CLINICAL STUDY PROTOCOL

Doxycycline for COPD in HIV-Infected Patients

IRB Protocol #1208012780

Amendment Version 12: 15 MAY 2018

Clinicaltrials.gov ID: NCT01744093

1. TITLE PAGE

Study Title:	Doxycycline for COPD in HIV-Infected Patients
Investigational Product:	Doxycycline
Indication Studied:	Chronic Obstructive Pulmonary Disease in HIV-Infected Patients
Sponsor:	National Institutes of Health
Development Phase:	Pilot Study
Principal Investigator:	Robert J. Kaner, MD
Compliance Statement:	The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and clinical research guidelines established by the Code of Federal Regulations (Title 21, CFR Parts 50, 56, and 312) and ICH guidelines. Essential study documents are currently archived in accordance with applicable regulations.
Amendment Dates:	**Original Submission - Amendment 0, v.08.06.12** **Amendment 1, v.07.31.13** **Amendment 2, v.07.22.14** **Amendment 3, v.10.07.14** **Amendment 4, v.11.20.14** **Amendment 5, v.01.29.15** **Amendment 6, v.06.27.15** **Amendment 7, v.09.16.15** **Amendment 8, v.12.04.15** **Amendment 9, v.01.28.16** **Amendment 10, v.05.27.16** **Amendment 11, v.05.12.17** **Amendment 12, v.05.15.18**

2. TABLE OF CONTENTS AND LIST OF TABLES

3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Placebo	An inert or innocuous substance used especially in controlled experiments testing the efficacy of another substance (as a drug)
Doxycycline	Name of the study "drug" in this protocol. Consists of an adenovirus vector expressing the human vascular endothelial growth factor gene.
AE	Adverse event
FEV1	Forced expiratory volume over 1 second
FVC	Forced vital capacity
HRCT	High-resolution computed tomography
PFT	Pulmonary function test
COPD	Chronic Obstructive Pulmonary Disease
HIV	Human immunodeficiency virus
MMP	Matrix metalloproteinases
DLCO	Carbon monoxide diffusing lung capacity
CD4 count	T-cell count
TLC	Total lung capacity
EKG	Electrocardiogram
SGRQ	St. George's Respiratory Questionanire
CAT	COPD assessment test
Bronchoscopy	Procedure to examine the airways of the lungs
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
ELF	Epithelial lining fluid
DGM	Department of Genetic Medicine
WCMC	Weill Cornell Medical College

4. SYNOPSIS

Name of Sponsor/Company: National Institutes of Health (NIH)

Name of Investigational Product: Doxycycline

Title of Study: "Doxycycline for COPD in HIV-Infected Patients"

Study Center(s): Weill Cornell Medicine-New York (WCMC-NY)/NewYork-Presbyterian Hospital, New York, NY

Principal Investigators:

Robert J. Kaner, MD	Weill Cornell Medical College-New York WCMC-NY/New York Presbyterian Hospital, New York, NY
Marshall J. Glesby, MD, PhD	Weill Cornell Medical College-New York WCMC-NY/New York Presbyterian Hospital, New York, NY

Investigators: Ronald G. Crystal, MD; Thomas Walsh, MD; Sarah O'Beirne, MD, PhD; Odelya Pagovich, MD; Grace Mammen, BA, CCRP; Denesy Mancenido, BA, CCRP; Jamico Jacinto, BS; Sandra Hyde

Study Period (years):
Active study: 2 years
Subject Participation: 24 weeks

Objectives:
Primary: To determine the safety, tolerability, and biologic effects of twice daily doxycycline for 6 months in HIV-infected subjects with COPD and/or emphysema.

Secondary: To characterize changes in FEV1, FVC, FEV1/FVC, DLCO over time; To characterizes change in respiratory symptoms over time (using the CAT COPD assessment test); To characterize change in BAL absolute cell number (AM, PMN, lymph); To characterize change in AM gene expression of MMPs and cytokines; To characterize change in BAL fluid MMP enzyme activity and cytokine levels; To characterize change in serum inflammatory markers (hsCRP, sIL-6, TNF-alpha, IL-1beta, procalcitonin); To determine doxycycline drug concentrations in serum, AM and ELF; To characterize change in quantitative bacterial burden in BAL fluid; To characterize frequency of doxycycline-resistant bacteria To describe adherence to study therapy by self-report and pill counts; To determine the incidence of non-elective hospitalization, classified as respiratory or non-respiratory; To determine the incidence of acute exacerbation of COPD (using the GOLD definition of change in symptoms requiring medication [we will use systemic steroids and/or antibiotics as the indicators]) To evaluate the role of biological mechanisms, such as oxidant stress, apoptosis, VEGF deficiency, etc., in relative gene expression of human alveolar macrophages and airway epithelial cells.

Study Design:
HIV-infected patients are at increased risk of COPD and emphysema may be accelerated in this population. While the pathogenesis of COPD in HIV-infected patients is almost certainly multifactorial, our preliminary data suggest that upregulation of MMPs may play a role in accelerating lung damage. Our overarching hypotheses are: (1) long-term doxycycline therapy will be safe and well tolerated; and (2) doxycycline will inhibit the activity of alveolar macrophage and epithelial lining fluid MMP. To determine this, we propose to conduct a randomized, double-blind, placebo-controlled pilot study of doxycycline 100 mg twice daily (2:1 doxycycline:placebo randomization) in 30 HIV-infected subjects with COPD and/or emphysema. We propose initially to conduct a pilot study as proof of concept of the biological effects of doxycycline, and to generate preliminary data that will facilitate the design of a larger Phase II clinical trial. For this study, the primary endpoint will be safety/tolerability, and secondary endpoints will include change in pulmonary function (FEV1) by spirometry, inhibition of MMP activity in a pan-MMP activity assay as well as individual MMPs assessed by gelatinase assays and doxycycline levels in serum, epithelial lining fluid and bronchoalveolar lavage cell pellets (consisting of alveolar macrophages, lymphocytes and some neutrophils).

Doxycycline (or matching placebo) will be given at a dose of 100 mg po twice daily for six months. This is a standard FDA-approved antibacterial dose for which there is abundant safety

data. While there are FDA-approved doses of doxycycline that are lower (e.g., 20-40 mg po daily for gingivitis or rosacea), we have selected a higher dose in this proof of concept trial in order to reduce the likelihood that we falsely conclude that the drug does not have the intended biological effects due to inadequate drug levels in the lungs. Pharmacokinetic data from subjects with cystic fibrosis support a 200 mg po daily dose of doxycycline to achieve appropriate lung levels.

Eligible subjects will have controlled HIV infection and a diagnosis of mild to moderate COPD and/or based on established criteria. Subjects will be randomized by the research pharmacist in a 2:1 fashion using permuted blocks of size 3 to receive doxycycline or placebo for 6 months and will undergo serial measures of lung function by spirometry as well as bronchoscopies at baseline and week 12 to collect specimens for translational studies aimed at characterizing the biological effects of the intervention. The bronchoscopy will be performed mainly as an outpatient procedure. The investigator may stop the procedure at any time if he/she feels that sufficient samples have been collected and/or it is not safe to continue the procedure. Subjects are expected to have an escort on the day of the bronchoscopy in order to undergo the bronchoscopy procedure. If the escort does not arrive or if no responsible adult is available to escort the subject home once the bronchoscopy procedure is complete, the subject will undergo observation by research staff until deemed stable. Follow-up calls will be performed one day (+/-3 days) and one week (+/-3 days) post-bronchoscopy. The randomization will be stratified on current versus former smoking status. The study is double-blinded, and only the research pharmacist will have knowledge of the treatment assignments, which is needed to dispense the study drug. Despite the pilot nature of this study, we have opted to conduct a randomized, placebo-controlled trial primarily to ensure that there is a control group to be able to interpret changes in biologic and physiologic endpoints that may vary based on the natural variation in the course of COPD and/or variability in the assays/assessments. We will randomize 2:1 in favor of active study drug to enable us to have a sufficient number of recipients of active drug within the constraints of a small study.

Number of subjects (planned): 30 subjects will be enrolled and will be randomized in a 2:1 fashion. Subjects for this study will be recruited from the referral base of the investigators from the population of individuals with COPD and documented HIV as defined by the protocol inclusion/exclusion criteria (see below).

Diagnosis and main criteria for inclusion:
Inclusion Criteria:
1. Males/Females ages 18-85 years
2. Documented HIV infection
3. CD4 cell count greater than 200 cells/mm3
4. HIV RNA less than 400 copies/ml
5. Stable antiretroviral therapy for greater than or equal to 12 weeks
6. Fulfills GOLD definition for COPD (post-bronchodilator FEV1/FVC less than 0.7) and/or has radiographic evidence of emphysema
7. Current or history of smoking with minimum 3 pack-year history
8. ALT and AST less than 3 x upper limit of normal
9. For women of childbearing potential: willingness to use 2 forms of birth control
10. Subjects on therapy for COPD must be on stable therapy for at least 4 weeks

Exclusion Criteria:

1. Pulmonary infection, COPD exacerbation, or acute opportunistic infection within 30 days of entry
2. Conditions associated with increased sedation of bronchoscopy risk, including but not limited to Gold class 3 or 4 COPD, requirement for home oxygen, hypercapneic respiratory failure, poorly control hypertension
3. Known allergy/intolerance to doxycycline, atropine, or any local anesthetic
4. Inability to provide informed consent
5. Pregnant or lactating women
6. Men must agree not to attempt to make a woman pregnant of participate in sperm donation during the study and for 6 weeks after discontinuing the drug
7. End stage renal disease
8. Cirrhosis
9. INR greater than 1.4
10. Platelets less than 80,000
11. Any condition including active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements or increase the risk of bronchoscopy
12. Active or planned participation in any other clinical trial or observational study without prior approval from the PI

Endpoints: The primary endpoint will be safety/tolerability, and secondary endpoints will include change in pulmonary function (FEV1) by spirometry, inhibition of MMP activity in a pan-MMP activity assay as well as individual MMPs assessed by gelatinase assays and doxycycline levels in serum, epithelial lining fluid and bronchoalveolar lavage cell pellets (consisting of alveolar macrophages, lymphocytes and some neutrophils).

Statistical Considerations: The overall sample size is 30 subjects, all of whom will be recruited at WCMC. We anticipate screening 70 subjects to randomize 30. The study investigates the safety/ tolerability (grade 2 or higher toxicity) of long-term use of doxycycline. A 20-30% rate of grade 2 or higher toxicity is expected. With 20 doxycycline recipients, we will have approximately 80% power (one-sided alpha = 0.05) to detect a true proportion of 20% (grade 2 or higher toxicity) while rejecting the null hypothesis of 45.5% or higher (in other words, ruling out the possibility of the event rate being any higher that 45.5%). Below are additional scenarios based on a true proportion of 5- 40% (P1), which demonstrate power greater than 80% for n = 20 doxycycline recipients under these assumptions.

Data will be entered into the REDCap, a PHP-based system developed by Vanderbilt University and currently managed and updated through a national consortium that includes the Weill Cornell CTSC. In collaboration with the CTSC's data management group, we will create a secure, HIPAA-compliant, web-based study-specific database system with customized full field validation capabilities, a full audit trail and user-based privileges.

We will compute summary statistics (e.g. mean, median, SD, interquartile range, minimum and maximum) for demographic/clinical variables and the primary outcome data (e.g. spirometry endpoints, adverse events, biological endpoints, measurable doxycycline levels in serum and

ELF) by study arm. We will compare changes from baseline in continuous variables between the two groups by 2-sample t-tests or the non-parametric Wilcoxon's rank sum test as appropriate. We will perform repeated measures ANOVA or Multivariate ANOVA for repeated measures data (e.g. spirometry) as deemed appropriate after checking assumptions of sphericity using Mauchly's Test. Given the moderate sample size for this data we will compute confidence interval for estimates from RMANOVA or MANOVA to assess their precision. The proportions of subjects in each arm experiencing adverse events will be summarized by grade and compared by Chi-squared or Fisher's exact tests or generalized Fisher's test (using Monte Carlo approximations) as appropriate. In a pre-specified subgroup analysis, we will explore whether the subset of subjects with emphysema by CT respond differently to doxycycline compared to those without emphysema.

Our research groups have a strong record of recruiting HIV+ subjects into clinical trials and observational studies, and specifically of recruiting HIV+ subjects with emphysema into studies requiring bronchoscopies. The HIV clinics at the New York Presbyterian Hospital/Weill Cornell Medical Center follow approximately 2,500 active patients (defined as patients with clinic visits within the past 6 months. We are presently conducting an observational study to evaluate a screening strategy for COPD in our HIV clinics, consisting of a questionnaire and peak flow measurement (IRB # 0912010782). Subjects who meet specific criteria, and a random selection of controls who do not, will undergo Spirometry (pre- and post-bronchodilator) to assess for fixed airway obstruction. We anticipate that this study will lead to the identification of approximately 65 subjects with new diagnoses of COPD, in addition to the clinic patients with established diagnoses. Furthermore, Dr. Kaner's research group has independently identified a significant number of HIV+ subjects with COPD and a few with emphysema and no airflow obstruction who had come to volunteer for a research bronchoscopy study.

5. INTRODUCTION

5.1. Background

In the context of improved survival from HIV infection itself, COPD (chronic obstructive pulmonary disease; a form of lung disease that includes emphysema, which makes breathing difficult) is emerging as an important cause of morbidity and perhaps ultimately mortality in this population. HIV-infected patients are at increased risk of COPD, likely due to multiple factors, including an increased presence of smoking, chronic inflammation and progression of immunodeficiency, oxidant stress (excessive levels of natural chemicals called oxidants and free radicals that can damage tissue), and respiratory infections. While natural history data on COPD are limited in the era of potent antiretroviral therapy, earlier data suggest that the course of emphysema may be accelerated in this population. Our preliminary data suggest that several matrix metalloproteinases (MMPs) derived from alveolar macrophages (a type of immune cell found in the lungs) have an increased cellular response in HIV-infected smokers, which could contribute to accelerated emphysema. MMPs are enzymes that break down the structural support of tissues, including the airways in the lung.

Based on these observations, we hypothesize that pharmacologic inhibition of MMPs by doxycycline will favorably modify the natural history of COPD in HIV-infected patients. To test

this hypothesis, we propose conducting a proof of concept pilot study as a prelude to a possible phase II randomized, placebo-controlled trial of doxycycline for COPD in HIV-infected patients should the proof of concept be successful. Our research team, led by a pulmonologist/researcher with expertise in HIV-associated COPD and an infectious diseases specialist/clinical trials expert, is well positioned to propose such a trial to the NIH-funded AIDS Clinical Trials Group (ACTG).

5.2. Rationale for Study

Our proposed study of doxycycline in HIV-infected subjects with COPD is highly innovative. There are very limited clinical data on the use of doxycycline for COPD in the general population, and there are no adequately controlled studies. Our pilot study will not only serve as the basis for designing a phase II trial but also will provide a unique opportunity to advance our scientific understanding of the biological and physiological effects of MMP inhibition in lung tissue. No study, to our knowledge, has evaluated the effects of doxycycline on MMPs and pro-inflammatory cytokines in the lung in humans. Our approach to use bronchoalveolar lavage to quantify the biological effects of doxycycline on MMP and cytokine gene and protein expression is novel and will advance knowledge significantly in this field. By correlating these biological effects with drug levels in epithelial lining fluid (ELF), alveolar macrophages (AM) and serum and changes in pulmonary function, we will be able to characterize the pharmacokinetic and pharmacodynamic profile of doxycycline in HIV-infected subjects with COPD.

Controlled HIV infection. We are requiring a CD4 count > 200 cells/mm3 and HIV RNA < 400 copies/ml to select for a patient population that is at low risk of pulmonary opportunistic infections.

This will also minimize any confounding factors related to large scale changes in CD4, reconstitution syndrome, etc that might occur in a cohort with higher viral loads who have more recently been initiated on antiretroviral therapy.

COPD definition - We will exclude advanced COPD (GOLD III/IV) in this proof of concept study in order to minimize risk to subjects from the bronchoscopies. We anticipate including more advanced COPD in a Phase II study, which will not necessarily include mandatory bronchoscopies. Since HIV+ smokers can have emphysema with a low number of pack-yr we will accept anyone with > 3 pack year history. We did not include a 2nd CT in the study design, because the time frame is too short to expect to see any favorable radiographic effects on preserving lung parenchyma to justify the additional radiation exposure.

5.3 Preliminary Studies

MMPs in HIV+ Subjects with Emphysema. Dr. Kaner has recently published on the pattern of increased MMP expression in HIV+ individuals with emphysema[18] RNA was successfully isolated from alveolar macrophages of HIV+ individuals with emphysema (and microarray data was deposited in GEO) and gene expression studies were performed and compared with the results of gelatinase assays and Western blots. These studies showed increased amounts and activation of MMP-2, -7, -9 and -12 in the epithelial lining fluid of HIV+ individuals with emphysema**.** Using a pan-MMP activity assay, 8/8 HIV+ subjects with emphysema had detectable MMP activity in ELF (data not shown). Further studies have addressed the potential

upstream cytokine regulation of MMPs by cytokines. Comparing the cytokine protein profile in ELF, HIV+ individuals, both smoking and nonsmoking show a broad spectrum expression of cytokines, that shares similarities with HIV negative individuals with emphysema, but not with HIV-negative nonsmokers and smokers with normal lung function. Interestingly, several of the cytokines identified are important in the Th17 pathway. Preliminary data further indicate that IL-23 (among other cytokines) upregulates human alveolar macrophage MMP-9 production via an intermediate step that involves T-lymphocytes (manuscript in preparation). These studies, along with the developing literature around the putative role of MMPs in the development of COPD and emphysema, provide a biologic rationale for the current proposal to test an MMP inhibitor in an effort to slow the progression of COPD and emphysema.

Pulmonary Function in HIV+ Subjects with COPD/Emphysema. In 2011, the Department of Genetic Medicine COPD human samples research core headed by Dr. Kaner performed 355 research bronchoscopies and the group has successfully performed thousands of research bronchoscopies over the last 15 years. In addition, the human samples core has independently (of Dr. Glesby's group) screened 262 HIV-infected current or ex-smokers for a COPD study, of whom 34 (13.0%) met the PFT criteria for this study (post-bronchodilator FEV1/FVC < 0.7 and/or DLCO < 80% predicted).

These individuals came for screening to volunteer for a research bronchoscopy. Specifically, 23 (68%) of 34 had post-bronchodilator FEV1/FVC < 0.7 and 26 (76%) had DLCO < 80%.

6. TRIAL OBJECTIVES AND PURPOSE

6.1. Primary Objectives

- To determine the safety and tolerability of twice daily doxycycline for 6 months in HIV-infected subjects with COPD and/or emphysema.

Secondary Objectives:

- To characterize changes in FEV1, FVC, FEV1/FVC, DLCO over time
- To characterizes change in respiratory symptoms over time (using the CAT – COPD assessment test)
- To characterize change in BAL absolute cell number (AM, PMN, lymph)
- To characterize change in AM gene expression of MMPs and cytokines
- To characterize change in BAL fluid MMP enzyme activity and cytokine levels
- To characterize change in serum inflammatory markers (hsCRP, sIL-6, TNF-α, IL-1β, procalcitonin)
- To determine doxycycline drug concentrations in serum, AM and ELF
- To characterize change in quantitative bacterial burden in BAL fluid
- To characterize frequency of doxycycline-resistant bacteria
- To describe adherence to study therapy by self-report and pill counts
- To determine the incidence of non-elective hospitalization, classified as respiratory or non-respiratory

- To determine the incidence of acute exacerbation of COPD (using the GOLD definition of change in symptoms requiring medication [we will use systemic steroids and/or antibiotics as the indicators])

7. INVESTIGATIONAL PLAN

7.1. Endpoints

The primary endpoint will be safety/tolerability, and secondary endpoints will include change in pulmonary function (FEV1) by spirometry, inhibition of MMP activity in a pan-MMP activity assay as well as individual MMPs assessed by gelatinase assays and doxycycline levels in serum, epithelial lining fluid and bronchoalveolar lavage cell pellets (consisting of alveolar macrophages, lymphocytes and some neutrophils).

7.2. Overall Study Design and Plan: Schedule of Assessments

SCREENING

- Physical examination including vital signs (blood pressure, pulse, and breathing rate) weight and height, and amount of oxygen in the blood
- Lung function tests (lung volumes, spirometry (post-bronchodilator) and diffusing capacity of the lung for carbon monoxide [DLCO])
- Blood will be drawn from a vein to check blood counts (to look for infection or anemia), HIV viral load (amount of virus in the blood), CD4 (T-cell) count, and blood chemistries (blood minerals, blood sugar level, kidney and liver function, PT/PTT, complete blood count, electrolytes, renal function, CMP, CBC+diff, c-reactive and carboxy hemoglobin). If documentation of an HIV test result is not available, we will do an HIV test at screening.
- Urine will be collected to measure protein levels, check kidney function, measure poisonous alkaloid from tobacco, the break-down product of nicotine from cigarette smoke, PGE collection and to evaluate toxicology for drugs
- For females capable of getting pregnant, urine will be collected to test for pregnancy. If you are pregnant, you will not be allowed to participate in this study
- Electrocardiogram, a non-invasive test that provides a tracing of the heart's electrical activity

PRE-ENROLLMENT (OPTIONAL)

- Physical examination including your signs (blood pressure, pulse, and breathing rate) weight and height, and amount of oxygen in the blood
- Blood will be drawn from a vein to check blood counts (to look for infection or anemia), HIV viral load (amount of virus in the blood), CD4 (T-cell) count, and blood chemistries (blood minerals, blood sugar level, kidney and liver function, PT/PTT, complete blood count, electrolytes, renal function, CMP, CBC+diff, c-reactive and carboxy hemoglobin). If documentation of an HIV test result is not available, we will do an HIV test at screening.
- For females capable of getting pregnant, urine will be collected to test for pregnancy. If you are pregnant, you will not be allowed to participate in this study
- A detailed x-ray of the lungs (High-resolution Computed Tomography, or HRCT) will be done if a scan of good quality was not done in the past 6 months

ENROLLMENT

- Physical examination including your signs (blood pressure, pulse, and breathing rate) weight and height, and amount of oxygen in the blood
- Lung function tests (lung volumes, spirometry (post bronchodilator))
- Assessment of any side effects or symptoms and if there have been any changes in any of the medications you may be taking
- EKG (optional; repeated only if 30 days have passed since last EKG)
- Blood will be drawn from a vein to check blood counts (to look for infection or anemia), blood chemistries (blood minerals, blood sugar level, kidney and liver function), stored serum and biomarkers
- Urine will be collected to evaluate toxicology for drugs
- Urine will be collected to measure poisonous alkaloid from tobacco and the break-down product of nicotine from cigarette smoke
- For females capable of getting pregnant, urine will be collected to test for pregnancy. If you are pregnant, you will not be allowed to participate in this study
- Study drug dispensing
- Respiratory symptom and smoking questionnaires
- Oral rinse to collect samples of the bacterial communities in the oral mouth and upper airway and to remove loose debris that may contaminate the airway samples.
- Bronchoscopy

WEEK 2

- Physical examination including your signs (blood pressure, pulse, and breathing rate) weight and height, and amount of oxygen in the blood
- Lung function tests (lung volumes, spirometry (post bronchodilator) and diffusing capacity of the lung for carbon monoxide [DLCO])
- Assessment of any side effects or symptoms and if there have been any changes in any of the medications you may be taking
- Blood will be drawn from a vein to check blood counts (to look for infection or anemia), blood chemistries (blood minerals, blood sugar level, kidney and liver function), stored serum and biomarkers
- For females capable of getting pregnant, urine will be collected to test for pregnancy. If you are pregnant, you will not be allowed to participate in this study

WEEK 6

- Physical examination including your signs (blood pressure, pulse, and breathing rate) weight and height, and amount of oxygen in the blood
- Assessment of any side effects or symptoms and if there have been any changes in any of the medications you may be taking
- Blood will be drawn from a vein to check blood counts (to look for infection or anemia), blood chemistries (blood minerals, blood sugar level, kidney and liver function), stored serum and biomarkers
- For females capable of getting pregnant, urine will be collected to test for pregnancy. If you are pregnant, you will not be allowed to participate in this study

- Study drug dispensing

WEEK 11 (OPTIONAL)
- Physical examination including your signs (blood pressure, pulse, and breathing rate) weight and height, and amount of oxygen in the blood
- Blood collection for safety labs (complete blood count, electrolytes, liver function and renal function (CMP, CBC+diff, c-reactive, carboxy hemoglobin, and PT/PTT).

WEEK 12
- Physical examination including your signs (blood pressure, pulse, and breathing rate) weight and height, and amount of oxygen in the blood
- Lung function tests (lung volumes, spirometry (post bronchodilator))
- Assessment of any side effects or symptoms and if there have been any changes in any of the medications you may be taking
- EKG
- Blood will be drawn from a vein to check blood counts (to look for infection or anemia), blood chemistries (blood minerals, blood sugar level, kidney and liver function), stored serum and biomarkers
- Urine will be collected to measure the break-down product of nicotine from cigarette smoke, for substances released from cells that are involved in inflammation, PGE collection, and to evaluate toxicology for drugs
- For females capable of getting pregnant, urine will be collected to test for pregnancy. If you are pregnant, you will not be allowed to participate in this study
- Study drug dispensing
- Oral rinse to collect samples of the bacterial communities in the oral mouth and upper airway and to remove loose debris that may contaminate the airway samples.
- Bronchoscopy

WEEK 18
- Physical examination including your signs (blood pressure, pulse, and breathing rate) weight and height, and amount of oxygen in the blood
- Assessment of any side effects or symptoms and if there have been any changes in any of the medications you may be taking
- Blood will be drawn from a vein to check blood counts (to look for infection or anemia), blood chemistries (blood minerals, blood sugar level, kidney and liver function), stored serum and biomarkers
- Urine will be collected to measure protein levels, check kidney function, measure poisonous alkaloid from tobacco and the break-down product of nicotine from cigarette smoke
- For females capable of getting pregnant, urine will be collected to test for pregnancy. If you are pregnant, you will not be allowed to participate in this study
- Study drug dispensing

WEEK 24
- Physical examination including your signs (blood pressure, pulse, and breathing rate) weight and height, and amount of oxygen in the blood

- Lung function tests (lung volumes, spirometry (post bronchodilator) and diffusing capacity of the lung for carbon monoxide [DLCO])
- Assessment of any side effects or symptoms
- Blood will be drawn from a vein to check blood counts (to look for infection or anemia), HIV viral load (amount of virus in the blood), CD4 (T-cell) count, and blood chemistries (blood minerals, blood sugar level, kidney and liver function, CMP, CBC+diff, c-reactive and carboxy hemoglobin), stored serum and biomarkers.
- For females capable of getting pregnant, urine will be collected to test for pregnancy. If you are pregnant, you will not be allowed to participate in this study

Table I: Schedule of Evaluations

	SCREENING	PRE-ENTRY (OPTIONAL)	ENTRY	WK 2	WK 6	WK 11 (OPTIONAL)	WK 12	WK 18	WK 24	PREMATURE DISCONTINUATION
Informed consent, medical history	X									
Clinical Assessments[1]	X	X	X*	X	X	X	X*	X	X	X
Safety labs[2]	X	X	X*	X	X	X	X*	X	X	X
Blood test[3]	X	X	X*			X	X*			
Other labs[4]	X									
HIV viral load	X						X		X	X
CD4 (T-cell) count	X						X		X	X
Research blood (collection priority)[5]			X	X	X		X	X	X	
Pregnancy test (urine)	X	X	X	X	X		X	X	X	
Urine toxicology (drug panel)	X		X				X			
PGE urine collection[6]	X**						X**			
Urinanalysis[7]	X									
Urine Metabolites[8]	X		X				X	X		
Spirometry	X		X				X		X	X
EKG	X		X				X			
Bronchoscopy[9]			X				X			
High resolution chest CT[10]		X	X*							
Oral rinse[11]			X				X			
Respiratory (CAT)/smoking questionnaires			X		X		X		X	X
Dispense study drug			X		X		X	X		
Pill count/adherence questionnaire				X	X		X	X	X	X

* Optional procedures if procedures were already conducted during the optional visits.

[1] Clinical assessments include full physical examination at screening, targeted physical examinations at other visits, ongoing assessments for adverse events and new clinical diagnoses and CAT review at each visit.

[2] Safety labs consist of complete metabolic panel, complete blood count, electrolytes, liver function and renal function (CMP, CBC+diff, c-reactive, carboxy hemoglobin).

[3] PT/PTT blood test

[4] Other Labs (Hepatitis panel (A, B and C), alpha-1 anti-trypsin, IgE)

[5] Research blood: Bronchoscopy visits (Week 0/Entry and Week 12), blood samples are collected for: doxycycline blood levels, EMP, DNA, serum (doxycycline assay, biomarker assay, DGM biobank), and monocytes. Non-bronchoscopy visits: (Week 2, Week 6, ~~and~~ Week 18 and Week 24) blood samples are collected for: doxycycline blood levels and serum ~~for~~ (doxycycline assay, biomarker assay and DGM biobank). Serum for biomarker assay will be frozen at -80°C for future assays (hsCRP, IL-6, TNF-α, IL-1β, procalcitonin). Should subject pre-maturely discontinue from the study, the subject will be invited to at least provide blood samples for the doxycycline blood assay.
[6] Prostaglandin E urine collection, 9 mL. **All subjects will be assessed for eligibility. PGE will be collected for any subject, who passes the screening as per the PI's recommendation, and has not taken NSAIDS within the past 7 days. Otherwise, subject will not be eligible for the PGE urine collection.
[7] Urine analysis will be collected to measure protein levels and kidney functions
[8] Urine metabolites will be collected to measure poisonous alkaloid from tobacco and the break-down product of nicotine from cigarette smoke
[9] Bronchoalveolar lavage; ≥ 50mL. To minimize any risk of bleeding to a subject after completion of the bronchoscopy procedure, subjects are advised not to take non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and/or ibuprofen, for up to 7 days post-procedure.
[10] CT-scans taken within the last 6 months may be acceptable. Subjects will not be asked to complete more than 2 HRCT scans in one year If subjects complete the maximum of two HRCT scans in one year, they will receive no more than 2200mrem of radiation, which is within the guidelines for radiation exposure for human subjects; it is also below the maximum permissible dose limits per year for radiation workers according to the Nuclear Radiation Committee (5000mrem/year).
[11] Oral rinse to collect samples of the bacterial communities in the oral mouth and upper airway and to remove loose debris that may contaminate the airway samples. Subjects will be asked to cough 5 times and then actively gargle 10 cc of sterile saline for 20 seconds. At the end of the gargle, the subject will be instructed to swish the saline in the mouth for 5 seconds. The subject will then spit the oral rinse into the ORAL specimen container, gently spitting into the RNALater.
[12] Refer to Table II for the acceptable "time windows" for the assessment days.

Table II: Assessment Windows

EVALUATION	SCREENING	PRE-ENTRY (Optional)	ENTRY	WK 2	WK 6	WK 11 (Optional)	WK 12	WK 18	WK 24	PREMATURE DISCONTINUATION
All other evaluations	N/A	N/A	N/A	±7 days	±7 days	N/A	N/A	±7 days	±7 days	±7 days
Bronchoscopy Visits	N/A	N/A	±14 days	N/A	N/A	N/A	±14 days	N/A	N/A	N/A

Table III: Bodily Fluid Collection

Bodily Fluid	Amount (mL per test)	Number of Visits	Total (mL) (To be taken over the course of 24 weeks)
Blood			
Doxycycline blood levels	12	6	72
Safety labs	36	6	216
Blood Test	3	2	6
Other lab	21	1	21

HIV viral load	7	3	21
CD4 (T-cell) count	4	3	12
Serum for biomarkers	18	4	72
Research blood	76	2	152
Total			**572**
Urine			
Urine Toxicology	12	3	36
Pregnancy	7	8	56
PGE Collection	9	2	18
Urinalysis	10	1	10
Urine Metabolites	4	2	8
Total			**128**
BAL fluid Total	50*	2	**100**
Oral rinse Total	10	2	**20**

*Administer 150mL fluid up to 300 mL max, for a minimum return of 50mL

8. SELECTION AND WITHDRAWAL OF SUBJECTS

8.1. Sample Population

Recruitment will be from the referral base of the responsible investigator and other physicians in the Division of Pulmonary and Critical Care Medicine, the NYPH Center for Special Studies (HIV clinic), and from community providers within the NY area.

8.2. Subject Inclusion Criteria

- Males/Females ages 18-85 years
- Documented HIV infection
- CD4 cell count greater than 200 cells/mm3
- HIV RNA less than 400 copies/ml
- Stable antiretroviral therapy for greater than or equal to 12 weeks
- Fulfills GOLD definition for COPD (post-bronchodilator FEV1/FVC less than 0.7) and/or has radiographic evidence of emphysema
- Current or history of smoking with minimum 3 pack-year history
- ALT and AST less than 3 x upper limit of normal
- For women of childbearing potential: willingness to use 2 forms of birth control
- Subjects on therapy for COPD must be on stable therapy for at least 4 weeks

8.3. Subject Exclusion Criteria

- Pulmonary infection, COPD exacerbation, or acute opportunistic infection within 30 days of entry
- Conditions associated with increased sedation of bronchoscopy risk, including but not limited to Gold class 3 or 4 COPD, requirement for home oxygen, hypercapneic respiratory failure, poorly control hypertension
- Known allergy/intolerance to doxycycline, atropine, or any local anesthetic
- Inability to provide informed consent
- Pregnant or lactating women
- Men must agree not to attempt to make a woman pregnant of participate in sperm donation during the study and for 6 weeks after discontinuing the drug
- End stage renal disease
- Cirrhosis
- INR greater than 1.4
- Platelets less than 80,000
- Any condition including active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements or increase the risk of bronchoscopy
- Active or planned participation in any other clinical trial or observational study without prior approval from the PI

8.4. Withdrawal Criteria

The investigators, physicians or sponsors may stop the study or remove the subject from the study at any time should they judge that it is in the subject's best interest to do so, if he/she experiences a study-related injury, if the subject needs additional or different medication, or if he/she does not comply with the study plan. They may remove the subject from the study for various other administrative and medical reasons. They can do this without the subject's consent.

9. STUDY CONDUCT

9.1. Study Overview

A subject's participation in this study is expected to be approximately 24 weeks. The day the subject begins taking the doxycycline will be designated as day 0 and enrollment. The study will include a bronchoscopy procedure at the enrollment/entry visit and week 12 visit.

9.2. Schedule of Assessments

The screening period begins at the time of consent/assent of the study subject. Subjects will provide written informed consent and/or assent before undergoing the screening procedures at the pre-vector time point. Please refer to **Table I** in **Section 7.2.**

10. SCREENING/BASELINE ASSESSMENTS

Screening is defined as the time of the signing of the informed consent form. Assessments and procedures that are to be performed after the informed consent form has been signed. See timeline of proposed clinical study for details.

11. ASSESSMENT OF EFFICACY

Efficacy will be evaluated through all assessments particularly those of lung function including: (1) primary – pulmonary function test; and (2) secondary – respiratory questionnaires.

11.1 Expected Risks

Risks and adverse effects related to the procedures and the study drug we are studying can be divided into the following categories: (1) those associated with taking doxycycline for a prolonged period of time; (2) those associated with undergoing evaluation procedures, including risks from undergoing a high resolution chest CT scan; and (3) those associated with undergoing a fiberoptic bronchoscopy.

(1) Risks associated with taking doxycycline

Doxycycline has a long track record of safety. The most common adverse events seen with doxycycline use are:

Gastrointestinal: anorexia, nausea, vomiting, diarrhea (including *Clostridium difficile* associated colitis), glossitis, dysphagia, enterocolitis, and inflammatory lesions (with monilial overgrowth) in the anogenital region. Esophagitis and esophageal ulcerations (rare). Hepatotoxicity (rare).

Skin: Photosensitivity manifested as an exaggerated sunburn reaction. Toxic epidermal necrolysis, Stevens-Johnson syndrome, erythema multiforme, maculopapular, erythematous rashes, and exfoliative dermatitis

Renal: Dose-related increases in blood urea nitrogen (BUN). Hypersensitivity reactions: urticaria, angioneurotic edema, anaphylaxis, anaphylactoid purpura, serum sickness, pericarditis, and exacerbation of systemic lupus erythematosus.

Blood: Hemolytic anemia, thrombocytopenia, neutropenia, and eosinophilia.

Other: Intracranial hypertension in adults. When given over prolonged periods, tetracyclines have been reported to produce brown-black microscopic discoloration of the thyroid gland. No abnormalities of thyroid function studies are known to occur.

Pregnancy Risk: Doxycycline is a category D drug. There are no adequate and well-controlled studies on the use of doxycycline in pregnant women. An expert review of the Teratogen Information System concluded that therapeutic doses during pregnancy are unlikely to pose a substantial teratogenic risk

(2) Risks associated with taking placebo

The risks associated with administering a placebo are that enrolled subjects will not receive active medication and possible worsening of COPD in HIV-infected patients. Enrolled subjects taking placebo will not receive any possible benefit associated with doxycycline.

(3) Risks associated with the tests and procedures done in this study

Blood Tests. These tests involve drawing blood from a vein in the arm by usual venipuncture. The risks associated with the venipuncture procedure will include pain or bruising where the needle is put in the vein, or very rarely, damage to the blood vessel. Rarely, people faint after blood drawing. The total amount of blood to be drawn will not exceed 150 ml (less than half of a pint), which will be used for general hematologic studies and/or general serologic/immunologic studies and/or biochemical analyses and/or HIV-1 serology. The amount of blood is within the acceptable limits for human research according to the guidelines for human research according to New York Presbyterian Hospital-Weill Cornell Medical College.

Pulmonary Function Tests. These tests involve breathing in and out of a pulmonary function testing machine and breath holding as required by the technician performing the tests. They are harmless but may occasionally cause slight soreness in the respiratory muscles and chest due to effort.

High Resolution Chest CT Scan. All study individuals will be asked to perform a high resolution CT scan of the chest. The amount of radiation a person receives during a CAT scan is between 700-1000 mrem,which is a safe level of exposure. In order to avoid the risk of undue radiation to a fetus, women will not be included in this study if they are pregnant. There is always a risk of damage to cells or tissue from being exposed to any radiation. The HRCT completed as part of this study, might involve unsuspected, incidental findings that might or might not be a sign of disease and might or might not lead to further medical work-up. This further medical work-up (if it is performed) might have associated risks, potentially cause anxiety, and may incur costs to the subject and/or the subject's insurer. A high resolution chest CT scan involves exposure to radiation and that exposure is increased the more often such tests are done. Radiation exposure at certain doses can potentially cause cancer. Any possible increase in cancer risk associated with participation in this study cannot be measured, is uncertain, and assumed to be quite low. The images from the HRCT will be analyzed to quantify emphysema.

Electrocardiogram. There are no risks associated with electrocardiography, per se. However, some people develop allergic reactions (rashes) on their skin to the electrode pads.

(4) Risks associated with a fiberoptic bronchoscopy

Fiberoptic Bronchoscopy. Dr. Kaner's group has well established procedures to reduce the risk of bronchoscopies. Potential complications from fiberoptic bronchoscopy include: (1) transient fever within 12 hours of the procedure; (2) bleeding within the nasopharynx due to trauma from passage of the bronchoscope; (3) allergic reactions to any of the pre-medications or local anesthesia; (4) cardiac arrhythmias due either to vagal stimulation or to toxicity of local anesthesia; (5) aspiration pneumonia (the presence of foreign fluid, blood, saliva, or stomach contents in the airways can occur when an individual consumes food prior to the bronchoscopy; as a result of vomiting; this risk is prevented by having all individuals fast for six hours prior to the procedures); (6) lack of oxygen and the associated shortness of breath; and (7) bronchospasm (the narrowing of the windpipes that causes shortness of breath). The most common of these problems are transient fever and bleeding and these are usually self-limiting. The medications used during the bronchoscopy cause drowsiness and may cause the subject's mouth and/or nose to feel dry. Risks associated with the medications include tachycardia and/or a transient lowering of blood pressure, which may be associated with light-headedness, dizziness, nausea, chills, rigors and in rare cases, vomiting. The problems affecting individuals with allergies to the medications used in bronchoscopy may be minimized by excluding individuals with these allergies from protocol. The difficulties with cardiac arrhythmias may be managed and anticipated by continuous cardiac monitoring throughout the procedures. In this context, bronchoscopy with bronchoalveolar lavage and/or bronchial brushing as performed in this manner is a safe procedure.

Since there has been a report of a death associated with fiberoptic bronchoscopy in a normal individual in Rochester, NY (reported in the New York Times, April 3, 1996), it is relevant to discuss the safety issues regarding this procedure and how our group insures the safety of study participants.

With regards to using bronchoscopic evaluation to obtain biologic specimens from the lung for research purposes, the Principal Investigator and his colleagues over the past 15 yr have performed more than 2,000 bronchoscopies with bronchoalveolar lavage and over 1000 with brushing, including individuals with lung disease, as well as normal volunteers in research studies. To ensure the safety of study participants, all procedures are carried out with the subject fully monitored (EKG, pulse oximeter) and with an intravenous line in place. A minimum of two physicians, including a fully trained pulmonary/critical care physician faculty member, or one physician and one physician assistant participate in each procedure, together with a minimum of one nurse. All procedures are carried out with a strict standard operating procedure, including guidelines for drug use and post-procedure monitoring. For example, the dose of topical lidocaine is limited to 5 mg/kg; (in contrast, in the New York Times reported death following the research bronchoscopy of a study individual in Rochester, NY, post-mortem studies revealed toxic levels of lidocaine in the blood. The individual had received > 1.5 gm lidocaine; significantly in excess of the amounts used in any bronchoscopic procedure). Based on the above approach, we have carried out multiple fiberoptic bronchoscopy procedures in many research patients and normal volunteers.

Over 1933 research patients and normal volunteers have had fiberoptic bronchoscopy procedures in our clinical protocols at New York Presbyterian Hospital-Weill Cornell Medical Center and The Rockefeller University. In our experience dealing with this population, we have encountered such adverse events as sore throat, cough, mild chest pain relating to the cough, mild hoarseness for several hours and transient, self-limiting fever and chills. One volunteer experienced an alteration in taste that lasted for approximately one month, which resolved without medical intervention. It is not known whether this event (alteration in taste) was related to the bronchoscopy procedure. There have been no other reports of this adverse event by any of

the other research participants. Examples of "serious adverse events" associated with this procedure reported in the literature are transient cardiac arrhythmia (0.9%), pneumothorax (0.4-4%), pneumonia (0.6%), and respiratory failure (0-0.2%). The pneumothorax risk only applies to individuals who are undergoing transbronchial biopsies which are NOT being done in this protocol.
Fiberoptic bronchoscopy performed several times are accepted tests in clinical medicine. Several of our previous clinical protocols in which fiberoptic bronchoscopy was performed several times on the same study individual found no adverse events or increased risks related to the number of bronchoscopies performed. The interval period for the bronchoscopies for those protocols varied from 1 day to several months. We are proposing here to do a second bronchoscopy 3 months after the initial one.

12. ASSESSMENT OF SAFETY

12.1 Adverse Events

Adverse events will be graded through use of the Division of AIDS Toxicity Grading Table. Any Grade 4 and Grade 5 clinical adverse events or laboratory abnormalities that are judged to be possibly, probably, or definitely related to blinded study treatment will be promptly (within 72 hours) reported to the IRB and FDA as indicated. Full details on recording and reporting adverse events are provided in Section **13.1.2.**

12.1a. Definitions

12.1.1a. Adverse Events

An adverse event is any untoward medical occurrence in a clinical study subject. No causal relationship with the study drug or with the clinical study itself is implied. An adverse event may be an unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the clinical study. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (eg., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be adverse events. Surgical procedures are not adverse events, but may constitute therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an adverse event, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol and the conditions(s) leading to these measures are not adverse events, if the condition(s) was (were) known before the start of study treatment. In the latter case the condition should be reported as medical history. Assessment of chronic obstructive pulmonary disease symptoms will be documented and analyzed as a measure of efficacy of the study treatment. These symptoms will not be reported as adverse events unless the symptom(s) worsen to the extent that the outcome fulfills the definition of a serious adverse event (see **13.1.2a** for definition), which then must be recorded as such. Adverse events are designated as "non-serious" or "serious."

Adverse events will be recorded by each site's study coordinator. Adverse events (AEs) will be entered by each site's coordinator into a cumulative table provided by the sponsor. Additionally, site coordinators will fill out a Department of Genetic Medicine case report form for each AE that occurs at their site. The cumulative table and associated case report forms will be sent to the sponsor on a regular basis, as agreed upon by the site and sponsor (e.g. every 2 months).

12.1.2a. Serious Adverse Event

A serious adverse event is an adverse event that results in any of the following outcomes:

- Death
- Is life-threatening (subject is at immediate risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires in-subject hospitalization (formal admission to a hospital for medical reasons) or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (ie. there is a substantial disruption of a person's ability to carry out normal life functions)
- Is a congenital anomaly/birth defect
- Requires intervention to prevent permanent impairment or damage
- Other serious (important) medical events that do not fit the other outcomes, but may jeopardize the subject and require medical or surgical intervention to prevent one of the other outcomes.

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

12.1b. Method, Frequency, and Time Period for Detecting Adverse Events and Reporting Serious Adverse Events

In the event of the occurrence of an adverse event that requires immediate medical intervention, Drs. Kaner or Glesby will be notified and will evaluate the subject. If the event is serious, the subject will be referred to the hospital's Emergency Department as appropriate. The data and safety monitoring plan is discussed in detail below. The safety data study will be monitored by Drs. Kaner and Glesby. Adverse events will be reported to the Weill Cornell IRB in accordance with institutional requirements.

If major adverse events occur and are related to the drug, no additional patients will be treated until the data is discussed with the IRB. If the research subject suffers a major bad effect caused by a research study related event, he/she will continue in the study as long as it does not interfere with his/her clinical care and welfare. It is also possible that the research subject will not be able to continue with the research study because of illness. All possibilities will be explored to prevent the subject's removal from the research study, as long as his/her welfare is ensured. It will be explained to the research subject that his/her safety and welfare comes first.

Serious adverse events (SAEs) will be assessed by the study team and principal investigator at each site. The site will inform the sponsor of the SAE within 24 hours, with whatever information is available at that time. Further information will be sent as it becomes available. The Department of Genetic Medicine SAE form will be completed by the coordinator, and reviewed and signed by the site PI.

12.1c. Definition of Severity

All adverse events will be assessed (graded) for severity and classified using the NIAID Division of AIDS Toxicity Grading Table. Any adverse events not covered by the DAIDS' criteria will be assessed and classified into one of four clearly defined categories as follows:

Mild: (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The adverse event does not interfere with the participant's normal functioning level. It may be an annoyance.

Moderate: (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The adverse event produces some impairment of functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.

Severe: (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The adverse event produces significant impairment of functioning or incapacitation.

Potentially Life-threatening: (Grade 4) Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death.

12.1d. Definition of Relationship to Study Drug

The blinded Principal Investigator or medically qualified designee must review each AE and make the determination of relationship to study drug using the following guidelines:

Not Related: The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident, and no temporal relationship exists between the study drug and the event.

Unlikely: The event does not follow a reasonable temporal sequence from drug administration and is readily explained by the subject's clinical state or by other modes of therapy administered to the subject.

Possibly Related: There is some temporal relationship between the event and the administration of the study drug and the event is unlikely to be explained by the subject's medical condition, other therapies, or accident.

Probably Related: The event follows a reasonable temporal sequence from drug administration and cannot be reasonably explained by the known characteristics of the subject's clinical state.

Definitely Related: The event follows a reasonable temporal sequence from administration of the medication, follows a known or suspected response pattern to the medication.

12.1e. Reporting Serious Adverse Events

Any serious adverse event or suspected unexpected serious adverse reaction must be reported to the Department of Genetic Medicine within 24 hours of the Investigator's recognition of the serious adverse events / suspected unexpected serious adverse reaction.

The site is required to fax a completed Serious Adverse Event Report Form (provided as a separate report form) within 24 hours. All additional follow-up evaluations of the serious adverse event / suspected unexpected serious adverse reaction must be reported and sent by facsimile to the Department of Genetic Medicine or its designee as soon as they are available. The Principal Investigator or designee at each site is responsible for submitting the IND safety report (initial and follow-up) or other safety information (eg, revised Investigator's Brochure) to the Institutional Review Board/Independent Ethics Committee and for retaining a copy in their files.

If the Investigator becomes aware of any serious adverse event or suspected unexpected serious adverse reaction occurring within 30 days after a subject has completed or withdrawn from the study, he or she should notify the Department of Genetic Medicine. After the 180 day final follow up visit, the Investigator should report any unexpected serious adverse events that are discovered during the yearly follow up phone calls to DGM.

Any serious adverse event or suspected unexpected serious adverse event considered possibly, probably or definitely related to treatment will be reported to the FDA and other Regulatory Competent Authorities as applicable via the MedWatch reporting system in accordance with FDA and other applicable regulations.

13. STUDY ADMINISTRATION

13.1 Regulatory and Ethical Considerations

13.1a. Regulatory Authority Approvals

This study will be conducted in compliance with the protocol; Good Clinical Practices, including International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines; Food and Drug Administration regulatory requirements and in accordance with the ethical principles of the Declaration of Helsinki. In addition, all applicable local laws and regulatory requirements relevant to the use of new therapeutic agents in the countries involved will be adhered to.

The Investigator will submit written reports of clinical study status to the Institutional Review Board annually or more frequently if requested by the Institutional Review Board. A final study notification will also be forwarded to the Institutional Review Board after the study is completed or in the event of premature termination of the study in accordance with the applicable regulations. Copies of all contact with the Institutional Review Board will be maintained in the study documents file. Copies of clinical study status reports (including termination) will be provided to the Department of Genetic Medicine.

13.1b. Ethics Committee Approvals

Before initiation of the study at each investigational site, the protocol, the informed consent and assent forms, the subject information sheet, and any other relevant study documentation will be submitted to the appropriate Institutional Review Board. Written approval of the study must be obtained before the investigational medicinal product is released to the Investigator and the study site may be opened for enrollment. Any necessary extensions or renewals of Institutional Review Board approval must be obtained, in particular, for changes to the study such as modification of the protocol, the informed consent and assent forms, the written information provided to subjects and/or other procedures. The Investigator will report promptly to the Institutional Review Board any new information that may adversely affect the safety of the

subjects or the conduct of the study. On completion of the study, the Investigator will provide the Institutional Review Board with a report of the outcome of the study.

13.1c. Subject Informed Consent

Signed informed consent must be obtained from each subject prior to performing any study related procedures. The subject will be provided with the consent form prior to arriving at the study site, to allow adequate time for review. Each subject should be given both verbal and written information describing the nature and duration of the clinical study. The informed consent process should take place under conditions where the subject has adequate time to consider the risks and benefits associated with his/her participation in the study. Subjects will not be screened or treated until the subject has signed an approved informed consent form written in a language in which the subject is fluent. If the subject is not able to provide written informed consent, a legally authorized representative may provide written informed consent where applicable local directives, guidelines or procedures allow.

The informed consent form that is used must be approved both by the Department of Genetic Medicine and by the reviewing Institutional Review Board. The informed consent should be in accordance with the current revision of the Declaration of Helsinki, current International Council on Harmonisation and Good Clinical Practice guidelines, and the Department of Genetic Medicine policy. The Investigator must explain to potential subjects or their legal representatives the aims, methods, reasonably anticipated benefits, and potential hazards of the trial and any discomfort it may entail. Subjects will be informed that they are free not to participate in the trial and that they may withdraw consent to participate at any time. They will be told that refusal to participate in the study will not prejudice future treatment. They will also be told that their records may be examined by competent authorities and authorized persons but that personal information will be treated as strictly confidential and will not be publicly available. Subjects must be given the opportunity to ask questions. After this explanation and before entry into the trial, consent should be appropriately recorded by means of the subject's dated signature. The subject should receive a signed and dated copy of the informed consent form, and, if applicable, the assent. The original signed informed consent should be retained in the study files. The Investigator shall maintain a log of all subjects (or their representatives), who sign the informed consent form and indicate if the subject was enrolled into the study or reason for non-enrollment.

13.1d. Payment to Subjects

Subjects will be reimbursed $50 for completion of the screening visit, $200 for a completed bronchoscopy and $50 for any additional non-bronchoscopy study visit.

13.2 Study Monitoring

Weill Cornell Medical College-New York (WCMC-NY)

The Clinical Operations and Regulatory Affairs (CORA) Core of the Department of Genetic Medicine will oversee and carry out the relevant monitoring and reporting of conduct of this trial. CORA Core provides the Weill Cornell clinical projects the infrastructure and expertise to conduct clinical gene therapy projects that follow the regulations and guidelines of the Food and Drug Administration (FDA) and Institutional Review Board (IRB). Included in the CORA Core are four components: Clinical Operations; Regulatory Affairs; Monitoring; and Data Management and Analysis. In addition, the CORA Core has the responsibility of reporting to the Weill Cornell Gene Therapy Data Safety Monitoring Board. The CORA Core also

participates in the Education Program at Weill Cornell, providing training in Clinical Operations/Regulatory Affairs relating to pulmonary diseases.

The Monitoring component is the verification arm of the CORA Core. It provides services that ensure that all aspects of the clinical projects maintain the quality critical for insuring compliance with the institutional oversight committees, federal regulations and GCPs. The responsibilities of the Monitoring component are divided into three categories: those pertaining to management of the Clinical Operations component (at Weill Cornell and at all other sites); those pertaining to the Regulatory Affairs component; and those pertaining to the Data Management and Analysis component. Monitoring will oversee the general activities of CORA, identify the responsibilities of CORA personnel involved in the clinical trial, and verify that all CORA staff is performing their specified study functions.

The function of the monitor involves several aspects: (1) perform the initiation visit - this visit takes place just before the study starts and the objective is to make sure the site is equipped to initiate the study and understand all the research procedures; (2) perform the routine monitoring visit - routine visits are all those made as needed to check site compliance with the protocol and regulatory requirements; and (3) perform the closeout visit - this is the final visit when the study has been completed or terminated. During this visit final arrangements are made for correcting and retrieving study records, writing final reports, and retrieving or otherwise disposing of surplus drug and clinical supplies. The monitor ensures the following relating to study drug: accountability of the study drug; acceptable storage area; sufficient supplies throughout the study; the drug is only administered only to subjects who are eligible to receive it; the protocol specified dose(s) is appropriately given; the receipt, use, and return of the drug are controlled and documented adequately; and the disposition of unused drug at the study site complies with applicable regulatory requirement(s).

13.3. Quality Assurance

The trial site may be subject to review by the Institutional Review Board and/or to quality assurance audits performed by Independent Contractors and/or to inspection by appropriate regulatory authorities. It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

13.4. Study Termination and Site Closure

The Department of Genetic Medicine reserves the right to discontinue the trial prior to inclusion of the intended number of subjects but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must contact all participating subjects immediately after notification. As directed by the Department of Genetic Medicine, all study materials must be collected and all case report forms completed to the greatest extent possible.

13.5. Records Retention

To enable evaluations and/or audits from regulatory authorities or the Department of Genetic Medicine, the Investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, case report forms and hospital records), all original signed informed consent, copies of all case report forms and detailed records of treatment disposition. The records should be retained by the Investigator according to the U.S. FDA's 21 Code of Federal Regulations. If the Investigator relocates,

retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to the Department of Genetic Medicine. The Investigator must obtain Department of Genetic Medicine's written permission before disposing of any records.

13.6. Data Safety Monitoring Committee

Weill Cornell Medical College has a Data Safety Monitoring Board, but its policy is to monitor phase I and II trials only if the risk to subjects is "unusually high" or if there is potential for a conflict of interest for the investigators to monitor safety. Given that the proposed intervention for the pilot study is likely to be very safe, the study will be monitored by the PIs (Drs. Glesby and Kaner). They will review adverse events in aggregate in a blinded fashion on a monthly basis. Adverse events will be reported to the Weill Cornell IRB as per their guidelines.

13.6.1 Adverse Event and Serious Adverse Event Submission Guidelines

Expected and/or Unrelated Adverse Events. All adverse events that are considered expected and/or unrelated to the study drug will be recorded in a cumulative table by the site coordinator. In addition, a DGM-AE case report form must be completed by the site coordinator, signed by the site PI, and sent to the sponsor on a regular basis, to be agreed upon (e.g. every 2 months). The study site will submit their local cumulative adverse event tables to their local oversight agencies, as required by local reporting guidelines. The sponsor is responsible to submitting the cumulative tables to the central FDA and central WCMC IRB with the annual reports.

Serious Adverse Events. All events that are considered serious, per the FDA's definition of serious adverse events, will be reported to the sponsor within 24 hours of occurrence. Additional information will be sent to the sponsor as it becomes available. Each site will be responsible for completing a DGM-SAE form, including assessing intensity, and submitting it to the sponsor, with the signature of the site PI. The Principal Investigator must then review and sign the SAE form. The site is responsible for submitting any SAEs which are reportable to their local regulatory agencies per the guidelines outlined by the agency. The sponsor is responsible for submitting the reportable SAEs to the FDA and central WCMC IRB, within the timeframe outlined by each agency.

13.7. Confidentiality of Information

The Department of Genetic Medicine affirms the subject's right to protection against invasion of privacy. Only a subject identification number, initials and/or date of birth will identify subject data retrieved by The Department of Genetic Medicine**.** However, in compliance with federal regulations, the Department of Genetic Medicine requires the investigator to permit the Department of Genetic Medicine's representatives and, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study. DGM will ensure that the use and disclosure of protected health information obtained during a research study complies with the HIPAA Privacy Rule, where this rule is applicable. The Rule provides federal protection for the privacy of protected health information by implementing standards to protect and guard against the misuse of individually identifiable health information of subjects participating in DGM-sponsored Clinical Trials. "Authorization" is required from each research subject, ie, specified permission granted by an individual to a covered entity for the use or disclosure of an individual's protected health information. A valid authorization must meet the

implementation specifications under the HIPAA Privacy Rule. Authorization may be combined in the Informed Consent document (approved by the Institutional Review Board/Independent Ethics Committee) or it may be a separate document, (approved by the Institutional Review Board/Independent Ethics Committee) or provided by the Investigator or Sponsor (without Institutional Review Board/Independent Ethics Committee approval). It is the responsibility of the investigator and institution to obtain such waiver/authorization in writing from the appropriate individual. HIPAA authorizations are required for U.S. sites only.

13.8. Study Publication

All data generated from this study are the property of DGM and shall be held in strict confidence along with all information furnished by DGM. Independent analysis, presentation, and/or publication of these data by any Investigator or any member of his/her staff are not permitted without prior written consent of the Department of Genetic Medicine. Written permission to the Investigator will be contingent on the review by DGM of the statistical analysis and manuscript and will provide for nondisclosure of DGM confidential or proprietary information. In all cases, the parties agree to submit all manuscripts or abstracts to all other parties 30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

REFERENCES

1. Krishnan S, Wu K, Smurzynski M, Bosch RJ, Benson CA, Collier AC, Klebert MK, Feinberg J, Koletar SL. Incidence rate of and factors associated with loss to follow-up in a longitudinal cohort of antiretroviral-treated HIV-infected persons: an AIDS Clinical Trials Group (ACTG) Longitudinal Linked Randomized Trials (ALLRT) analysis. HIV Clin Trials 2011; 12: 190-200

2. Friis-Moller N, Sabin CA, Weber R, D'Arminio MA, El-Sadr WM, Reiss P, Thiebaut R, Morfeldt L, de WS, Pradier C, Calvo G, Law MG, Kirk O, Phillips AN, Lundgren JD. Combination antiretroviraltherapy and the risk of myocardial infarction. N Engl J Med 2003; 349: 1993-2003

3. Weber R, Sabin CA, Friis-Moller N, Reiss P, El-Sadr WM, Kirk O, Dabis F, Law MG, Pradier C, de WS, Akerlund B, Calvo G, Monforte A, Rickenbach M, Ledergerber B, Phillips AN, Lundgren JD. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. Arch Intern Med 2006; 166: 1632-1641

4. Palella FJ, Jr., Baker RK, Moorman AC, Chmiel JS, Wood KC, Brooks JT, Holmberg SD. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr 2006; 43: 27-34

5. Sutherland ER and Cherniack RM. Management of chronic obstructive pulmonary disease. N Engl J Med 2004; 350: 2689-2697

6. Global initiative for chronic obstructive lung disease. 2011. http://www.goldcopd.org/guidelinesglobal-strategy-for-diagnosis-management.html [Last accessed:3-15-2012]

7. Kochanek KD, Xu J, Murphy SL, Minino AM, Kung HC. Deaths: preliminary data for 2009. (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2009) 4: 1-51.

8. Diaz PT, King MA, Pacht ER, Wewers MD, Gadek JE, Nagaraja HN, Drake J, Clanton TL. Increased susceptibility to pulmonary emphysema among HIV-seropositive smokers. Ann Intern Med 2000; 132: 369-372

9. Morris A, Crothers K, Beck JM, Huang L. An official ATS workshop report: Emerging issues and current controversies in HIV-associated pulmonary diseases. Proc Am Thorac Soc 2011; 8: 17-26

10. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic obstructive lung disease. N Engl J Med 1968; 278: 1355-1360

11. McDonough JE, Yuan R, Suzuki M, Seyednejad N, Elliott WM, Sanchez PG, Wright AC, Gefter WB, Litzky L, Coxson HO, Pare PD, Sin DD, Pierce RA, Woods JC, McWilliams AM, Mayo JR, Lam SC, Cooper JD, Hogg JC. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. N Engl J Med 2011; 365: 1567-1575

12. Greenlee KJ, Werb Z, Kheradmand F. Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted. Physiol Rev 2007; 87: 69-98

13. Russell RE, Culpitt SV, DeMatos C, Donnelly L, Smith M, Wiggins J, Barnes PJ. Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 2002; 26: 602-609

14. Atkinson JJ, Lutey BA, Suzuki Y, Toennies HM, Kelley DG, Kobayashi DK, Ijem WG, Deslee G, Moore CH, Jacobs ME, Conradi SH, Gierada DS, Pierce RA, Betsuyaku T, Senior RM. The role of matrix metalloproteinase-9 in cigarette smoke-induced emphysema. Am J Respir Crit Care Med 2011; 183: 876-884

15. Morris A, George MP, Crothers K, Huang L, Lucht L, Kessinger C, Kleerup EC. HIV and chronic obstructive pulmonary disease: is it worse and why? Proc Am Thorac Soc 2011; 8: 320-325

16. Crothers K, Huang L, Goulet JL, Goetz MB, Brown ST, Rodriguez-Barradas MC, Oursler KK, Rimland D, Gibert CL, Butt AA, Justice AC. HIV infection and risk for incident pulmonary diseases in the combination antiretroviral therapy era. Am J Respir Crit Care Med 2011; 183: 388-395

17. Giantsou E and Powrie D. Chronic obstructive pulmonary disease in adults with human immunodeficiency virus infection: a systematic review. Health 2011; 3: 218-227

18. Kaner RJ, Santiago F, Crystal RG. Up-regulation of alveolar macrophage matrix metalloproteinases in HIV1(+) smokers with early emphysema. J Leukoc Biol 2009; 86: 913-922 [PMC2752014]

19. Periostat prescribing info. http://www.onlinepharmacycatalog.com/drugsmedications/antibiotics/doxycycline/periostat-prescribing-information.pdf . [Last accessed:3-15-2012]

20. Lindeman JH, bdul-Hussien H, van Bockel JH, Wolterbeek R, Kleemann R. Clinical trial of doxycycline for matrix metalloproteinase-9 inhibition in patients with an abdominal aneurysm: doxycycline selectively depletes aortic wall neutrophils and cytotoxic T cells. Circulation 2009; 119: 2209-2216

21. Dalvi PS, Singh A, Trivedi HR, Ghanchi FD, Parmar DM, Mistry SD. Effect of doxycycline in patients of moderate to severe chronic obstructive pulmonary disease with stable symptoms. Ann Thorac Med 2011; 6: 221-226

22. Bhattacharyya P, Nag S, Bardhan S, Acharya D, Paul R, Dey R, Ghosh M, Dey R, Saha I. The role of long-term doxycycline in patients of idiopathic pulmonaryfibrosis: The results of an open prospective trial. Lung India 2009; 26: 81-85

23. Beringer PM, Owens H, Nguyen A, Benitez D, Rao A, D'Argenio DZ. Pharmacokinetics of doxycycline in adults with cystic fibrosis. Antimicrob Agents Chemother 2012; 56: 70-74

24. Cazalis J, Bodet C, Gagnon G, Grenier D. Doxycycline reduces lipopolysaccharide-induced inflammatory mediator secretion in macrophage and ex vivo human whole blood models. J Periodontol 2008; 79: 1762-1768

25. Fujita M, Ye Q, Ouchi H, Harada E, Inoshima I, Kuwano K, Nakanishi Y. Doxycycline attenuated pulmonary fibrosis induced by bleomycin in mice. Antimicrob Agents Chemother 2006; 50: 739-743

26. Pawlowska M, Gajda M, Pyka-Fosciak G, Toton-Zuranska J, Niepsuj A, Kus K, Bujak-Gizycka B, Suski M, Olszanecki R, Jawien J, Korbut R. The effect of doxycycline on atherogenesis in apoEknockout mice. J Physiol Pharmacol 2011; 62: 247-250

27. Dodd JW, Hogg L, Nolan J, Jefford H, Grant A, Lord VM, Falzon C, Garrod R, Lee C, Polkey MI, Jones PW, Man WD, Hopkinson NS. The COPD assessment test (CAT): response to pulmonary rehabilitation. A multicentre, prospective study. Thorax 2011; 66: 425-429

28. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 2004; 3: Article3-

29. Agwuh KN and MacGowan A. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J Antimicrob Chemother 2006; 58: 256-265

30. Brown DL, Desai KK, Vakili BA, Nouneh C, Lee HM, Golub LM. Clinical and biochemical results of the metalloproteinase inhibition with subantimicrobial doses of doxycycline to prevent acute coronary syndromes (MIDAS) pilot trial. Arterioscler Thromb Vasc Biol 2004; 24: 733-738

31. Kannengiesser M, Kaltenbach M, Stille W, Reifart N, Haase J. Influence of doxycycline on clinical and angiographic outcome following percutaneous coronary intervention. J Interv Cardiol 2004; 17: 447-453

32. Guidance for Industry Chronic Obstructive Pulmonary Disease: Developing Drugs for Treatment. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm071575.pdf . [Last accessed: 3-15-2012]

33. Kaner RJ and Crystal RG. Compartmentalization of vascular endothelial growth factor to the epithelial surface of the human lung. Mol Med 2001; 7: 240-246 [PMC1950032]

34. Hope WW, Kruhlak MJ, Lyman CA, Petraitiene R, Petraitis V, Francesconi A, Kasai M, Mickiene D, Sein T, Peter J, Kelaher AM, Hughes JE, Cotton MP, Cotten CJ, Bacher J, Tripathi S, Bermudez L, Maugel TK, Zerfas PM, Wingard JR, Drusano GL, Walsh TJ. Pathogenesis of Aspergillus fumigatus and the kinetics of galactomannan in an in vitro model of early invasive pulmonary aspergillosis: implications for antifungal therapy. J Infect Dis 2007; 195: 455-466

35. Kang-Birken SL, Castel U, Prichard JG. Oral doxycycline for treatment of neurosyphilis in two patients infected with human immunodeficiency virus. Pharmacotherapy 2010; 30: 119e-122e