD patients treated (at least 2 weeks) with EGCG and analyzed in Chapman Lab. PYD/DPD collagen crosslinks will be measured and normalized using PYD EIA kit and creatinine kit from Quidel.

9 EVALUATIONS BY VISIT

Part 1 (single visit in non diseased participants):

Review the inclusion and exclusion criteria with the subject and obtain written informed consent and HIPAA authorization and assent.
Assign the subject a unique number.
Record demographics data.
Perform and record vital signs.
Collect baseline blood.
Administer EGCG dose.
Collect blood at 0.5, 2, and 4 hours post-EGCG administration

Part 2 (two visits in ILD participants)

Visit 1 (during pre-surgical clinic visit)

Review the inclusion and exclusion criteria with the subject and obtain written informed consent and HIPAA authorization and assent.
Assign the subject a unique number.
Record demographics data and concomitant medications.
Record vital signs.
Collect baseline blood and urine.
Deliver EGCG (study medication) and instruct on administration and dosing.
Provide subject diary.

Visit 2 (during surgical admission)
Record any Adverse Experiences and/or Review subject diary for adverse experiences and dosing compliance.
Record vital signs.
Collect blood and urine pre-operatively.
Collect Tissue Sample post -op

10 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

10.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

10.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

10.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

10.3 Medical Monitoring

Dr. Harold Collard, Jeffrey Golden or study personnel should be contacted directly at the below number to report medical concerns or questions regarding safety.

Phone: 415-353-2593

11 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

11.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

Subject withdrawal of consent

Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment

Protocol violation requiring discontinuation of study treatment

Lost to follow-up

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

12.3 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in

the subject's source documents. Subjects who discontinue study treatment early (i.e., they withdraw prior to Visit 2) should have an early discontinuation visit.

12.4 Replacement of Subjects

Subjects who withdraw from the study treatment will be replaced.
Subjects who withdraw from the study will be replaced.

12 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

Failure to meet inclusion/exclusion criteria

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

13 DATA SAFETY MONITORING (OPTIONAL SECTION – INCLUDE WHEN APPROPRIATE)

The Independent Monitor for this study is Dr. John Fahy. Dr. Fahy is not associated with this research project and thus works independently of the PI. Dr. Fahy is not a part of the key personnel involved in this grant, and is qualified to review the patient safety data generated by this study because of his expertise in the area of pulmonary medicine and clinical trials.

Study progress and safety will be reviewed monthly (and more frequently if needed). Progress reports, including patient recruitment, retention, and AEs/SAEs, will be provided to the monitor following each of the monthly reviews. An Annual Report will be compiled and will include a list and summary of AEs/SAEs. In addition, the Annual Report will address (1) whether AE/SAE rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely. The Annual Report will be sent to the monitor and will be forwarded to the IRB and NHLBI. The IRB will review progress of this study on an annual basis.

14 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

14.1 Data Sets Analyzed

All eligible patients who receive at least one dose of the study dietary supplement (the Safety Population) will be included in the safety analysis.

14.2 Demographic and Baseline Characteristics

N/A

14.3 Analysis of Primary Endpoint

Post-treatment reductions in PYD/DPD levels, LOXL2 protein and activity will be assessed using non-parametric as appropriate.

14.4 Analysis of Secondary Endpoints

N/A

14.5 Interim Analysis

N/A

14.6 Sample Size and Randomization

Based on variability in prior dosing studies of EGCG in human volunteers, 5 volunteers per dose is at the minimum end to assess reliability of sufficient blood levels of EGCG, detected by LC/MS, in doses of 450 to 750 mg once orally.

For blood and urine biomarker analyses, we are not sure of the needed population size but because each patient serves as their own control we believe 20 subjects should reveal whether there is any change in urinary PYD/DPD levels after 2 weeks of EGCG. To prevent selection bias and produce comparable groups, patients will be randomized into EGCG-treated or untreated groups.

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the paper CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study

documents to be collected by the Sponsor (or designee), but will be identified by a site number, subject number and initials.

If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

15.2 Data Management Procedures

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

15.3 Data Quality Control and Reporting

After data have been entered into the study records, they will be double checked by study personnel for accuracy. All changes to the study database will be documented.

15.4 Archival of Data

The data is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained.

15.5 Availability and Retention of Investigational Records

A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

15.6 Monitoring

N/A.

15.7 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation.

16 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. The research results will be made available to the subject at request. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

16.1 Protocol Amendments

Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

16.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

16.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

16.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

16.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.