COVER PAGE

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A Phase I Trial of Inhaled Iloprost for the Prevention of Lung Cancer in Former Smokers

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IND# Exempt
Agent(s)/Supplier: Inhalational Iloprost (Systematic (IUPAC) name: 5-{(E)-(1S,5S,6R,7R)-7-hydroxy-6[(E)-(3S, 4RS)-3-hydroxy-4-methyl-1-octen-6-inyl]-bi-cyclo[3.3.0]octan-3-ylidene}pentanoic acid) supplied by Bayer-Schering/Actelion Pharmaceuticals (USA)/Bayer/Actelion Pharmaceuticals
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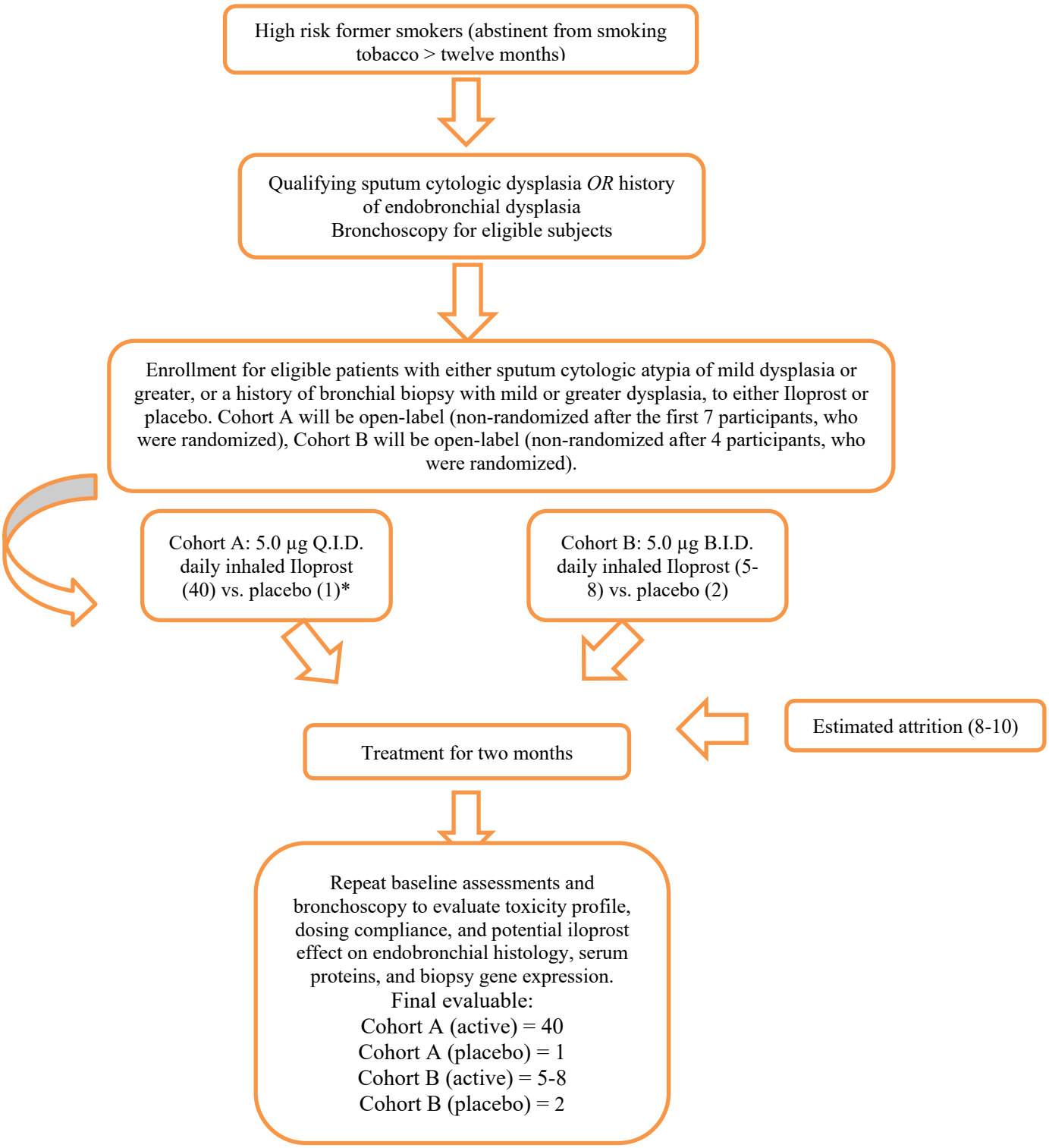
SCHEMA

A Phase I Trial of Inhaled Iloprost for the Prevention of Lung Cancer in Former Smokers



*See section 3 for further details

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objectives

The primary objective of this study is to evaluate the toxicity of inhalational iloprost administered to patients daily for 2 months, given QID.

1.2 Secondary Objectives

The secondary objectives are to evaluate the:

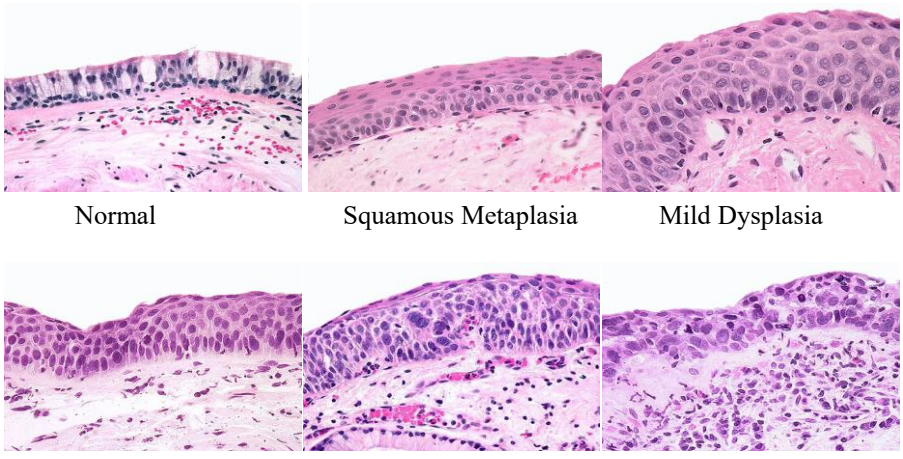
- Compliance with QID dosing regimen
- Effect on endobronchial histology
- Effect on expectorated sputum cytology by both standard cytologic analysis and an automated three dimensional morphologic analysis. Effect on serum proteins, including ADIPOQ, C9, ATP5B, CHST15, SHBG, HNRNPA2B1, quantitated by a highly multiplexed aptamer based analysis measuring approximately 1500 proteins. The associations with individual proteins and dysplasia on baseline bronchoscopy found in the Phase IIb oral iloprost trial will be tested in this study as a validation.
- Effect on endobronchial brushing and biopsy gene expression of PPARγ, GSTmu, Ces1, FosL1, cytochrome p4502e1, stearoyl coA desaturase 1, TNF superfamily member 9, TGFβ, Jun and a 46 gene panel associated with dysplasia persistence, using Affymetrix arrays. The 46 gene panel previously associated with dysplasia persistence will be tested in this study as a validation. Endobronchial brushing gene expression panels previously reported as associated with dysplasia will be tested in this study for validation.
- Improvement in COPD as measured by ABG (improved ventilation perfusion matching), pulmonary function testing, 6 minute walk distance, quality of life (St. George's respiratory questionnaire, COPD assessment test [CAT])
- Whether the in vitro response of cultured airway epithelial progenitor cells to iloprost is a predictor of in vivo response in study subjects.

2. BACKGROUND

2.1 Study Disease

Squamous Cell Carcinoma: The increased risk of developing lung cancer in ex-smokers (even many years after smoking cessation) and the fact the majority of lung cancers in the U.S. arise in former smokers [1], underscores the need for novel chemopreventive strategies [2]. Squamous cell carcinoma of the lung (SCC) is one of the four major subtypes of lung cancer and is the second most common form of Non-Small Cell Lung Cancer, or NSCLC, (adenocarcinoma being the most common [3]**) and is commonly associated with tobacco smoke exposure**. SCC typically arises from the central airways (including the trachea and segmental bronchi), and

Figure 1*: Histology of Bronchial Squamous Epithelium*



Normal | Squamous Metaplasia | Mild Dysplasia

Moderate Dysplasia | Severe Dysplasia | Carcinoma ***in situ***

histological studies have clearly shown a series of predictable pathologic changes preceding invasive bronchogenic carcinoma [4,5].

Typical human bronchial epithelial changes induced by tobacco smoke are shown in **Figure 1**. Autofluorescence bronchoscopy (AFB) can be utilized to reproducibly detect these lesions. When compared to routine white light bronchoscopy, AFB more reliably detects pre-malignant lesions in the central airways. Many patients in the trials confirming the superiority of AFB were recruited to trials conducted at the University of Colorado Denver and the Denver VA Medical Center [6]. Our subsequent trials have proven that AFB is the preferred modality for detecting angiogenic squamous dysplasia (ASD) [7], a central airway lesion characterized by capillary blood vessels projecting into metaplastic or dysplastic squamous bronchial epithelium.

Eicosanoids are a family of bioactive lipids produced from arachidonic acid (AA) liberated by the action of cytosolic phospholipases A2 ($cPLA_2$). AA is metabolized through three major pathways, cyclo-oxygenase (COX), lipoxygenase (LO) and cytochrome P450, to produce a family of bioactive lipids which have both pro- and anti-tumorigenic activities in lung cancer. Metabolic products downstream of COX include prostaglandins, prostacyclins and thromboxanes. Prostaglandins have been most extensively studied in relation to carcinogenesis. In NSCLC, increased production of prostaglandin E_2 has been shown to promote carcinogenesis, progression and metastasis, through effects directly on the cells that become cancer cells, as well as upon the tumor microenvironment. Inhibition of COX activity decreases prostaglandin production and prevents lung cancer in some, but not all, animal models [8,9]. However, non-specific COX inhibition (most notably with aspirin) has not been proven to reproducibly prevent lung cancer in large human trials with cancer incidence as the endpoint [10].

In 2011, a meta-analysis of all randomized trials of daily aspirin versus no aspirin has been published. Eight eligible trials, comprised of 25,570 patients and 674 cancer deaths, were analyzed and demonstrated that those on aspirin had reduced deaths due to cancer (OR = 0.79; 95% CI 0.68-0.92, p = 0.003). In the seven trials in which individual patient data was available, the protective effect of aspirin for all cancers (HR = 0.62; 95% CI 0.47-0.82, p = 0.001) was only apparent after 5 years follow-up. In this analysis of 5 years or greater follow-up, there was a trend towards protection from lung cancer (HR = 0.68; 95% CI 0.42-1.10, p = 0.11). In three trials, longer follow-up, out to 20 years, was available. In these trials, there were 1634 cancer deaths in 12,659 participants and the analysis demonstrated significant reduction in lung cancer death (HR = 0.68, $p < 0.01$) with 0-10 years follow-up. The curves for lung cancer death with and without aspirin begin to separate at 4 years follow-up and the protective effect increases to yield a HR = 0.71 ($p < 0.002$) with 20 years follow-up[11] . The effect on lung cancer death risk is confined to adenocarcinoma and is not found in small cell or squamous cell lung cancer. This analysis suggests that for chemoprevention trials to demonstrate efficacy, 5 years of treatment and long follow up periods may well be required.

Lung targeted prostacyclin synthase overexpression increases lung prostacyclin levels and protects mice from multiple chemical, including tobacco smoke, lung carcinogenesis models[12,13]. The prostacyclin analog, iloprost, is also effective in murine lung chemoprevention [14]. Furthermore, oral iloprost, a stable prostacyclin analog, administration improves bronchial epithelial premalignant dysplasia in former, but not current smokers[15]. Therefore, additional investigation of the potential of iloprost for lung cancer chemoprevention is needed. The University of Colorado Cancer Center investigators have extensive experience in identifying current and former smokers at high risk for the development of lung cancer[16]. For the previous oral iloprost and the ongoing pioglitazone chemoprevention trials, former smokers have been recruited from the Denver Veterans Affairs Medical Center and University of Colorado Hospital Pulmonary clinics, using inclusion criteria of either mild or greater sputum atypia or a history of endobronchial dysplasia on a previous bronchoscopy with 58% and 57% respectively having endobronchial dysplasia on the subsequent study bronchoscopies[15]. Analysis of recruitment data from the ongoing pioglitazone trial demonstrates that approximately 63% of former smokers in our recruitment population have at least mild sputum atypia and 23% of former smokers in our recruitment population go on trial.

2.2 Study Agent

Iloprost: This will not be the first trial administering inhaled iloprost, a stable prostacyclin analog, to humans. It is an FDA approved drug for the acute and chronic treatment of pulmonary hypertension (PH), and has clinically been used in subjects for many years. However, inhaled iloprost dosing for PH is 6-9 times per day, a regimen that would not be acceptable for a chemopreventive indication. We therefore propose to study the drug at a 4x per day (QID) dosing regimen, and believe that this is an acceptable scheduling for a chemopreventive intervention. The initial dose for the subject will be 2.5 µg per inhalation and they will be directly observed in clinic for side effects. They will then begin on the 5 µg dose. If this is not tolerated, the dose level will de-escalate to 2.5µg. There is preclinical data to suggest that cyclical dosing may be more efficacious for preventing cancer[17,18] and our clinical endpoint is different than those in pulmonary hypertension studies where continuous vasodilatation of the pulmonary vasculature is the goal. As previously described, increasing prostacyclin levels in the lung by targeted overexpression of prostacyclin synthase has been demonstrated to have a strong chemopreventive effect in multiple murine chemical carcinogenesis models, including a tobacco smoke exposure model[12,13]. This effect has been demonstrated to be mediated by PPARγ, not the cell surface prostacyclin receptor[14]. A Phase II trial in humans with endobronchial dysplasia as the primary endpoint was carried out in high risk current and former smokers[15]. In this trial, no improvement in endobronchial dysplasia was seen in current smokers, but significant improvements were seen in former smokers. Therefore, we hypothesize that the effect of continued smoking overcomes any chemopreventive actions of iloprost.

Because Iloprost is an FDA approved agent, and has been evaluated in several phase III trials, there is an advanced understanding of the toxicities associated with its use. In general, Iloprost is well tolerated, even at the more frequent dosing schedules of 6-9 times per day used for the treatment of primary pulmonary hypertension. Listed in **Table 1** are common toxicities associated with the use of inhaled Iloprost, as summarized in the FDA package insert. Additional data from a placebo controlled randomized controlled trial in which inhaled iloprost dose was µg 6-9 times daily demonstrates similar toxicities, including increased cough (27% iloprost, 18% placebo), flushing (27% iloprost, 12% placebo) headache (13% iloprost, 9% placebo) and syncope (8% iloprost, 5% placebo)[19]. These will be measured by and captured on case report forms based on standard Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (National Cancer Institute 2009).

Table 1: Adverse Events in Phase 3 Clinical Trial

Adverse Event	Ventavis n = 101	Placebo n = 102	Placebo subtracted %
Vasodilation (flushing)	27	9	18
Cough increased	39	26	13
Headache	30	20	10
Trismus	12	3	9
Insomnia	8	2	6
Nausea	13	8	5
Hypotension	11	6	5
Vomiting	7	2	5
Alk phos increased	6	1	5
Flu syndrome	14	10	4
Back pain	7	3	4
Tongue pain	4	0	4
Palpitations	7	4	3
Syncope	8	5	3
GGT increased	6	3	3
Muscle cramps	6	3	3
Hemoptysis	5	2	3
Pneumonia	4	1	3

2.3 Rationale

Background:
Extensive work conducted by our group has shown that prostaglandin I_2 (PGI_2, prostacyclin) inhibits lung carcinogenesis in pre-clinical models[12]. Transgenic mice with selective pulmonary PGI_2 synthase (PGIS) overexpression exhibited significantly reduced lung tumor multiplicity and incidence in response to either chemical carcinogens or exposure to tobacco smoke [12,13], suggesting that manipulation of the arachidonic acid pathway downstream from COX is an attractive target for lung cancer prevention. Our group has also shown that iloprost, a long-lasting prostacyclin analogue that is currently FDA approved for the treatment of pulmonary hypertension, inhibits lung tumorigenesis in mice, both in a chemopreventive and chemotherapeutic setting [14]. Studies using mice deficient in the cell surface prostacyclin receptor (IP) have demonstrated that chemoprevention is not dependent on IP expression [14]. Additionally, prostacyclin analogues, like iloprost, activate the peroxisome proliferator activated receptor (PPARγ) response element[14]. PPARγ over expressing mice have similar resistance to chemical carcinogenesis as do prostacyclin over expressing mice, supporting the concept that part of the chemopreventive activity of prostacyclin analogs is mediated by PPARγ [12-14]. These studies led to the implementation of a double-blind, placebo controlled clinical chemoprevention trial in which patients at high risk for lung cancer (as defined by tobacco exposure, sputum cytologic atypia, and endobronchial dysplasia) were treated with oral iloprost for 6 months. This trial demonstrated significant improvement of endobronchial dysplasia, but this was evidently only in former smokers [15]. This is the first chemoprevention trial to show improvement of its primary endpoint, endobronchial histology. Importantly, the iloprost trial utilized oral iloprost, a compound that is no longer produced due to a complicated manufacturing process and lack of additional patent life. However, an inhaled version (delivered by a special nebulizer manufactured by Accredo) has been developed and is currently FDA approved for the treatment of pulmonary hypertension. Importantly, delivery via inhalation offers the highly desirable advantage of selective delivery to the target organ, thereby minimizing systemic delivery and associated systemic toxicity. When used for the treatment of pulmonary hypertension, Iloprost is given 6-9 times per day. This high frequency dosing schedule is not optimal for a chemoprevention strategy, as compliance would not be expected to be high. However, cyclical treatment with another prostacyclin analog, cicaprost, has been demonstrated to be effective in suppressing metastasis[17,18]. Therefore, we propose to study inhaled iloprost in a phase I study with a lower dosing frequency and novel endpoints and biomarkers that are relevant to lung cancer chemoprevention. The proposed project will allow us to investigate a new and novel delivery method for a medication proven to have positive effects in a prior phase II study.

Hypothesis for primary and secondary objectives:
Overall Hypothesis: Inhalational iloprost will be well tolerated and will activate pathways in the pulmonary epithelium relevant to lung carcinogenesis and chemoprevention efficacy.

Target population:
We propose to enroll only high risk former smokers, as our original oral iloprost trial did not demonstrate improvement in current smokers. Entry criteria are based on our positive oral iloprost study[14] and will consist of: age > 18; history of tobacco exposure with >/= 20 pack years; former smoker (no smoking in the prior 12 months); at least mild sputum cytologic atypia and/or prior history of endobronchial dysplasia. Subjects from prior chemoprevention trials will need to be off investigational agents for at least 12 months.

Agent:
Inhalational Iloprost (Systematic (IUPAC) name: 5-{(E)-(1S,5S,6R,7R)-7-hydroxy-6[(E)-(3S, 4RS)-3-hydroxy-4-methyl-1-octen-6-inyl]-bi-cyclo[3.3.0]octan-3-ylidene}pentanoic acid) supplied by Bayer-Schering/Actelion Pharmaceuticals (USA)

Endpoints:

Primary Objective:
The primary objective of this study is to evaluate the toxicity of inhalational iloprost administered to patients daily for 2 months, given QID.

Secondary Objectives:

The secondary objectives are to evaluate the

- Compliance with QID dosing regimens
- Effect on endobronchial histology
- Effect on expectorated sputum cytology by both standard cytologic analysis and an automated three dimensional morphologic analysis.
- Effect on serum proteins, including ADIPOQ, C9, ATP5B, CHST15, SHBG, HNRNPA2B1, quantitated by a highly multiplexed aptamer based analysis measuring approximately 1500 proteins.
- Effect on endobronchial brushing and biopsy gene expression of PPARγ, GSTmu, Ces1, FosL1, cytochrome p4502e1, stearoyl coA desaturase 1, TNF superfamily member 9, TGFβ, Jun and a 46 gene panel associated with dysplasia persistence.
- Improvement in COPD as measured by ABG (improved ventilation perfusion matching), pulmonary function testing, 6 minute walk distance, quality of life (St. George's respiratory questionnaire, COPD assessment test [CAT]).
- Whether the in vitro response of cultured airway epithelial progenitor cells to iloprost is a predictor of in vivo response in study subjects. .

Choice of techniques for endpoint evaluation:

Toxicity: Toxicity will be assessed at study visits using the NCI criteria and recorded in case report forms.

Compliance: Compliance will be measured by interrogation of the I-neb AAD device. The manufacturer, Accredo, has developed software that interfaces with the AAD device and provides dosing information. In addition, patients are requested to complete a drug diary which is reviewed at each study visit.

Effect on endobronchial histology: Biopsies will be formalin fixed, paraffin embedded, cut into 4 μM sections, mounted on glass slides and stained with hematoxylin and eosin. Bronchial histology will be graded from using the World Health Organization criteria and assigned a score according to the following system: 1, normal; 2, reserve cell hyperplasia; 3, squamous metaplasia; 4, mild dysplasia; 5 moderate dysplasia; 6, severe dysplasia; 7, carcinoma in situ; 8, invasive carcinoma[4]. All biopsies will be graded by Dr. Daniel Merrick, the study pathologist, in a blinded fashion.

Automated Three Dimensional Morphologic Analysis of Sputum Cells: Sputum cytology is currently the only way to non-invasively measure bronchial epithelial dysplasia. However, conventional sputum cytology has not been formally evaluated as a marker of endobronchial dysplasia. VisionGate, Inc., has developed an automated system for the three dimensional analysis of epithelial cells in sputum (The LuCED® test. This automated system has shown promising preliminary results in the diagnosis of lung cancer from sputum[20]. The analytic software can potentially detect dysplasia, however this is a research focus in the development of the technology. Sputum is collected at the beginning and end of the trial and conventional cytology scored by the study pathologist. We will also process sputum from the same timepoints for the automated three dimensional morphologic analysis. The results of automated analysis will be compared to the scoring by the study pathologist. In addition, changes in the cytologic grade as determined by the automated system will be compared to changes in endobronchial histology. The NCI has agreed to supply VisionGate with sputum aliquots and de-identified clinical information for this purpose. If the three dimensional morphologic analysis of

sputum cells accurately mirrors endobronchial histology, this could lead to elimination of the need for bronchoscopy in future chemoprevention trials.

Serum protein quantification by aptamer based analysis: Exploratory analysis of serum or plasma from the oral iloprost chemoprevention trial has identified several proteins which are correlated with dysplasia on baseline bronchoscopy; determining which, if any, of these are validated in an independent test set will be a primary goal of this exercise. In addition, we will perform exploratory analyses for serum proteins correlated with iloprost administration and histologic response.

Biospecimens, including bronchial biopsies, urine and blood, were banked at enrollment and completion on each subject in the Phase II iloprost trial. The expression of endobronchial miRNAs has been published and an analysis of eicosanoid levels in urine has been completed[21]. Recently, serum taken at enrollment and trial completion has been analyzed on the 125 subjects who completed this trial using the highly multiplexed aptamer based assay for serum proteins developed by SomaLogic, Inc. The assay detects > 1,500 proteins in a 50 µl serum sample with a mean detection limit of 40 fM[22]. Our study design is to run the protein analysis on enrollment and completion sera from all subjects in one batch; this has been completed. For data analysis, we have chosen to initially break the baseline analysis into a training set (2/3) and a blinded test set (1/3) for correlation of protein abundance with baseline parameters including age, sex, pulmonary function and dysplasia grade. After this is completed, data analysis will proceed on completion samples as well in a similar test set/training set design with the goal of discovering biomarkers that predict response to iloprost. At present, only the baseline training set has been analyzed. While these results are highly preliminary and are to be considered confidential, there are some highly tantalizing findings. Six proteins tracked with maximum dysplasia grade on the baseline bronchoscopy. The uncorrected p values are shown in **Table 2**. When adjusted for false discovery, q values for most are approximately 0.3 (likelihood of having been discovered by chance 30%). The exception to this is SHBG, for which the q value = 0.05.

Protein	KS Distance*	p.value	Fold Change**
ATP5B	0.56	0.0009	1.35
CHST15	-0.52	0.0024	0.82
C9	0.52	0.0027	1.14
SHBG	-0.50	0.0036	0.52
ADIPOQ	-0.50	0.0036	0.71
HNRNPA2B1	0.39	0.0420	1.60
*Negative values are lower as Max Path increases			
**Relative to Max Path 1&2			

Table 2. p values and fold changes (dysplasia grades 1+2 vs. 5+6) for proteins with possible correlation with baseline maximum dysplasia.

Protein functions are shown in **Table 3**. Of interest, C9 has been found to correlate with the presence of lung cancer and is a member of the SomaLogic classifier for diagnosing lung cancer. HNRNPA2B1 has a long history as a potential lung cancer diagnostic biomarker and has recently been demonstrated to regulate EMT in lung cancer cell lines[23,24,25]. Adiponectin is an adipocyte derived protein that has been shown to be negatively correlated with cardiovascular disease, diabetes and multiple forms of cancer. Adiponectin null mice exhibit accelerated tumor growth[26]. Adiponectin has been widely described as upregulated by PPAR gamma ligands[27], like iloprost. We are eager to determine if low adiponectin levels, associated with baseline higher dysplasia, are upregulated by iloprost and track with improvement in dysplasia.

Protein	Function
ATP5B	Mitochondrial ATP synthase
CHST15	Chondroition sulfate sulfotransferase
SHBG	Sex hormone binding globulin; promotes clearance of steroid hormones
C9	Complement C9
ADIPOQ	Adiponectin; inhibits cancer growth
HNRNPA2B1	Ribonucleoprotein; promotes EMT

Table 3. Proteins and function.

Endobronchial brushing gene expression: Recent chemoprevention trials have included complementary gene expression analysis of endobronchial brushings taken from the right mainstem bronchus[28]. These studies built on previous studies of similar analyses of gene expression which discriminated smokers and non-smokers, as well as patients with and without lung cancer[29]. The underlying hypothesis for this approach is that a field effect

exists in the airway epithelium reflecting exposure and disease. We propose exploratory analysis of endobronchial gene expression as a measure of exposure to inhaled iloprost. We have previously published statistically significant alterations in gene expression in the alveolar type II cells isolated from prostacyclin synthase overexpressor mice (**Table 4**) and these provide multiple candidates which we anticipate may also exhibit similar changes in human airway epithelium upon iloprost exposure[13].

Endobronchial biopsy gene expression: We propose to analyze gene expression alterations induced by inhaled iloprost in dysplastic lesions sampled by biopsy as well as in the general airway field as sampled by brushing. Dr. Daniel Merrick, a pathologist at University of Colorado Denver, and colleagues have developed a 46 gene classifier for discriminating persistent dysplasias from those which spontaneously regress (**Figure 2**). We will carry out exploratory analyses to determine if gene expression changes occur with iloprost exposure that may potentially be informative of specific mechanisms by which iloprost mediates improvement in dysplasia.

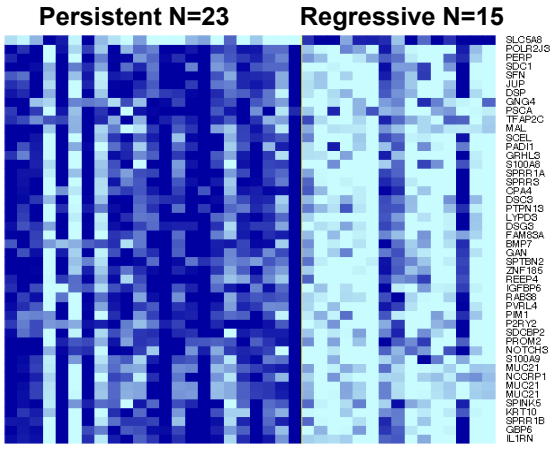


Figure 2. Heat Map of 46 Gene "Classifier" for Prognostic "Persistent" versus "Regressive" Dysplasia. Affymetrix Gene 1.0ST array data from N=23 persistent (moderate or worse) dysplasia compared to N=15 regressive (spontaneous) dysplasia samples. 46 gene classifier developed from genes with FDR = 0.02 (P values, 10^{-4}. Permutation Test P value for leave one out cross-validation demonstrated p<0.0004.

Performance of the Compound Covariate Predictor Classifier:

Class	Sensitivity	Specificity	PPV	NPV
Persistent	0.783	0.733	0.818	0.688
Regressive	0.733	0.783	0.688	0.818

Table 4: Gene Expression

Overexpressed	Fold Change	Underexpressed	Fold Change
PPARγ	1.8	Cytochrome p450, 2e1	10.4
Glutathione S-transferase, mu1	1.4	Stearoyl-coenzyme A desaturase 1	2.6
Carboxylesterase 1 (Ces1)	2.7	TNF superfamily member 9	2.5
Prostacyclin Synthase (PGIS)	12.9	TGF-β	2.1
Fos-Like antigen (FosL1)	1.6	Jun oncogene	1.4

Chronic obstructive lung disease endpoints: Pulmonary function testing and 6 minute walk data will be measured by pulmonary function staff at Denver VAMC. The St. Georges Respiratory Questionnaire and COPD Assessment Test will be administered by clinical trial coordinators.

Airway epithelial progenitor cell culture and response to iloprost: Bronchial biopsies will be taken from an area suspicious for dysplasia and an area that appears normal. These will be cultured as described and transferred to an air liquid interface culture in which dysplasia is recapitulated (Ghosh et al, in preparation). Cultures will be treated with either vehicle or iloprost and assessed for both self renewal and differentiation to ciliated and secretory cells.

3. SUMMARY OF STUDY PLAN

1. Subjects at high risk for the development of lung cancer as defined by a 20 pack-year or greater smoking history and the presence of mild or greater sputum atypia or mild or greater endobronchial dysplasia on a previous bronchoscopy will be recruited for the study from the Denver Veterans Affairs Medical Center clinics. Subjects seen at University of Colorado Hospital and National Jewish Health clinics may also be referred to the study, but will be enrolled as a non-veteran at the Denver Veteran's Affairs Medical Center if eligibility is met.
2. This was an open label dose de-escalation trial. We initially proposed to study two separate iloprost dosing regimens (QID and BID) with 20 enrolled subjects in each iloprost dosing regimen group (estimated attrition of 20% during the study would result in 16 evaluable participants per group). In Cohort A, the first 7 subjects were randomized to iloprost versus placebo, with one participant receiving placebo, but the remaining 14 subjects will not be randomized and instead will be given active iloprost. This is due to lack of availability of the placebo. Within the BID group (Cohort B), we randomized 4 participants, until revision for protocol v3.8 was been approved, due to the expiration of placebo in December 2018. After approval of protocol v3.8, we registered all proceeding participants to cohort B as open label (without randomization). Thus, 7 participants were enrolled to cohort B: 5 active treatment BID, and 2 placebo. During the approval of protocol v3.9, we expect that up to 3 more patients will be enrolled into Cohort B open label. Thus, we expect Cohort B will have 5-8 patients on active agent, and 2 patients on placebo.
3. An interim analysis was conducted after completion of Cohort A and discussed on March 26, 2019. As toxicity was minimal with the QID regimen and compliance was good (65% achieved 80% compliance or more), a decision was made to discontinue Cohort B and enlarge Cohort A to a maximum of 40 participants on active agent, in order to better assess any effects of inhaled iloprost on secondary biomarkers. We expect up to 147 people will participate in the "pre-screening" portion of this study.
4. Subjects will be required to complete a physical exam, labs, smoking history assessment, bronchoscopy, sputum, EKG, PFT, exhaled carbon monoxide testing, 6-minute walk test, and questionnaires at the initial visit. During treatment, subjects will complete physical exams, labs, carbon monoxide readings, toxicity assessments, and drug compliance at the scheduled 30 and 60 day visits. At the end of treatment, subjects will repeat earlier screening assessments to determine the outcomes of the primary and secondary objectives. If our trial plans to enroll up to 50 subjects (2 treatment arms with 21 subjects on arm A and 20-29 subjects on arm B), we anticipate this taking about 36 months to complete enrollment.
5. Subjects will be seen at 30 and 60 day visits and monitored for toxicity at those times. A telephone call will be placed at 15 days after initiating treatment and subjects will be interviewed for toxicity, compliance and any problems with the protocol. A 90 day visit is also made to evaluate for resolution of any symptoms which occur while on trial or development of new symptoms after study drug withdrawal.

Participants will be accrued and monitored for toxicity. Toxicity will be graded according to the NCI CTCAE version 4.0. If grade 2, 3 or 4 toxicity thought to be at least possibly due to drug is experienced by a subject, then modifications based on specific dose schedules will be made as listed in Section 5.6.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Participants must have either sputum cytologic atypia of mild dysplasia or greater or a history of bronchial biopsy with mild or greater dysplasia within the past 12 months.

4.1.2 Participants must have a smoking history of 20 pack-years or greater.

4.1.3 Participants must have the ability to safely undergo bronchoscopy in the judgment of the investigators.

4.1.4 Participants must be age 18 - 85 years. Because no dosing or adverse event data are currently available on the use of Iloprost in participants <18 years of age, children are excluded from this study but will be eligible for future pediatric trials, if applicable.

4.1.5 Participants must have ECOG performance status ≤1. *Note: See Appendix A for ECOG Performance Status Table.*

4.1.6 Participants must have normal organ and marrow function as defined below:

Leukocytes	≥3,000/microliter
Platelets	≥100,000/microliter
Total bilirubin	≤2.0 mg/dl
AST (SGOT)/ALT (SGPT)	≤2.5 × institutional ULN
Creatinine	≤2.0 mg/dl

4.1.7 The effects of Iloprost on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because prostacyclins are known to be teratogenic, women of child-bearing potential and men having intercourse with a woman of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately. *Note: Women are considered to be of child-bearing potential if they are not surgically sterile or are under the age of 65 and have menstruated within the last two years.*

4.1.8 Participants must be able to understand and willing to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 Participants must not have used any tobacco product in the past year.

4.2.2 Participants must not be currently receiving or have previously received thiazolidinedione treatment unless sputum atypia or endobronchial dysplasia are documented again after thiazolidinedione treatment and within 12 months of entry.

4.2.3 Participants must not have been treated with iloprost at any time. *Note: participants on the placebo arm of previous iloprost trials are eligible, but participants on the placebo arm of cohort A of this study may not be enrolled in cohort B.*

4.2.4 Participants must not have used any other investigation agent within the last six months.

4.2.5 Participants must not have a history of allergic reactions attributed to compounds of similar chemical or biologic composition of Iloprost.

4.2.6 Participants must not have uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that in the opinion of investigators would jeopardize patient safety of data integrity. *Note: Individuals who are HIV positive will not necessarily be excluded, will be considered on a case-by-case basis, but will be required to meet criteria related to patient safety and data integrity, as assessed by investigators.*

4.2.7 Participants must not have a current or prior invasive malignancy within the past 6 months. Participants may enroll prior to biopsy result report, unless there are findings at bronchoscopy suggesting an invasive malignancy. History of the following curatively treated cancers during any time prior to screening is allowed: non-melanoma skin cancer, cervical carcinoma in situ, and bladder carcinoma in situ.

4.2.8 Participants must not have received either chemotherapy or radiotherapy within the previous 6 months. *Note: Participants receiving long-term adjuvant hormonal therapy (such as tamoxifen or aromatase inhibitors for breast cancer) are allowed*.

4.2.9 Women must not be pregnant or breastfeeding. Iloprost is a prostacyclin agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with iloprost, breastfeeding should be discontinued if the mother is treated with iloprost.

4.2.10 As iloprost inhibits platelet function, patients must not be taking anticoagulants, with the exception of aspirin or other non-steroidal anti-inflammatory medications.

4.2.11 Due to risk for hypotension in patients on vasodilators or antihypertensive medications, participants must not have blood pressure <95 mm Hg systolic.

4.3 Inclusion of Women and Minorities

Members of all races, ethnic groups and genders are eligible for this trial.

4.4 Recruitment and Retention Plan

Participants will be recruited through the Pulmonary Clinics at the Denver Veterans Affairs Medical Center and by referral from the University of Colorado Hospital associated clinics. Subjects who have participated in clinical trials carried out through the University of Colorado Specialized Program of Research Excellence (SPORE) in Lung Cancer will be reviewed and contacted if they appear to meet entry criteria. In addition, advertisements will be posted at National Jewish Health with study contact information. During the study, at monthly visits, compliance with inhalations will be monitored and subjects will be encouraged to maintain compliance. In order to cover costs to subjects associated with participation on this study, subjects will be compensated $250 at the completion of the second bronchoscopy. Any participants who have a study biopsy with either invasive carcinoma of the lung or carcinoma in situ will be removed from the study.

5. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. The initial treatment will be a 2.5 μg inhaled dose administered under direct observation in the clinic; if this is tolerated, subjects will receive a 5 μg dose per treatment thereafter. Reported adverse events (AEs) and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

Iloprost will be administered 5 μg QID daily for 2 months.

Cohort A: 40 subjects will be enrolled into the QID daily treatment with iloprost and 1 will be enrolled into the QID daily treatment with placebo. All subjects will be treated for 60 days. Study arm assignment to iloprost or placebo was made in a randomized fashion for the first 7 participants, but then due to unavailability of placebo, the remaining participants will be assigned to active Iloprost.

Cohort B: 5-8 subjects will be enrolled into the BID daily treatment with iloprost and 2 were enrolled into the BID daily treatment with placebo. All subjects will be treated for 60 days.

Cohort A was enrolled to completion (at the time, 20 subjects) before initiation of Cohort B enrollment. After an interim analysis, Cohort B was discontinued and Cohort A expanded to 40 subjects on active agent (open label).

5.2 Iloprost Administration

Inhaled iloprost or matched placebo is delivered by a specialized nebulizer made by Accredo called the I-neb Adaptive Aerosol Delivery (AAD), which each subject will be taught to self-administer. This is the only FDA approved device for inhalational iloprost. It is a lightweight and fully portable device with a rechargeable battery. Each treatment takes 4-10 minutes and the device is able to measure compliance and delivered dose. An initial treatment with a total dose of 2.5 μg iloprost (for those receiving active drug), or placebo, is given under supervision. During this visit the subject will be trained on the proper use of the AAD device. If clinically tolerated, subsequent doses will be 5 μg. Intolerance will be defined as coughing lasting more than 10 minutes, a systolic blood pressure decrease of more than 20 mm Hg or flushing lasting more than 10 minutes. Subjects will be given a 5-6 week supply of ampules containing either iloprost or placebo at study visits and will receive treatment for 60 days. We will ask subjects to administer treatments approximately every twelve hours for the BID regimen and approximately every 4-6 hours while awake for the QID regimen.

5.3 Run-in Procedures

N/A

5.4 Contraindications

None, other than pregnancy or unprotected intercourse for females of childbearing potential, and for males having intercourse with females of childbearing potential without the use of effective contraception.

5.5 Concomitant Medications

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a test or procedure (eg. PFT or biopsy) do not need to be recorded in CRFs.

5.6 Dose Modification

An initial treatment with a total dose of 2.5 μg iloprost (for those receiving active drug) or placebo is given under supervision and during this visit the subject will be trained on proper use of the I-neb AAD device. If safely tolerated (intolerance will be defined as coughing lasting more than 10 minutes, a systolic blood pressure decrease of more than 20 mm Hg or flushing lasting more than 10 minutes), subsequent doses will be 5 μg. Participants will be accrued and monitored for toxicity. Toxicity will be graded according to the NCI CTCAE version 4.0. If grade 2, 3 or 4 toxicity thought to be at least possibly due to drug is experienced by a subject, then modifications based specific doses schedules will be made as follows. Note 50% dose level indicates BID from QID for Cohort A, and QD from BID for Cohort B.

Toxicity			**Attribution to study drug**		
Grade	**Unrelated**	**Unlikely**	**Possible**	**Probable**	**Definite**
1	**C**	**C**	**C**	**C**	**C**
2	**C**	**C**	**S-R0**	**S-R0**	**S-R1**

3	**S-R2**	**S-R2**	**S-R1**	**W**	**W**
4	**S-R2**	**S-R2**	**W**	**W**	**W**

C = Continue drug
S-R0 = Hold drug until toxicity reaches grade 1 or lower, then restart drug at full dose. If toxicity resumes, hold drug again until toxicity reaches grade 1 or lower, and then restart at 50% dose level. No further reductions will be permitted and patients with returning toxicities will be removed from the study.
S-R1 = Hold drug until toxicity reaches grade 1 or lower, then reduce drug by 50% and maintain the reduced dose. No further reductions will be permitted and patients with returning toxicities will be removed from the study.
S-R2 = Hold drug until toxicity reaches grade 1 or lower, then reduce drug by 50% for 2 weeks. If toxicity is no more than grade 1, restart drug at full dose. If toxicity resumes after reintroduction of full dose, hold drug again until toxicity reaches grade 1 or lower and then reduce drug by 50% and maintain this dose. No further reductions will be permitted and patients with returning toxicities will be removed from the study.
W = Withdrawal

Participants may miss doses. Compliance is an outcome measure for this phase I study. Missed doses will not be made up. Compliance will be assessed at each visit. For analysis of secondary endpoint responses, we will define the efficacy compliance threshold as the initiation of 80% of treatments. For any Grade 1 toxicities (related or unrelated) or unrelated grade 2, 3, or 4 toxicities, subjects will remain on protocol treatment unless otherwise directed by the study physician.

5.7 Adherence/Compliance

5.7.1 Compliance is an outcome measure for this trial, and will be determined at the monthly clinic visit and also by interrogating the I-neb AAD device. For analysis of secondary endpoint responses, we will define the efficacy compliance threshold as the initiation of 80% of treatments.

5.7.2 The Accredo I-neb device measures compliance. This will be monitored at monthly visits and subjects will be encouraged to maintain compliance. Patients will be asked to keep a study drug diary (see Appendix B). We will contact study subjects by phone after 15 days from the first day of agent consumption to assess compliance.

6. PHARMACEUTICAL INFORMATION

6.1 Study Agent (IND Exempt)

Iloprost (5-{(E)-(1S,5S,6R,7R)-7-hydroxy-6[(E)-(3S, 4RS)-3-hydroxy-4-methyl-1-octen-6-inyl]-bi-cyclo[3.3.0]octan-3-ylidene}pentanoic acid; Ventavis®) is a synthetic analog of prostacyclin formulated as an inhalation solution and approved by FDA for the treatment of pulmonary arterial hypertension (PAH) to improve a composite endpoint of exercise tolerance, symptoms, and lack of deterioration. Studies establishing effectiveness were performed predominantly in patients with idiopathic or inherited PAH (65%) or PAH symptoms associated with connective tissue disease (23%). [Ventavis® Prescribing Information 5/2013]

Iloprost Inhalation Solution is a clear, colorless, sterile solution containing iloprost formulated for inhalation via the I-neb® AAD® (Adaptive Aerosol Delivery) System. It is supplied in 1 mL single-use glass ampules containing 10 µg/mL. Approximately half the total dose is adequately delivered to the lung, so administration of 1 mL of the 10 µg/mL solution results in an effective dose of 5 µg.

Bayer/Actelion will supply Iloprost and Placebo inhalation solution ampules to the Denver Veterans Affairs Medical Center in coded packaging.

6.2 Reported Adverse Events and Potential Risks

Safety data on iloprost inhalation solution were obtained from 215 patients with PH in two 12-week clinical trials and two long-term extensions [19]. Patients received inhaled iloprost for periods of from one day to more than three years; the median exposure was 15 weeks. Forty patients completed 12 months of open-label treatment with iloprost [Ventavis® Prescribing Information 5/2013]. Inhaled iloprost has not been studied in individuals with COPD or severe asthma.

The most common (>3% placebo rate adjusted) adverse events (AEs) noted in the phase 3 registration study were vasodilation (flushing) (18%); cough increased (13%); headache (10%); trismus (masticatory muscle spasm) (9%); insomnia (6%); nausea, hypotension, vomiting, and alkaline phosphatase increased (each 5%); flu syndrome, back pain, and tongue pain (each 4%); and palpitations, syncope, GGT increased, muscle cramps, hemoptysis, and pneumonia (each 3%). Cases of thrombocytopenia, dizziness, diarrhea, mouth and tongue irritation, nasal congestion, dysgeusia (loss of taste), hypersensitivity, and rash have also been reported [Ventavis® Prescribing Information 5/2013]. Only syncope identified as a serious adverse event (SAE) occurred more frequently with inhaled iloprost (5%) than with placebo (0%) among 203 patients[19]. These were not associated with clinical deterioration or premature withdrawal from the study.

SAEs reported in premarketing studies with inhaled iloprost and not included in the most common AE listings were congestive heart failure, chest pain, supraventricular tachycardia, dyspnea, peripheral edema, and kidney failure. Fatal cases of cerebral and intracranial hemorrhage have been reported.

In a study in healthy subjects (n=160), inhaled doses of iloprost solution were given every 2 hours, beginning at 5 µg and increasing up to 20 µg after a total of 6 inhalations (total cumulative dose of 70 µg) or up to the highest dose tolerated in a subgroup of 40 subjects. Thirteen subjects (32%) failed to reach the highest scheduled dose (20 µg). Five were unable to increase the dose because of mild to moderate transient chest pain/discomfort/tightness, usually accompanied by headache, nausea, and dizziness. The remaining eight subjects discontinued for other reasons [Ventavis® Prescribing Information 5/2013].
Inhaled iloprost may cause bronchospasm, and patients with a history of hyperreactive airway disease may be more sensitive. Inhaled iloprost has not been evaluated in patients with COPD, severe asthma, or acute pulmonary infections. In patients with hepatic impairment, the dosing interval may need to be extended depending on the subject's response (*e.g*., 3−4 hours). Iloprost should not be administered in patients with systolic blood pressure below 85 mm Hg, because hypotension leading to syncope has been observed. Iloprost may also cause pulmonary venous hypertension and should be discontinued if pulmonary edema is present [Ventavis® Prescribing Information 5/2013].

A pilot study using an oral tablet formulation of iloprost and a placebo was performed to explore the potential of the drug to improve endobronchial dysplasia in former smokers[15]. A total of 152 subjects (75 iloprost, 77 placebo) was randomized to one 50 µg tablet bid (100 µg total) or placebo, then escalated to a maximum of three tablets bid (300 µg total) by one additional tablet per month for a total of six months of treatment. Safety was monitored twice during this interval. In the iloprost group, 73% of subjects completed dose escalation compared with 89% of the placebo group (p=0.02). The following AEs were described (and are largely identical to those reported with inhaled iloprost): headache (52% iloprost *vs*. 23% placebo), flushing (23% *vs.* 8%), nausea (16% *vs.* 8%), myalgia (17% *vs.* 1%), fatigue (11% *vs*. 2%), neuropathic pain (9% *vs*. 1%), and dysguesia (8% *vs.* 0%). No SAEs were reported, and there was no significant difference in the number of grade 3 AEs between groups. Another placebo-controlled trial of oral iloprost (100 or 200 µg bid for six weeks) to control Raynaud's symptoms in systemic sclerosis patients reported similar AE rates and types (*e.g*., headache, flushing, nausea, flu-like syndrome, diarrhea, and trismus)[30].

Iloprost has the potential to increase the hypotensive effect of vasodilators and antihypertensive agents. There is a potential for increased risk of bleeding, particularly in patients maintained on anticoagulants. During clinical trials, iloprost was used concurrently with anticoagulants, diuretics, cardiac glycosides, calcium channel blockers, analgesics, antipyretics, non-steroidal anti-inflammatory drugs, corticosteroids, and other medications.

Intravenous infusion of iloprost had no effect on the pharmacokinetics of digoxin. Acetylsalicylic acid did not alter the clearance (pharmacokinetics) of iloprost [Ventavis® Prescribing Information 5/2013].

Iloprost has been shown to be teratogenic in rats. In developmental toxicity studies in pregnant Han-Wistar rats, continuous intravenous administration of iloprost at a dose of 0.01 mg/kg daily (serum levels not available) led to shortened digits of the thoracic extremity in fetuses and pups. In comparable studies in pregnant Sprague-Dawley rats at oral doses of iloprost clathrate (13% iloprost by weight) up to 50 mg/kg/day (C_{max} of 90 ng/mL), in pregnant rabbits at intravenous doses up to 0.5 mg/kg/day (C_{max} of 86 ng/mL), and in pregnant monkeys at doses up to 0.04 mg/kg/day (serum levels of 1 ng/mL), no digital anomalies or other gross structural abnormalities were observed fetuses/pups. However, in gravid Sprague-Dawley rats, iloprost clathrate (13% iloprost) significantly increased the number of non-viable fetuses at a maternally toxic oral dose of 250 mg/kg/day, and it was found to be embryolethal in 15 of 44 litters at an intravenous dose of 1 mg/kg/day in Han-Wistar rats. Fertility of males or females was not impaired in Han-Wistar rats at intravenous doses of iloprost up to 1 mg/kg/day [Ventavis® Prescribing Information 5/2013]. There are no adequate, well-controlled clinical studies in pregnant women. Inhaled iloprost solution should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

It is not known whether iloprost is excreted in human milk. In studies with Han-Wistar rats, higher mortality was observed in pups of lactating dams receiving iloprost intravenously at 1 mg/kg daily. In Sprague-Dawley rats, higher mortality was also observed in nursing pups at a maternally toxic oral dose of 250 mg/kg/day of iloprost clathrate (13% iloprost by weight). In rats, passage of low levels of iloprost or metabolites to milk was observed (less than 1% of iloprost dose given intravenously). No disturbance of postnatal development and reproductive performance was seen in animals exposed during lactation.

Iloprost was not mutagenic in bacterial and mammalian cells in the presence or absence of extrinsic metabolic activation. It did not cause chromosomal aberrations *in vitro* in human lymphocytes and was not clastogenic *in vivo* in NMRI/SPF mice. There was no evidence of a tumorigenic effect of iloprost clathrate (13% iloprost by weight) in Sprague-Dawley rats dosed orally for up to 8 months at doses up to 125 mg/kg/day (serum C_{max} of 45 ng/mL), followed by 16 months at 100 mg/kg/day, or in Crl:CD-1®(ICR)BR albino mice dosed orally up to 24 months at doses up to 125 mg/kg/day (serum C_{max} of 156 ng/mL) [Ventavis® Prescribing Information 5/2013].

In humans, iloprost administered intravenously has linear pharmacokinetics over the dose range of 1 to 3 ng/kg/min. The half-life is 20 to 30 minutes. Following inhalation of iloprost (5 µg), patients with pulmonary hypertension have peak plasma levels of approximately 150 pg/mL. Iloprost was generally not detectable in plasma 30 minutes to 1 hour after inhalation. The absolute bioavailability of inhaled iloprost has not been determined. Following intravenous infusion, the apparent steady-state volume of distribution was 0.7 to 0.8 L/kg in healthy subjects. Iloprost is approximately 60% protein-bound, mainly to albumin, and this ratio is concentration-independent in the range of 30 to 3000 pg/mL. Clearance in normal subjects was approximately 20 mL/min/kg. The main metabolite is tetranor-iloprost, which is found in the urine in free and conjugated form. In animal experiments, tetranor-iloprost was pharmacologically inactive [Ventavis® Prescribing Information 5/2013].

6.3 Availability

Iloprost inhalation solution and matching placebo will be manufactured and supplied to the Denver Veterans Affairs Medical Center by Actelion Pharmaceuticals US, Inc. (South San Francisco, CA, 94080).

6.4 Agent Distribution

Iloprost solution and placebo will be directly supplied by Bayer/Actelion to the investigators at the Denver Veterans Affairs Medical Center. The Pharmacist at the Denver Veterans Affairs Medical Center will label the study medication.

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, will maintain a record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF). The Investigator will maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to the Denver Veterans Affairs Pharmacy Service, Research Section (Stephen Bartlett). Included on receipt record: from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record: quantities and dates study agent was dispensed to and returned by each participant.

6.6 Packaging and Labeling

Iloprost solution and matching placebo will be packaged by Bayer/Actelion and sent directly to the Denver Veterans Affairs Medical Center.

Iloprost solution and placebo will be packaged by Actelion Pharmaceuticals. Cartons will be labeled with a study label containing the study identifier, descriptions of the contents, and instructions and blanks for entering the dates of first and last use. The cartons each consist of 30 x 1mL clear glass single-use ampules that contain 10 mcg iloprost per 1 mL, or matching placebo. The carton will include the sponsor/caution label instructing participants that the drug is for investigational use only and is to be stored at temperatures between 20°C and 25°C (68°F and 77°F) and out of the reach of children. The Research Pharmacy at the Denver Veterans Administration Medical Center will provide blinded labeling that will include the Study Number, Subject Number, and the subject's initials.

6.7 Storage

Iloprost inhalation solution is supplied in 1 ml clear glass single-use ampules and should be stored in a secure location at temperatures between 20°C and 25°C (68°F and 77°F) with excursions permitted to 15°C-30°C (59°F-86°F). Placebo will be provided in a similar ampule, and all investigational ampules will be stored and distributed by the Denver VA Research Pharmacy.

6.8 Registration/Randomization

The Lurie Cancer Center Clinical Trials Management System (CTMS) will be the database of record. The study coordinator must upload (via CTMS), a signed and complete informed consent along with HIPAA authorization and a completed registration form for each participant identified as eligible to be entered into the study.

When possible, the study coordinator will notify an NCPC Quality Assurance Monitor and/or send an email to ncpc@northwestern.edu prior to registering a participant. Prior notification is required for participant randomizations outside the normal business hours of Monday-Friday 9:00am-5:00pm CT Participants must not start protocol treatment prior to registration in CTMS.

After the approval of amendment 3.8, this trial became a non-randomized trial.

6.9 Blinding and Unblinding Methods

Study participants will receive a prescription, blinded, from the investigational pharmacy at Denver Veterans Affairs Medical Center. Unblinding will only occur when it is deemed medically necessary and will only take place after consultation with the NCI, DCP Medical Monitor:

DCP Medical Monitor
Eva Szabo, M.D., Chief

NCI/Division of Cancer Prevention
Telephone (240) 276-7011
Fax (240) 276-7848
Email: szaboe@mail.nih.gov

If the medical monitor is unavailable or if after hours consultation is needed, please contact Dr. Seema Khan, at telephone: 1-312-503-4236, or via cell phone: 312 307-3646. If Drs. Szabo and Khan are unavailable, the research pharmacy may unblind if necessary for patient safety. In the case of SAEs, the investigators should perform what they deem medically appropriate.

6.10 Agent Destruction/Disposal

All drug will be destroyed on site by the Denver pharmacy. Destruction records will be kept.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

	Pre-screening Visit	Screening Visit (within 6 weeks of registration unless otherwise noted)	Visit 1 (Day1)	Phone call (Day 15 +/- 3 days)	Visit 2 (Day 30 +/-5 days)	Visit 3 (day 60 +/-5 days)	Visit 4 (Day 90 +/-5 days)	Follow up[1]
Informed Consent	X (Pre-Screening)	**X (Full Consent)						
Inclusion/Exclusion Criteria Review	X	X						
PHYSICAL								
History and Physical		X			X	X	X	
Vitals[2]		X			X	X	X	
Prior/concurrent medication review		X		X	X	X		
Smoking History		X			X	X	X	
Performance Status		X			X	X	X	
Toxicity Assessment				X	X	X	X	
LABORATORY VALUES								
CBC/Platelets [3]		X			X	X		
Research Blood[4]		X				X		
Chemistry Panel[5]		X			X	X		
Serum Pregnancy Test[6]		X						
MISC. PROCEDURE								
Bronchoscopy/ Biopsies/brushings[7]		X				X		
Sputum[8]	X	*X				X		
Urine (Research)		X				X		
EKG		X[9]						
Full PFT		X				X		
Carbon Monoxide (CO)		X			X	X		
6-Minute Walk Distance		X				X		
Questionnaires[10]		X				X		
BODE INDEX		X				X		
TREATMENT								
Inhaled Iloprost TREATMENT								
Drug Dispensing			X		X			
Ampule Count and I-neb download					X	X		
Drug Diary			X		X	X		

[1] To be done until resolution of all toxicities and then annually (interrogation of National Death Index, Colorado Cancer Registry, and review of electronic medical record) for up to five years from end of treatment. Follow up should be done per institution's Standard of Care (SOC).
[2] Vitals to include weight, blood pressure, pulse, respiration rate, and temperature. Height is to be taken only at screening.
[3] Hematology includes assessment of hemoglobin, platelets count, a complete red blood cell count, and total white blood cell count (WBC).
[4] Research Blood: Two yellow top citrates, one purple top EDTA, and one red top tube will be collected.
[5] Serum chemistries include assessment of total serum protein, albumin, bilirubin, AST, ALT, GGT, CO2, alkaline phosphatase, BUN, creatinine, sodium, potassium, calcium, phosphorus, and glucose.
[6] Only applicable to women of child-bearing potential.
[7] Patients are to be called 24 and 72 hours post bronchoscopy to assess for adverse events and instructed to call within 30 days, if febrile, or with increased sputum production, or increasing shortness of breath or any perceived deterioration of respiratory condition. Patient communication log to be completed (Appendix G)
[8] To be obtained within three months of registration. *An entry sample will be completed by subjects who qualify by bronchial dysplasia. Sputum collection will be performed over a six day period or by induction.
[9] Screening EKG to be performed within two months of registration
[10] St. George, CAT, and BORG questionnaires
**Signed full trial informed consent to be obtained prior to ordering baseline/screening testing in no specified timeframe prior to registration.

7.2 Baseline Testing/Pre-study Evaluation

7.2.1 Pre-screening Visit

7.2.1.1 Signed pre-screening informed consent.

7.2.1.2 Sputum cytology: Sputum cytology will be obtained on enrolled subjects. Entry consists of two stages, with subjects qualifying with either **sputum cytologic atypia, or a history of endobronchial dysplasia, for the full study. Qualifying sputum must be submitted less than 3 months prior to bronchoscopy, and all subjects will provide an entry sputum sample (for example, sputum samples will be collected from subjects who have a history of endobronchial dysplasia).** Subjects will be asked to perform an early morning sputum collection over a six day period. Those who are not able to provide spontaneous sputum will have a sputum induction with hypertonic saline performed in the Denver VAMC PFT laboratory. The sputum will be collected in a container containing fixative. Incoming sputum specimens will be processed by a cytotechnologist using the two slide pull technique. Two slides will be stained by the Papanicolaou method and screened by both a cytotechnologist and by the study pathologists.

7.2.2 Screening Visit

All screening assessments below to be performed within 6 weeks prior to registration unless otherwise noted.

1. Signed full trial informed consent (to be obtained prior to ordering baseline/screening testing in no specified timeframe prior to registration).

2. Physical exam including height, weight, vitals and ECOG performance status.

3. Medical history including smoking history and concomitant medications.

4. Laboratory analysis to include: CBC (hemoglobin, a complete red blood cell count, total white blood cell count (WBC), platelet count, and serum chemistries (total serumprotein, albumin, bilirubin, AST, ALT, GGT, CO2, alkaline phosphatase, BUN, creatinine, sodium, potassium, calcium, phosphorus, and glucose).

5. Full pulmonary function testing within 6 months prior to registration.

6. EKG within 2 months prior to registration.

7. Serum pregnancy test in females of childbearing potential. Women are considered to be of child-bearing potential if they are not surgically sterile or under the age 65 and have menstruated within the last two years.

8. Exhaled Carbon Monoxide (CO) measurement

9. Six-Minute Walk Distance (6MWD). See Appendix C.

10. St. George Respiratory and CAT (COPD Assessment Test) Questionnaire. See Appendix D and E.

11. BODE Index will be calculated. See Appendix F.

12. **Blood draw and urine collection (see section 10 for further processing details).**

13. **Bronchoscopy is scheduled either at or after this screening visit, unless an autofluorescence and white light bronchoscopy with the full research bronchoscopy procedures and biopsies has been performed within 3 months prior to study entry. Registration will occur after completion of bronchoscopy with biopsies, but is not dependent on biopsy results.**

Bronchoscopy: Study subjects will undergo autofluorescence (AFB) and/or white-light bronchoscopy prior to starting treatment.

Endobronchial biopsies: Biopsies will be taken from six predetermined sites. In addition, biopsies will be taken from any sites that appear suspicious under white light or autofluorescence.

Endobronchial brushings: The cytology brushes will be placed in a microcentrifuge tube containing 1.0ml RNAprotect cell or RLT+ reagent or saline reagent, labeled with a non-identifiable subject ID and time-point (enrollment or post-treatment), snap-frozen in liquid nitrogen and stored at <-80C.

Research Blood: Two yellow top citrates, one purple top EDTA, and one red top tube will be collected and stored for later analysis.

Urine collection: Urine will be collected and adjusted to a final concentration of 2N glacial acetic acid and stored at –80 ° C.

Sputum cytology: Subjects who qualify by bronchial dysplasia will complete an entry sputum sample. Subjects will be asked to perform an early morning sputum collection over a six day period. Those who are unable to provide spontaneous sputum (or who have an unsatisfactory specimen) will have a sputum induction with hypertonic saline performed in the Denver VAMC PFT laboratory.

All screening assessments must be completed prior to registration.

7.3 Evaluation During Study Intervention

7.3.1 **Visit 1 (Day 1)**

After all eligibility criteria confirms the subject eligible to participate in this study, the patient will be registered. The study drug will be dispensed at Visit 1. The instructions will be reviewed with the participant, the first dose will be taken, and the participant will be observed for 30 minutes. Patients will be given a drug diary and instructed to complete daily.

7.3.2 **Day 15 Phone call (+/- 3 days)**

Patients will receive a telephone call to review compliance and side effects.

7.3.3 **Visit 2 (Day 30 [+/- 5 days])**

1. Physical exam including weight, vitals and ECOG performance status.
2. Medical history including smoking history and concomitant medications
3. Laboratory analysis to include: CBC, platelet count, and serum chemistries (total serum protein, albumin, bilirubin, AST, ALT, GGT, CO2, alkaline phosphatase, BUN, creatinine, sodium, potassium, calcium, phosphorus, and glucose).
4. Exhaled Carbon Monoxide (CO) measurement
5. Toxicity Assessment
6. Ampule count and diary review.
7. Interrogation of I-neb AAD device to determine compliance. Accredo has developed software that interfaces with the AAD device and can provide dosing information.

8. Drug dispensing.

7.4 Evaluation at Completion of Study Intervention

7.4.1 **Visit 3 (Day 60 [+/- 5 days])**

1. Physical exam including weight, vitals and ECOG performance status.

2. Medical history including smoking history and concomitant medications

3. Laboratory analysis to include: CBC, platelet count, and serum chemistries (total serum protein, albumin, bilirubin, AST, ALT, GGT, CO2, alkaline phosphatase, BUN, creatinine, sodium, potassium, calcium, phosphorus, and glucose).

4. Full pulmonary function testing

5. Exhaled Carbon Monoxide (CO) measurement

6. Ampule count and diary review.

7. Interrogation of I-neb AAD device to determine compliance.

8. Six-Minute Walk Distance (6MWD). See Appendix C.

9. St. George Respiratory and CAT (COPD Assessment Test) Questionnaire. See Appendix D and E.

10. BODE Index will be calculated. See Appendix F.

11. Research Blood: Two yellow top citrates, one purple top EDTA, and one red top tube will be collected and stored for later analysis.

12. Bronchoscopy, sputum, and urine collection (see section 10 for further processing details).

Note that Visit 3 may be completed over a two-day time frame as long as all testing remains within the protocol specified time frame for Visit 3 (Day 60+/- 5days).

Bronchoscopy: Study subjects will undergo autofluorescence and/or white-light bronchoscopy.

Endobronchial biopsies: Biopsies will be taken from six predetermined sites. In addition, biopsies will be taken from any sites that appear suspicious under white light or fluorescence.

Endobronchial brushings: Cytology brushes will be placed in a microcentrifuge tube containing 1.0 ml RNAprotect cell reagent or RLT+ reagent or saline, labeled with a non-identifiable subject ID and time-point (enrollment or post-treatment), snap-frozen in liquid nitrogen and stored at <-80C.

Research Blood: Two yellow top citrates, one purple top EDTA, and one red top will be collected.

Urine collection: Urine will be collected and adjusted to a final concentration of 2N glacial acetic acid and stored at –80 ° C.

Sputum cytology: Sputum cytology will be obtained. Subjects will be asked to perform an early morning sputum collection over a six day period immediately after the 60 day study visit. The sputum will be collected in a container with fixative. Incoming sputum specimens will be processed by a cytotechnologist using the two slide pull technique. Two slides will be stained by the Papanicolaou method and screened by both a cytotechnologist and by the study pathologists.

7.5 Post-intervention Follow-up Period

7.5.1 **Visit 4 (Post-intervention Day 90 [+/- 5 days])**

1. Physical exam including weight, vitals and ECOG performance status.
2. Medical history including smoking history and concomitant medications
3. Toxicity Assessment

7.5.2 Follow up

Patients who have developed drug-related toxicities will have follow-up clinic visits per institutional SOC until the toxicity resolves.

7.6 Methods for Clinical Procedures

Bronchoscopy: Study subjects will undergo autofluorescence and white-light bronchoscopy.

Endobronchial biopsies: Biopsies will be taken from six predetermined sites as described in 10.2. In addition, biopsies will be taken from any sites that appear suspicious under white light or fluorescence.

Endobronchial brushings: Cytology brushes will be placed in a microcentrifuge tube containing 1.0 ml RNAprotect cell reagent or RLT+ reagent or saline, labeled with a non-identifiable subject ID and time-point (enrollment or post-treatment), snap-frozen in liquid nitrogen and stored at <-80C.

Research Blood: Two yellow top citrates, one purple top EDTA, and one red top will be collected.

Urine collection: Urine will be collected and adjusted to a final concentration of 2N glacial acetic acid and stored at –80 ° C.

Sputum cytology: Sputum cytology will be obtained. Subjects will be asked to perform an early morning sputum collection over a six day period. The sputum will be collected in a container with fixative. Incoming sputum specimens will be processed by a cytotechnologist using the two slide pull technique. Two slides will be stained by the Papanicolaou method and screened by both a cytotechnologist and by the study pathologists.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

Toxicity data for participants according to active drug (iloprost) will be collected.

8.2 Secondary Endpoints

Secondary Endpoints will be evaluated in a batch fashion analyzed once the targeted number of study subjects has completed the protocol.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, and medical contraindication. If screening biopsy shows either invasive carcinoma or carcinoma in situ, the participant will be taken off study and managed according to participant and physician decision.

Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events. Patients will not be replaced.

8.4 Off-Study Criteria

Participants may go 'off-study' for the following reasons:

- Adverse Event
- Death
- Disease Progression
- Discovery of invasive carcinoma or carcinoma in situ on screening biopsy
- Lost to follow-up
- Other
- Participant Withdrawal
- Participant Refused Follow-up
- Physician Decision
- Protocol Defined Follow-up Completed
- Protocol Violation
- Study Complete
- Ineligible

8.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

A number of exploratory secondary endpoints will be assessed to guide future chemoprevention studies.

Endobronchial histology: Oral iloprost administration resulted in improvement in endobronchial histology in former smokers[14]. We will utilize the same procedures for tissue acquisition, fixation and pathologic examination as described.

Effect on serum proteins: An exploratory analysis of the association of serum protein abundance with baseline dysplasia revealed six proteins (ADIPOQ, C9, ATP5B, CHST15, SHBG, HNRNPA2B1) with the potential to predict endobronchial dysplasia. These were quantitated using a highly multiplexed aptamer based assay system developed by SomaLogic, Inc.[16]. The current study provides an opportunity to validate these associations, potentially leading to a means to identify high risk individuals with bronchial dysplasia non-invasively.

Effect on endobronchial brushing and biopsy genomics: Endobronchial brushings have previously been reported as potential predictive biomarkers for chemoprevention response to myo-inositol[28]. We propose to carry out exploratory analyses of gene expression biomarkers for chemopreventive response to inhaled iloprost. Dr. Daniel Merrick has analyzed gene expression patterns discriminating dysplastic lesions that persist or progress from those that regress spontaneously, with the identification of a 46 gene predictive panel. We propose to test whether this predictive panel can be validated in this Phase I inhaled iloprost study. In addition, DNA from bronchial epithelial samples will be stored for future analysis of somatic mutations.

Parameters of COPD severity: COPD shares a major causative agent (tobacco smoke) with lung cancer and is a biomarker for risk of developing lung cancer[31]. Ideally, lung cancer chemopreventive agents would also have beneficial effects on COPD. Iloprost has anti-inflammatory properties and may potentially modulate lung

function. Inhaled iloprost improves V/Q matching, reduces pulmonary hypertension and could have a favorable effect on patients with COPD[32]. We propose exploratory analysis of pulmonary function, 6 minute walk test and quality of life measurements, similar to those being carried out in a current Phase II pioglitazone chemoprevention trial.

The conduct of early phase cancer trials provides the opportunity to evaluate systemic drug effect markers. Clinical samples will therefore be captured and stored for purposes of future molecular fingerprinting at the level of both the proteome as well as at the level of gene expression (i.e. RNA) from blood samples. Therapy-induced changes in cellular function will be expected to result from changes in gene expression and/or protein expression. Advances in technology now permit protein and expressed gene (RNA) compartments to be analyzed on a broad scale, or to be fingerprinted. Pre- and post-treatment plasma and RNA from peripheral white blood cells will therefore be collected and stored for future batch analysis. Blood plasma proteins will be characterized by mass spectrometric analysis, while expressed genes will be characterized by using RNA to probe gene arrays. Samples will be banked, for future processing. Resultant data relating to the molecular effects of this drug, as well as future drugs, will be used to populate a library. This data library, which will not have any patient identifier information in it, will contain information which will cross classes of drugs, as well as target cohorts (i.e., cohorts at risk for different cancer types).

9.2 Comparable Methods

Endobronchial histology: The methods proposed for tissue processing and pathologic grading are the only ones to have previously successfully demonstrated improvement in endobronchial histology with a chemopreventive agent, oral iloprost.

Effect on serum proteins: The aptamer based highly multiplexed protein assay system developed by SomaLogic, Inc. is highly sensitive and reproducible, with a mean limit of detection of 40 fM. At a cost of $300 per patient (pretreatment and post treatment samples), this is a highly cost effective assay.

Effect on endobronchial brushing and biopsy gene expression: RNA sequencing, DNA sequencing, and gene promoter methylation will be used for these assays. In order to discriminate between somatic and germ line mutation, DNA sequencing will be performed on peripheral blood DNA in selected instances.

Parameters of COPD severity: Full PFTs, 6 minute walk test, and the St. George's respiratory questionnaire and COPD assessment test are all well recognized standard tests that can be reproducibly performed either in the Denver VAMC Pulmonary Function Laboratory or by our study coordinators.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

10.1.1. Tissues for study specific biomarkers

Specimens will be processed and banked at the SPORE Tissue Bank located on the University of Colorado Anschutz Medical Campus.

The address is:
SPORE Lab
University of Colorado Denver
RC1 South
12801 E. 17th Avenue Room L18-5400F
Aurora, CO 80045

Specimens for blood sample laboratory analysis for the Screening visit, and Visits 2 and 3 will be analyzed at:
Denver Veterans Administration Medical Center
Department of Pathology and Lab Medicine
1055 Clermont Street, #113
Denver, CO 80220

10.1.2. Tissue Banking

All biospecimens will be considered the property of the National Cancer Institute and will not be used for assays other than specified in this protocol without NCI approval. Biospecimens will be stored in the University of Colorado SPORE Tissue Bank, a Department of Veterans Affairs approved tissue bank, and supplied to the National Cancer Institute as needed after approval of supplemental amendments. Plasma from blood and DNA from peripheral white blood cells, will be collected at baseline (just prior to treatment), and at the end of treatment, and will be stored in the SPORE Tissue Bank for future proteomic and genetic analysis. This analysis will be performed in the future, and will seek to evaluate the effects of therapeutic intervention by comparing pre- and post-treatment samples, and measuring changes in protein expression and epithelial mutation. Samples will be de-identified through established SOPs no information will be conveyed back to individual subjects, nor will any link to individual subjects be sought.

10.2 Collection and Handling Procedures

Bronchoscopy: Study subjects will undergo autofluorescence and/or white-light bronchoscopy after being NPO for a minimum of six hours prior to the procedure. The patient will be monitored with continuous electrocardiographic, respiratory and oximetric monitoring, and with intermittent blood pressure monitoring. A complete endobronchial inspection will be performed and suspicious areas identified under fluorescence or white light will be biopsied. A Bronchoscopy Patient Communication Log Form is attached (Appendix G) to follow-up the patients after the bronchoscopy is performed. Patients are called within 24 hours, 72 hours, and instructed to call within 30 days, if febrile, or with increased sputum production, or increasing shortness of breath or any perceived deterioration of respiratory condition.

Endobronchial biopsies: Biopsies will be taken from six predetermined sites: upper lobe orifices and superior segment orifices (left and right), carina between left upper lobe division and lingular orifices and right middle lobe orifice. In addition, biopsies will be taken from any sites that appear suspicious under white light or fluorescence. On follow-up bronchoscopies, all sites from the original bronchoscopy will be rebiopsied, along with any abnormal appearing areas under white light or fluorescence. Biopsies will be formalin-fixed and paraffin-embedded for hematoxylin and eosin staining, as well as for immunohistochemical staining. One or more biopsies may be cultured or snap frozen for protein, RNA or DNA analysis. Depending on patient tolerance, and operator discretion, endobronchial biopsies may be terminated at any time; however, an attempt will be made to biopsy between 6 and 12 sites on each patient. Additional sites may be biopsied if deemed clinically indicated but more than 12 endobronchial biopsies will not be routinely taken. The number and location of biopsies may be altered according to bronchoscopist's discretion and patient tolerance.
Endobronchial brushings: Endobronchial brushings will be taken from either the right and/or left, usually corresponding with certain biopsy sites (normal and abnormal appearing areas). Additionally, the right mainstem bronchus will be brushed when possible, or if not a possibility, a brushing will be completed in the left mainstem bronchus. Cytology brushes will be placed in a microcentrifuge tube containing 1 ml RNAprotect cell reagent or RLT+ reagent or saline, labeled with a non-identifiable subject ID and time-point (enrollment or post-treatment), snap-frozen in liquid nitrogen and stored at <-80ºC. The number and location of brushings may be altered according to bronchoscopist's discretion and patient tolerance.

Research Samples for Biomarkers: Two yellow top citrates, one purple top EDTA, and one red top tube will be collected and banked for future studies.

Two 8.5 ml Vacutainer Citrate (yellow top) tubes. Draw blood into tubes and immediately mix thoroughly with anticoagulant by gently shaking. Refrigerate immediately until transfer to processing lab. Upon arrival at processing lab, centrifuge both tubes at 400 x G for 20 minutes. Remove the buffy coat from one tube (approx 1ml) to a 2ml bar-coded cryovial. Remove the buffy coat from the second tube (approx 1ml) to a 2ml bar-coded cryovial and add 700ul RLT+ reagent (Qiagen, cat. 1053393). Invert several times to mix. Freeze both buffy coat vials immediately. Remove the plasma remaining in each tube and place in individual 15mL conical vials. Centrifuge the conical vials at 4000 rpm for 10 minutes, and aliquot the spun plasma into 0.5mL bar-coded cryovials, as many as the sample allows, a maximum of 10 per tube. Plasma must be clear before freezing; no cells or debris should be present. Freeze all immediately at –80C.

One 10.0 ml Vacutainer Serum (red top) tube. Draw blood into tube and immediately mix thoroughly by inverting 8 to 10 times. Allow clot formation for 30mins at room temp. Centrifuge at 4000 rpm for 10 mins at room temp.

Urine collection:
Urine will be collected a screw-top jar and aliquoted into six 3 ml cryovials. Any remaining urine is to be discarded. Seal the cap tightly. Freeze urine sample at –80˚C for future studies.

Sputum cytology: Sputum cytology will be obtained. Subjects will be asked to perform an early morning sputum collection over a six day period. For those unable to provide a spontaneous expectorated specimen, sputum induction with hypertonic saline will be performed in the DVAMC PFT lab. The sputum will be collected in a container containing fixative. Incoming sputum specimens will be processed by a cytotechnologist using the two slide pull technique. Two slides will be stained by the Papanicolaou method and screened by both a cytotechnologist and by the study pathologists.

One 10.0 ml EDTA Vacutainer (purple top) tube. Collect 10ml of peripheral blood for gene array analysis. Draw blood into tube and immediately mix thoroughly with anticoagulant by gently shaking. Refrigerate immediately until transfer to processing lab. Upon arrival at processing lab, centrifuge at 400 x G for 20 minutes. Remove the buffy coat ACD tubes (approx 1ml) to a 2ml bar-coded cryovials. Remove the plasma remaining and place in a 15mL conical vial. Centrifuge the conical vial at 4000 rpm for 10 minutes, and aliquot the spun plasma into 0.5mL bar-coded cryovials, as many as the sample allows, a maximum of 10. Plasma must be clear before freezing; no cells or debris should be present. Freeze all immediately at –80C.

10.3 Research Samples for Genetic/ Genomic Analysis:

10.3.1 Description of the scope of genetic/ genomic analysis

RNA sequencing, DNA sequencing, and gene promoter methylation will be performed on samples from selected airway biopsies and brushings. In order to discriminate between somatic and germ line mutation, DNA sequencing will be performed on peripheral blood DNA in selected instances.

10.3.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

All tissue and blood samples collected under this study will be coded. DNA and RNA isolated from these tissues will all be similarly coded. Only the site Principal Investigator and primary research coordinators involved with this study at the University of Colorado will have access to the code.

To facilitate genetic research, and for the purpose of publication of research work, data from genetic and genomic studies may be deposited in appropriate public databases. Coded data will be deposited in a manner that the patient's identity cannot be traced and patients will have the opportunity to opt out of having their information deposited in appropriate public databases at the time of informed consent.

10.3.3 Management of Results

The analyses performed in University of Colorado and National Jewish Health laboratories under this protocol are for research purposes only; they are not nearly as sensitive as the tests that are performed in a laboratory that is certified to perform genetic testing for clinical purposes and are not meant to substitute for clinical care at any time. Changes observed unrelated to our research may or may not be valid. Therefore, we do not plan to inform participants of the results of testing on the tissue or blood that is performed in research labs.

However, in the unlikely event that clinically relevant incidental findings are discovered, subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Subjects who still remain on the study will be contacted by the site Principal Investigator and be provided with a referral to a certified genetic health professional if the subject desires. Any costs associated with follow-up of an actionable gene variant will be responsibility of the subject.

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

10.4 Shipping Instructions

None. All samples will be stored and processed on site.

10.5 Tissue Banking

Biospecimens are collected either immediately prior to bronchoscopy or during bronchoscopy and stored in the Colorado SPORE in Lung Cancer Tissue Bank and Biomarkers Core (TBBC) according to established standard operating procedures (SOPs).

Biologic specimens collected during the conduct of each clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense, provided that this is a VA-approved tissue bank. If no such VA-approved tissue bank is identified, specimens will remain in the SPORE Tissue Bank for future distribution.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value

of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible, by the treating physician's discretion.

A list of AEs that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in §6.2, Pharmaceutical Information, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are completed must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for adverse event reporting.

- AE verbatim term
- System Organ Class (SOC)
- Common Terminology Criteria for Adverse Events v4.0 (CTCAE) AE term
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a serious adverse event (SAE)
- Whether or not the subject dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

ADL

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.1.4 Assessment of relationship of AE to treatment

The possibility that the adverse event is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such. If a participant is taken off study due to discovery of invasive carcinoma or carcinoma in situ, these participants will be followed according to standard of care.

11.2 Serious Adverse Events

11.2.1 DEFINITION: Fed. Reg. 75, Sept. 29, 2010 defines SAEs as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).*
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality/birth defect
- Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed.

11.2.2 Reporting SAEs to DCP

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE form found at http://prevention.cancer.gov/sites/default/files/uploads/clinical_trial/SAE-Form.pdf.

11.2.2.2 Contact the DCP Medical Monitor by phone or email within 24 hours of knowledge of the event.

DCP Medical Monitor
Eva Szabo, M.D.
Chief, LUACRG, DCP, NCI
9609 Medical Center Dr., Rm 5E-102, MSC 9781
Bethesda, MD 20892-9781 (for FedEx, use Rockville, MD 20850)
Telephone (240) 276-7011
Fax (240) 276-7848
Email: szaboe@mail.nih.gov

Include the following information when calling the Medical Monitor:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug and expectedness

11.2.2.3 The Lead Organization and all Participating Organizations will email written SAE reports to the DCP Medical Monitor within 48 hours of learning of the event using the paper SAE form. The written SAE reports will also be emailed (safety@ccsainc.com) to DCP's Regulatory Contractor, CCS Associates (phone: 650-691-4400). The written SAE reports will also be emailed (ncpc@northwestern.edu) to the Lead Organization (phone: 312-695-0562) if sent by a Participating Organization.

SAEs that are a response to the medicinal product should be reported to Bayer Actelion (email: DrugSafety.GPV.US@bayer.com, phone: 1-888-842-2937). The accrual site is responsible for determining which SAEs should be reported to Bayer Actelion.

11.2.2.4 The DCP Medical Monitor and regulatory staff will determine which SAEs require FDA submission.

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.3 Follow-up of SAE

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE form in the appropriate format. Follow-up information should be sent to DCP as soon as available. Follow up information should be sent to the DCP and Lead Organization as soon as available.

12. STUDY MONITORING

12.1 Consortia 2012 Data Management

Data will be managed by the study statistician, Dr. Jovanovic, according to standard operating procedures, which meet the guidelines of DCP Requirements for Data Management and which follow the Data Management Plan that Northwestern University has on file with the Division of Cancer Prevention, NCI. The Consortia 2012 Data Management Plan, submitted as part of a contract agreement with the NCI (HHSN261201200035I), was approved.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRFs) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDEs). The approved CRFs will be used by Northwestern University to create the electronic CRFs (e-CRFs) screens in the Lurie Cancer Center Clinical Trials Management System. Site staff will enter data into the e-CRFs for transmission to DCP according to DCP standards and procedures.

12.3 Source Documents

All source documents will be collected and stored at the study site. Any data recorded directly on CRFs that constitute no prior written or electronic record of data, will be specifically identified as source data.

12.4 Data and Safety Monitoring Plan

A comprehensive Data Safety and Monitoring Plan has been submitted by Northwestern University, has been approved by the DCP, and is on file there. Any future changes will be forwarded for review.

12.5 Sponsor, FDA, or Biomarker Development Partner Monitoring

The NCI, DCP (or their designee), pharmaceutical and technology collaborator (or their designee), or FDA may monitor/audit various aspects of the study. Collaborators with a research agreement with the University of Colorado (the enrollment site) may also monitor/ audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as IRB records and other regulatory documentation, will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)/ Material Transfer Agreement (MTA)/ Master Research Agreements

Agreements will be negotiated between the University of Colorado and Bayer/Actelion as well as Accredo. An additional Master Research Agreement has been negotiated between the University of Colorado and VisionGate, Inc. to allow for use of sputa samples for analysis by automated three dimensional morphologic analysis.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This is a Phase I toxicity and compliance study. The primary endpoints are clinical toxicity and compliance. Secondary endpoints are the response of airway histology, serum protein profiling, endobronchial brushing gene expression and gene expression of dysplastic lesions.

13.2 Randomization/Stratification

This is an open label trial. We initially proposed to study two separate iloprost dosing regimens (QID and BID) with 20 enrolled subjects in each iloprost dosing regimen group (estimated attrition of 20% during the study would result in 16 evaluable participants per group). In Cohort A, the first 7 subjects were randomized to iloprost versus placebo, with one participant receiving placebo, but the remaining 14 subjects will not be randomized and instead will be given active iloprost. This is due to lack of availability of the placebo. Within the BID group (Cohort B), we randomized 4 participants, until revision for protocol v3.8 was approved, due to

the expiration of placebo in December 2018. After approval of protocol v3.8, we registered all proceeding participants to cohort B as open label (without randomization). Thus, 7 participants were enrolled to cohort B: 5 active treatment BID, and 2 placebo. During the approval of protocol v3.9, we expect that up to 3 more patients will be enrolled into Cohort B open label. Thus, we expect Cohort B will have 5-8 patients on active agent, and 2 patients on placebo.

An interim analysis was conducted after completion of Cohort A and discussed on March 26, 2019. As toxicity was minimal with the QID regimen and compliance was good (65% achieved 80% compliance or more), a decision was made to discontinue Cohort B and enlarge Cohort A to a maximum of 40 participants on active agent, in order to better assess any effects of inhaled iloprost on secondary biomarkers. We expect up to 147 people will participate in the "pre-screening" portion of this study.

We expect 20% attrition during the study (for insufficient samples and non-compliance), for a final evaluable sample of 32 active QID.

13.3 Accrual and Feasibility

In a typical week, there are 5-6 subjects identified who would qualify for chemoprevention trials, and about 1/3 of these subjects are agreeable to submit a screening sputum cytology sample and consider entering trials. We plan to enroll a total of up to 51 subjects to allow for attrition of 8-10 patients. We anticipate this taking about 24-36 months to complete. We also have subjects who participated in prior trials who have provided consent to be contacted for future studies.

13.4 Primary Objective, Endpoint(s), Analysis Plan

For primary objectives we will provide descriptive statistics (mean, SD, median, max, min and range) for toxicity dropouts and compliance.

In order to assess the rates of ***toxicity, dropouts and compliance*** we will use approximate 95% confidence intervals. With n=40, and 20% dropout rate, we get n=32, the approximate half-width of the 95% confidence interval around the mean rate in a group is equal to or smaller **than 2 x 0.5/sqrt(32) = 0.18.** This is compatible with the difference of 18% observed in Phase III study (ref 35). Although we will not be able to study each toxicity per se, due to small number of subjects and their rare appearance, we will be able to assess a total number of toxicities per subject in treatment and placebo groups. Comparison between group B and B-control will follow the confidence interval approach outlined above for comparison of groups A and B. Group A will not be compared to B-control.

13.5 Secondary Objectives, Endpoints, Analysis Plans

For Secondary outcomes we will provide descriptive statistics for a) Effect on endobronchial histology, with the primary parameter being worst histology at matched sites; additional outcomes will be average histology, dysplasia index and response, as described[15]) b) Effect on expectorated sputum cytology, both by standard analysis and by an automated three dimensional technique (Detecting dysplasia by LuCED is a research effort. The endpoint assessment will be limited to a binary indication of dysplasia by LuCED compared to cases with confirmed dysplasia by histology) c) Effect on serum proteins as quantitated by aptamer based analysis, d) Effect on endobronchial brushing gene expression, focusing on prostacyclin-targeted pathways, and e) improvement in COPD as measured by ABG (improved ventilation perfusion matching), pulmonary function testing, 6 minute walk distance, and quality of life (St. George's respiratory questionnaire, COPD assessment test [CAT]). Project statistician Dr. Jovanovic will supervise all of the analyses of primary and secondary outcomes.

When measuring global gene expression a concern is multiple testing and the generation of false positives, given the thousands of statistical tests needed to survey the entire genome. We will control for multiple testing by applying a strict false discover rate (FDR) of less than 1% to all gene lists used to define inhaled iloprost exposure. Iloprost-specific profiles will be further refined through bioinformatics analysis. Ingenuity pathway analysis (IPA) (www.ingenuity.com) will be used to identify over-represented pathways and networks contained within the affected genes using a Fisher's Exact Test p-value of less than 0.01. We will also use IPA to identify master regulators associated with our expression profiles, based on the over-representation of their target genes.

Our ultimate goal is to identify biomarkers that will indicate a positive reactivity to iloprost from an endobronchial sample. The ideal biomarkers for this purpose would be ones that are easy to interpret and have a biological basis for their use. To these ends, we will use strict statistical criteria, correcting for multiple testing, to select our genes of interest. Our selected biomarkers will also demonstrate a strong biological connectivity to other genes affected by iloprost exposure, as evidence of being associated in the top significant pathways and networks as assessed by IPA. Any proposed transcriptional biomarker will be validated in an additional cohort using RT-PCR. The transcribed proteins for any promising transcriptional markers will also be explored as potential signatures for iloprost exposure, based on the ease and relative quickness of this method of detection for clinical purposes.

13.6 Reporting and Exclusions

Compliance is an outcome directly measured as the fraction of prescribed inhalations actually administered. In previous similar chemoprevention studies we have conducted, dropout rate has been approximately 15% (similar in both placebo and active drug arms). Non-compliance will not result in removal from study. The primary analysis of secondary endpoints will be on an intention to treat basis. Further secondary statistical analyses may be conducted based on varying degrees of compliance.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time they take study drug through the day 90 study visit (study visit 4 on our calendar and corresponds to a one month visit after completing the 60 day treatment period), even if they have been taken off study for any reason.

13.8 Evaluation of Response

All participants included in the study will be assessed for response to intervention, even if there are major protocol deviations or if they are ineligible. All of the participants who met the eligibility criteria (with the possible exception of those who did not receive study agent) will be included in the main analysis. All conclusions regarding efficacy will be based on all eligible participants.
Sub-analyses may be performed on the subsets of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of intervention, major protocol violations, etc.). However, sub-analyses may not serve as the basis for drawing conclusions concerning efficacy, and the reasons for excluding participants from the analysis should be clearly reported. For all measurements of response, the 95% confidence intervals should be provided.

13.9 Interim Analysis

N/A

13.10 Ancillary Studies

N/A

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Signed and dated current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations. CVs or biosketches do not need to be updated for participating study staff after drug shipment authorization (DSA).

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 Lab certification (*e.g.*, CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.2.4 Documentation of Good Clinical Practice training for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.5 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 Signed Investigator's Brochure/Package Insert acknowledgement form

14.2.7 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator for each site and initialed by all study personnel listed on the form

14.2.8 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent. A Model Consent Form for Use of Tissue for Research is available through a link in the DCP website.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the IRB at each Organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by NCI, DCP, the Consortium Lead Organization's IRB, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to the DCP Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates
2001 Gateway Place, Suite 350 West
San Jose, CA 95110
Phone: 650-691-4400
Fax: 650-691-4410

E-mail Submissions: regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review, which will then be electronically forwarded to the DCP Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

14.6.1 Non-physical risks of genetic research

Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related DNA and RNA sequencing or disease tendencies. Patients will be clearly informed that the data related to DNA sequencing and genetic analysis is coded, investigational and will not be shared with patients, family members or health care providers.

This includes the risk that data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the patients, family members or health care providers, this risk will be included in the informed consent document.

15. FINANCING, EXPENSES, AND/OR INSURANCE

All research related costs associated with participating in this study will be paid for, and will not be the responsibility of the participant

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CONSENT FORM

Department of Veterans Affairs	Informed Consent Form	
Valid Through:	**R&D Stamp:**	**COMIRB Approval Stamp/Date:**
Protocol Version Date: 4/19/2019 (v3.9) **Consent Version Date: 4/19/2019**		

Subject Name: _____Date:_____

Title of Study: NWU2013-02-01: A Phase I Trial of Inhaled Iloprost for the Prevention of Lung Cancer in Former Smokers

Principal Investigator: York E. Miller, M.D. **VAMC**: 554_____

VA Investigator: York E. Miller, M.D. **COMIRB#__14-1247_____**

You are being asked to be in a research study. This form provides you with information about this study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to take part.

Why is this study being done?

This study is designed to learn more about the safety and effectiveness of an investigational drug called inhaled iloprost (Ventavis®). The U.S. Food and Drug Administration (FDA) is the U.S. government agency that reviews the results of research and decides if a drug can be sold in the U.S. Inhaled iloprost is approved by the FDA for treating a disease of the lungs, called pulmonary hypertension. However, Inhaled iloprost may also be effective at preventing lung cancer in people at higher risk for developing lung cancer, such as yourself, a former smoker who has been found to have abnormal cells in your sputum or airways. Because inhaled iloprost has not yet been demonstrated to be effective, nor approved for preventive treatment in lung cancer, it is considered investigational.

When lung cells are looked at under a microscope and tested in the lab, they often show changes that tell us they can turn into cancer. These signs or cell changes are called biomarkers. We want to learn if inhaled iloprost can stop these cells from turning into cancer. We also want to find out what side effects patients might have if they take inhaled iloprost for 2 months.

You are being asked to be in this research study because you are at risk to develop lung cancer. People who are at risk for lung cancer and choose not to participate in a study are usually followed closely by their doctor to watch for the development of cancer. Your participation in this study is voluntary. If you decide not to participate in this research study, it will not affect the treatment that your doctors would normally give you. If you decide not to take part in this study, you have other choices. For example:

- You may choose to have the usual approach described above.
- You may choose to take part in a different study, if one is available.
- Or you may choose to do nothing

We plan to enroll up to 51 participants this treatment part of the study.

Inhaled iloprost will be called "the study drug" for the rest of this consent form.

How long will I be in this study?

Your first screening visit will take about 6 hours for all the procedures to be completed. If you are eligible for the study, you will be on the study drug for 2 months. The study doctors will continue to watch you for side effects and follow your condition for 1 month after you have stopped the study drug. You will be in the study for a total of about 3 ½ months.

What happens if I join this study?

If you join the study, you will be asked to do the following after you sign the consent form.

Before the Study (Screening period)

You will first go through several tests and procedures to make sure that you are eligible to take part in the study. The following list of exams, tests, and procedures except for the bronchoscopy are medical treatments that you will get because you are in this research study. The National Cancer Institute (NCI) will pay for those. If your sputum shows moderate or greater changes to normal cells the bronchoscopy would be a test that you would have to get for your condition even if you were not in this study. Some of the blood, urine, and precancer tissue, as described below, will be taken for the study during screening. These samples are required in order for you to take part in this study because the research on the samples is an important part of the study for researchers to learn how to diagnose and treat lung cancer.

- Physical exam (including your height, and weight)
- Vital signs (including blood pressure, heart rate, and temperature)
- Review of your medical history (including a review of any medications you are currently taking, and smoking history)
- Performance status evaluation (what type of daily activities you can do)
- Research Blood tests: The total blood drawn will be 2½ ounces (approximately 5 tablespoons of blood). The blood tests performed will determine your eligibility, follow your safety, and stored for research
- Research Pregnancy test for women who are able to become pregnant
- Urine sample
- Electrocardiogram (EKG) – a painless tracing of your heart's rhythm
- Sputum Sample: A sputum sample will be done at this visit only if you have not provided us with a sample within the last 3 months. You will collect six coughed sputum samples. Each sample will consist of coughing up sputum (once a day for six days). You will be instructed on how to collect and handle these samples. If you are not able to cough up sputum you may have a sputum induction with hypertonic saline performed in the Denver VA PFT laboratory.
- Bronchoscopy:
    - This is a test to view the lung airways to diagnose lung disease.
    - A bronchoscope is a flexible device used to see the inside of the lungs.
    - You will get some medicine to help you relax and to dry your mouth, nose and airway. Your nose, throat, vocal cords and windpipe will be sprayed with Lidocaine (numbing medicine) to keep you from coughing and to numb your airways.
    - During the procedure a biopsy in your airways will be obtained (from at least six areas, approximately 1-2 mm in size each), and brushes will also be done to sample the cells that line the airways.

- The bronchial biopsy results will take one to three weeks to be reported. In the highly unlikely event that a finding, such as invasive cancer or carcinoma in situ, with clinical importance is discovered you will be taken off of study and you and your healthcare provider will be alerted to this.
- Investigators may do genetic or genomic research studies on the tissue obtained in the biopsies taken during your bronchoscopy. This tissue may be used for the analysis of your RNA and DNA. RNA and DNA are the 'instruction book' your body uses in all the cells in your body. These tests will help investigators to test whether your airway cells respond to iloprost in the laboratory.
- You will not be able to drive yourself home after the test so you will need to make plans for a ride home.
- You will receive a telephone call about 24 hours and 72 hours after your test to see how you are feeling.
- You will receive instructions to call us if you develop any of the following complications within 30 days after the bronchoscopy:
  - fever
  - increased sputum
  - increased shortness of breath, and any feeling that your breathing condition has gotten worse

- Pulmonary Function Test (PFT): This breathing test looks at how well your lungs work. You will blow at a fast and long pace into a mouthpiece on a recording device (spirometer).
- Carbon Monoxide Measurement: You will breathe normally into a mouthpiece on a recording device (smokerlyzer) in order to test the levels (amount) of carbon monoxide in your body.
- Six-Minute Walk Distance (6MWT): A 6-minute Walk Distance test is used to find out how much you are able to exercise. The test will measure how far you can walk on a flat surface in 6 minutes.
- Questionnaire: You will be asked to complete 4 separate questionnaires which have questions about your lung/respiratory (breathing in/out) problems. You are free to skip any questions that you prefer not to answer.

During the Study

If the exams, tests, and procedures show that you can be in this study, and you choose to take part, then you will need the following research-related tests and procedures during the treatment period.

Study Drug Treatment

What are the study groups?

- **This study has just one group – Iloprost inhaled four times per day**

Participants will be asked to take the study drug for 2 months.

Visit 1

You will meet with the study coordinator to receive the study drug and learn how to use the nebulizer to take the study drug.

- You will receive the study drug by using a nebulizer. The nebulizer is a device used to reduce liquid forms of the drug into a fine spray that you breathe in (inhale). The nebulizer is lightweight, small, and has an internal rechargeable battery like a cell phone.

- You will be trained on how to take the study drug, and how to use and clean the nebulizer. You will also view a video about using the nebulizer.
- It is important to note that the study drug solution should not be allowed to come in contact with the eyes. You will be advised what to do in the event some solution comes in contact with your eyes.
- You will take first dose in the clinic in a private room under the supervision of the study coordinator and/or study physician.
- You will be observed for 30 minutes after your first dose.
- You will take the study drug every 4-6 hours.
- Each treatment will take about 10-15 minutes.
- You will be sent home with the study drug and nebulizer to use daily as instructed, and with a drug diary to record taking each dose.

Day 15

You will receive a telephone call to review any medications you are currently taking and any side effects that you have had within the past 15 days.

Visit 2 (Month 1 end)

You will be asked to bring in all unused study medication, the nebulizer, and the drug diary to your appointment.

You will have the following tests/procedures:

- Physical exam (including your height, and weight)
- Vital signs (including blood pressure, heart rate, and temperature)
- Review of your medical history (including a review of any medications you are currently taking, and smoking history)
- Performance status evaluation (what type of daily activities you can do)
- A review of any side effects that you have had within the past month
- Blood draw. ½ ounce for routine blood tests (approximately 1 tablespoon)".
- Research Carbon Monoxide Measurement

Visit 3 (Month 2 end)

You will have the following tests/procedures:

- Physical exam (including your height, and weight)
- Vital signs (including blood pressure, heart rate, and temperature)
- Review of your medical history (including a review of any medications you are currently taking, and smoking history)
- Performance status evaluation (what type of daily activities you can do)
- A review of any side effects that you have had within the past month.
- Research Blood draw. The total blood drawn will be 2 ½ ounces (approximately 5 tablespoons of blood).

- Research Carbon Monoxide Measurement:
- Urine sample
- Sputum Sample
- Research Bronchoscopy:
- Pulmonary Function Test (PFT)
- Six-Minute Walk Distance (6MWT)
- Questionnaire

Visit 4 (Month 3 end)

You will have the following tests/procedures:

- Physical exam (including your height, and weight)
- Vital signs (including blood pressure, heart rate, and temperature)
- Review of your medical history (including a review of any medications you are currently taking, and smoking history)
- Performance status evaluation (what type of daily activities you can do)
- A review of any side effects that you have had within the past month.
- Research Carbon Monoxide Measurement

Further follow-up may be required if you experience side effects from study participation.

What are the possible discomforts or risks?

Any procedure has possible risks and discomforts. The procedures in this study may cause all, some, or none of the risks or side effects listed below. Rare, unknown, or unforeseeable (unexpected) risks also may occur.

Discomforts you may experience while in this study include the following:

Bronchial biopsy and brushings: The bronchial biopsy and brushings are very safe. However, no test is entirely free of any risk. The risks are as follows:

Main Risks:

- Bleeding from biopsy sites
- Infection

Uncommon Risks:

- Arrhythmias (abnormal heart beat)
- Breathing difficulties
- Fever
- Low blood oxygen
- Abnormal collection of air in the lung which may cause difficulty in breathing (Pneumothorax)
- Sore throat
- Chest pain
- Heart attack

Rare Risks when conscious sedation is used:

- Change in blood pressure
- Slower heart rate
- Nausea
- Headache
- Vomiting

When a biopsy is taken, there is a risk of severe bleeding (hemorrhage). There is a risk of choking if anything (including water) is swallowed before the numbing medicine wears off.

Sometimes antibiotics and/or corticosteroids are needed if you are showing signs of a lung infection. It is not expected that patients will have all of these side effects. Other side effects may occur which were not seen before. Side effects are usually temporary and manageable.

IV Infusion during bronchoscopy: Some pain can be expected when a needle is placed in your arm to infuse (give) fluids, in addition to minor discomfort of having the needle taped to your arm. A bruise may form. The risk of temporary clotting of the vein is about 1 person out of 100, and the risk of infection of a bruise or major blood loss occurs in less than 1 person out of 1,000. To lessen these discomforts, only trained doctors and technicians will be able to place the intravenous line.

Other risks may include:

Venipuncture (blood draw): You will feel pain when the needle goes into the vein. A bruise may form at the site. A total of 5.5 ounces will be taken over the course of this study.

Sputum samples: The risks of collecting sputum that you produce spontaneously are very small. If you are given a solution to increase your coughed sputum there is a small but significant risk of chest tightness, breathing difficulty, or severe respiratory distress. If this happens, you will be given a breathing treatment, and you will be watched in the office or clinic until you feel better, and if necessary, transferred to the Emergency Department. Severe shortness of breath or chest tightness is a very rare occurrence.

Pulmonary Function Test (PFT): There is a small risk of fainting, dizziness, and lightheadedness.

EKG: This is a test to check the electrical activity of your heart. There are usually no risks. Because this exam only checks electrical impulses and does not give electricity, there is no risk of shock. An EKG is done without going into the body or using dyes or x-rays, so there should be no pain or risk. You may feel discomfort similar to pulling a band aid when the electrodes placed on your chest are removed.

Six-Minute Walk Test: Changes in blood pressure, heart rate, and fainting can happen, and in very rare cases, heart attack or stroke. Trained staff and emergency equipment are available if any of these things happen.

Side Effects of the Study Drug:

COMMON, SOME MAY BE SERIOUS In 100 people receiving, more than 20 people may have:
• Red face (flushing) • Increased cough • Headache

OCCASIONAL, SOME MAY BE SERIOUS

In 100 people receiving, from 4 to 20 people may have:

- Tightness of jaw muscles that makes it hard to open the mouth
- Muscle cramps
- Pain in the chest, back, tongue or mouth
- Difficulty sleeping
- Low blood pressure, which may cause symptoms of dizziness, lightheadedness, or feeling faint.
- Increased low blood pressure effect when taking blood pressure lowering medications
- Nausea
- Vomiting
- Abnormal lab tests related to the liver
- "Flu-like" symptoms such as fever, chills, body aches, muscle pain
- Irregular heartbeat
- Bleeding, including nose bleed, spitting up blood
- Infection in the lungs

POSSIBLE, SOME MAY BE SERIOUS

The frequency of some individual side effects has not yet been determined:

- Allergic reaction which may cause itching, rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat
- Feeling short of breath or wheezing without allergic reaction. This is more common in people who have a history of asthma.
- Diarrhea
- Nasal congestion
- Changes in taste
- Heart failure which may cause shortness of breath, swelling of ankles, and tiredness
- Swelling in the hands or feet
- Kidney failure
- Abnormal blood test related to how well your blood clots (prevents bleeding)
- Increased risk of bleeding, particularly in patients who are on blood thinners or medications to prevent blood clotting
- Bleeding in the brain that can be life threatening
- Potential for harm to fetus and to infants who are nursing
- Potential for higher blood levels in patients with liver abnormalities.

You should not get pregnant, breastfeed, or father a baby while in this study. If any of these occur, you should report it to the study staff as soon as possible. Iloprost could be very damaging to an unborn baby. Check with the study doctor about what types of birth control, or pregnancy prevention, to use while in this study. If you become pregnant, the particular treatment or procedures involved in the study may involve risks to the embryo or fetus which are currently unclear.

Risks associated with information gained from genetic/ genomic testing: Any genetic or genomic testing that will be done with your samples collected as part of this study are done for research purposes only. These tests will be done at an unknown time in the future and we do not plan to contact you about the results of these tests. There is a very small possibility that we can discover something medically relevant that you should be told about. In this case, the principal investigator will make every attempt to contact you and refer you to a certified genetic health professional if you desire. Any cost associated with follow-up related to this medically relevant information will be your responsibility. **It is very important to remember that any results we might find do not substitute for clinical care at any point in time.** You may also experience anxiety or stress if we find anything medically relevant from genetic or genomic testing that you should be told about and that this information could be shared with others. Any data related to genetic or genomic testing is coded, used for research only, and will not be shared with family members, health care providers, or insurance companies.

The study may include risks that are unknown or unforeseeable or unexpected at this time.

Risks of the usual care you receive (procedures done as standard of care) are not risks of the research and are not included in this consent form. You should talk with your health care providers if you have any questions about the risks of usual care.

What are the possible benefits of the study?

This study is designed to learn more about Lung Cancer. This study is not designed to treat any illness or to improve your health.

Who is paying for this study?

This research is being paid for by funding through the National Cancer Institute (NCI).

Will I receive money for participation on the study?

You will be paid up to $250.00 at your completion of the trial, at your Visit 4. This money will help to cover any costs associated with participating on this study, such as travel, food, and time away from work. If you withdraw from the study before Visit 4, the payment will be less, based on the number of study visits that you completed.

The following payments will be paid for each visit:
Screening Visit 1 (includes bronchoscopy): $100
Visit 1: $25
Visit 2: $25
Visit 3 (includes bronchoscopy): $100
Total for four visits: $250

It is important to know that payment for participation in a study is taxable income.

Will I have to pay for anything?

There will be no cost to you for participation in this study. However, some veterans are required to pay co-payments for medical care and services provided by the VA. These co-payment requirements will continue to apply to medical care and services provided by the VA that are not part of this study but are standard of care (usual care you receive at the VA). If you decide to participate in this study, you cannot be charged nor your insurance billed, for research-related interventions or procedures that are required by the study protocol.

Do I have to take part in this study?

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled.

If you do not take part or leave this study, you will still receive your normal medical care. If you leave this study, the only medical care that you will lose is the medical care you are getting as part of this study. You might be able to get the same kind of medical care outside of the study. Ask your study doctor about your options. If you want to withdraw from the study, you should contact the investigator to discuss what follow-up care could be most helpful to you.

If there are any new findings during the study that may affect whether you want to continue to take part in it, you will be told about them.

Can I be taken out of the study?

The study doctor may decide to stop your participation without your permission, if he or she thinks that being in the study may cause you harm, or for any other reason. Also, the sponsor may stop the study at any time.

What happens if I am injured or hurt during the study?

Every reasonable safety measure will be used to protect your well-being. The Eastern Colorado Health Care System will provide necessary medical care and treatment for any injury that is a result of participation in this study for veterans and **non-veterans**. Compensation for such an injury may be permitted by applicable federal laws and/or regulations. The VA is not required to provide treatment for injuries in research studies if the injuries are caused by your non-compliance with study procedures.

You should inform your care provider(s) if you decide to participate in this research study. If you have questions about an injury related to the research, call Dr. York Miller at 303-393-2869.

Who do I call if I have questions?

The researcher carrying out this study at the VA is Dr. York Miller. You may ask any questions you have now. If you have any questions later you may call Dr. York Miller at 303-393-2869. You will be given a copy of this form to keep.

If you have questions regarding your rights as a research subject, concerns or complaints about this research study, please call the Colorado Multiple Institutional Review Board (COMIRB) office at 303-724-1055. This is the Board that is responsible for overseeing the safety of human participants in this study. If you want to verify that this study is approved, please contact the VA Research Office at 303.399.8020, ext. 2755. Information can also be found at http://www.clinicaltrials.gov.

Who will see my research information?

Taking part in this study will involve collecting private information about you. We will keep all research records that contain your identifiable health information confidential to the extent allowed by law. Paper records about you will be kept in locked filing cabinets. We will also keep an electronic copy of your study records on a secure server accessible only from a password protected computer database.

We will try to keep your medical records confidential, but it cannot be guaranteed. Records that identify you (including your medical records and the consent form signed by you), may be looked at or portions of your records copied that identify you by others. These include:

- Federal agencies such as the Food and Drug Administration (FDA), the General Accountability Office (GAO), the Office of the Inspector General, Office for Human Research Protections (OHRP), and the Office of Research Oversight (ORO) that protect research subjects like you, may also copy portions of records about you.

- People at the Colorado Multiple Institution Review Board
- The investigator and research team for this study
- The sponsor (National Cancer Institute), study monitors or agents for the sponsor
- Officials at the institution where the research is being conducted, and officials at other institutions involved in this study who are in charge of making sure that we follow all of the rules for research
- Our local VA Research and Development Committee
- Colorado Statue tumor Registry and Vital Statistics (for the purpose of learning whether or not you develop lung cancer).
- UCDenver and its Integrated Clinical Trials Management System
- Northwestern University
- The National Cancer Institute will obtain information from this clinical trial under data collection authority Title 42 U.S.C. 285.
- VisionGate, Inc. (a biotechnology company developing a test to distinguish cancer cells from non cancer cells using sputum samples)

Information about you will be combined with information from other people taking part in the study. We might talk about this research study at meetings. We might also print the results of this research study in relevant journals. But we will always keep the names of the research subjects, like you, private.

Where can I get more information?

You may visit the NCI website at http://cancer.gov/ for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at: 1-800-4-CANCER (1-800-422-6237).

We will ask you to sign a different form that talks about who can see your research records. That form is called a HIPAA Authorization form. It will mention companies and universities who will see your research records.

You have the right to request access to your personal health information from the Investigator. To ensure proper evaluation of test results, your access to these study results may not be allowed until after the study has been completed.

The HIPAA Authorization form that you will also be asked to sign will state when or if it expires. However, you may withdraw this authorization for use and disclosure of your personal health information by providing written request to the Investigator. If you withdraw the HIPAA Authorization form, the Institution, the Investigator, the research staff, and the research Sponsor will no longer be able to use or disclose your personal health information from this study, except so far as that they have already relied on this information to conduct the study.

Is there other information I need to know?

- Your samples collected under this protocol may be used for genetic or genomic studies and data from these studies will be deposited in the database of Genotypes and Phentoypes (dbGaP) as a requirement of the National Cancer Institute. Any information in the database will be coded and will not contain any identifiable information about you, such as your name, phone, or address, but we still need your permission to include your data in the database of Genotypes and Phentoypes.

☐ Yes, I give my permission for study investigators to deposit de-identified data from genetic and genomic studies using my research samples in the database of Genotypes and Phentoypes . ____Initials

☐ No, you may not deposit de-identified data from genetic and genomic studies using my research samples in the database of Genotypes and Phentoypes . __Initials

- ***Re-contact.*** We may wish to re-contact you in the future for additional studies you may be eligible to participate in. These studies may be within the VA or outside the VA. I give my permission for my study doctor (or someone he or she chooses) to contact me in the future to ask me to take part in more research.

☐ Yes, I am interested in being contacted to participate in future studies. ____Initials

☐ No, I am not interested in being contacted to participate in future studies. __Initials

Agreement to be in this study

I have read this form or it has been read to me. A member of the research team has explained the study to me and answered my questions. I have been told about the risks or discomforts and possible benefits of the study. I have been told of other choices of treatment available to me.

I have been told that I do not have to take part in this study. My refusal to participate will involve no penalty or loss of rights to which I am entitled. I may withdraw from this study at any time without penalty or loss of VA or other benefits to which I am entitled.

The results of this study may be published, but my records will not be revealed unless required by law.

In case there are medical problems or questions, I have been told I can call Dr. York Miller at 303-393-2869 during the day and at 303-393-2869 after hours. If any medical problems occur in connection with this study, the VA will provide the necessary medical care.

I choose to participate in this study. A copy of this consent form will be placed in my medical record. If I am not a veteran, a health record will be created for me in the VA computerized patient record system (CPRS) to include my research records.

Subject's Signature: _____ Date:_____

Print name: _____

Consent form explained by: _____ Date: _____

Print name: _____

VA Additional Consent for Blood and Tissue for Research

The study investigators would like to keep some of the sputum, blood, urine, and tissue that is taken during the study but is not used for other tests. If you agree, the sputum, blood, urine, and tissue samples will be kept and may be used in future research to learn more about lung cancer. The research that is done with your sputum, blood, urine, and tissue samples is not designed to specifically help you. It might help people who have lung cancer and other diseases in the future. Reports about research done with your samples will not be given to you or your doctor. These reports will not be put in your health records. The research using your sputum, blood,

urine, and tissue samples will not affect your care.

The choice to let the study investigators keep the sputum, blood, urine, and tissue samples for future research is up to you. The specimens will be kept at the SPORE (Specialized Program of Research Excellence) Tissue Bank at the University of Colorado, Denver and accessed by study investigators. Your samples will be labeled with a code that doesn't identify you directly (or list the specific minimal identifiers needed). No matter what you decide to do, it will not affect the care that you will receive as part of the study. If you decide now that your sputum, blood, urine, and tissue samples can be kept for research, you can change your mind at any time and contact your study doctor to let him or her know that you do not want Dr. York Miller to use your sputum, blood, urine, and tissue samples any longer, and they will no longer be used for research. Otherwise, they may be kept until they are used up, or until Dr. York Miller decides to destroy them.

In the future, people who do research with your sputum, blood, urine, and tissue samples may need to know more about your health. While the study investigators may give those reports about your health, they will not give them your name, address, phone number or any other information that will let the research know who you are.

Sometimes sputum, blood, urine, and tissue samples are used for genetic research (about diseases that are passed on in families). Even if your sputum, blood, urine, and tissue samples are used for this kind of research, the results will not be told to you and will not be put in your health records. Your sputum, blood, urine, and tissue samples will only be used for research and will not be sold. The research done with your samples may help to develop new products in the future, but there is no plan for you to be paid.

A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you, based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information obtained from this research when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

The possible benefits of research from your sputum, blood, urine, and tissue include learning more about what causes lung cancer and other diseases, how to prevent them and how to treat them. The greatest risk to you is the release of information from your health records. Dr. York Miller will protect your records so that your name, address and phone number will be kept private. The chance that this information will be given to someone else is very small. There will be no cost to you for any sputum, blood, urine, and tissue collected and stored by Dr. York Miller.

Please read each sentence below and think about your choice. After reading each sentence, circle "yes" or "no." If you have questions, please talk to your doctor or nurse. Remember, no matter what you decide to do about the storage and future use of your sputum, blood, urine, and tissue samples, you may still take part in the study.

I give my permission for my sputum, blood, urine, and tissue to be stored in a central tissue bank at the SPORE Tissue Bank for future use by the study investigators:

1. I give my permissions for my sputum, blood, urine, and tissue samples to be kept by Dr. York Miller for use in future research to learn more about how to prevent, detect, or treat Lung Cancer.

☐ Yes ☐ No _____Initials

2. I give my permissions for my sputum, blood, urine, and tissue samples to be used for research about

other health problems (for example: causes of heart disease, osteoporosis, and diabetes).

☐ Yes ☐ No _____Initials

I agree to take part in the study having to do with research on sputum, blood, urine, and tissue as indicated above.

Signature_____ Date_____
Print Name:_____

Consent form explained by:_____ Date:_____
Print Name: _____

Witness Signature:_____Date_____
Print Name: _____

Witness of Signature ☐
Witness of consent process ☐

APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	**Descriptions**	**Percent**	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B

Patient Diary

Participant Name_______________
Participant ID__________________
Dose_________________________
Frequency_____________________

DAY ___ DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________
DAY ___ DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________	**DAY ___** DATE: ___/____/____ TIME: ____________ TIME: ____________ TIME: ____________ TIME: ____________ Please list any new symptoms or reasons for missed doses: ________________ ________________ ________________ ________________ ________________ ________________ ________________ ________________

Participant Signature: ____ Date: _____________

APPENDIX C

Six Minute Walk Test Recording Sheet

SIX MINUTE WALK TEST 1 OF 2

INSTITUTION	PARTICIPANT ID	VISIT DATE
_____	_____	__ __ / __ __ / __ __ __ __

Predicted Maximum Heart Rate (220-age):	_____
Bronchodilator: Time since last dose (HH:MM):	_____
Supplemental Oxygen Used?	_____
Gait Aid Used?	_____
Rest: SpO2 (%)	_____
Rest: Heart Rate (bpm)	_____
Rest: Dyspnea	_____

1 Minute: SpO2 (%)	_____
1 Minute: Heart Rate (bpm)	_____
1 Minute: Dyspnea	_____
1 Minute: Rests	_____
2 Minutes: SpO2 (%)	_____
2 Minutes: Heart Rate (bpm)	_____
2 Minutes: Dyspnea	_____
2 Minutes: Rests	_____
3 Minutes: SpO2 (%)	_____
3 Minutes: Heart Rate (bpm)	_____
3 Minutes: Dyspnea	_____
3 Minutes: Rests	_____
4 Minutes: SpO2 (%)	_____
4 Minutes: Heart Rate (bpm)	_____
4 Minutes: Dyspnea	_____
4 Minutes: Rests	_____
5 Minutes: SpO2 (%)	_____
5 Minutes: Heart Rate (bpm)	_____
5 Minutes: Dyspnea	_____
5 Minutes: Rests	_____
6 Minutes: SpO2 (%)	_____
6 Minutes: Heart Rate (bpm)	_____
6 Minutes: Dyspnea	_____
6 Minutes: Rests	_____

SIX MINUTE WALK TEST 2 OF 2

INSTITUTION	PARTICIPANT ID	VISIT DATE
_____	_____	__ __ / __ __ / __ __ __ __

Comments:

Recovery 1 Minute: SpO2 (%)	_____
Recovery 1 Minute: Heart Rate (bpm)	_____
Recovery 1 Minute: Dyspnea	_____
Distance (miles):	_____
Limiting factor to the test: SOB	_____
Limiting factor to the test: Low SpO2?	_____
Limiting factor to the test: Leg fatigue?	_____
Limiting factor to the test: Other	_____
Other: Specify	_____

_____

_____

_____

_____

Signature: _____ Date: _____

APPENDIX D

Your name:

Today's date:

How is your COPD? Take the COPD Assessment Test™ (CAT)

This questionnaire will help you and your healthcare professional measure the impact COPD (Chronic Obstructive Pulmonary Disease) is having on your wellbeing and daily life. Your answers, and test score, can be used by you and your healthcare professional to help improve the management of your COPD and get the greatest benefit from treatment.

For each item below, place a mark (X) in the box that best describes you currently. Be sure to only select one response for each question.

Example I am very happy 0 X 2 3 4 5 I am very sad

			SCORE
I never cough	0 1 2 3 4 5	I cough all the time	
I have no phlegm (mucus) in my chest at all	0 1 2 3 4 5	My chest is completely full of phlegm (mucus)	
My chest does not feel tight at all	0 1 2 3 4 5	My chest feels very tight	
When I walk up a hill or one flight of stairs I am not breathless	0 1 2 3 4 5	When I walk up a hill or one flight of stairs I am very breathless	
I am not limited doing any activities at home	0 1 2 3 4 5	I am very limited doing activities at home	
I am confident leaving my home despite my lung condition	0 1 2 3 4 5	I am not at all confident leaving my home because of my lung condition	
I sleep soundly	0 1 2 3 4 5	I don't sleep soundly because of my lung condition	
I have lots of energy	0 1 2 3 4 5	I have no energy at all	
		TOTAL SCORE	

Last Updated: February 24, 2012

Signature: _____ Date: _____

APPENDIX E

THE ST. GEORGE'S HOSPITAL RESPIRATORY QUESTIONNAIRE (SGRQ)

This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you most problems, rather than what the doctors and nurses think your problems are.

Please read the instructions carefully and ask questions if you do not understand anything. Do not spend too long deciding on your answers.

Before completing the rest of the questionnaire:

Please checkmark one box to show how you describe your present health:

Very good	Good	Fair	Poor	Very poor
☐	☐	☐	☐	☐

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St. George's Respiratory Questionnaire
PART 1

Questions about how much chest problem you have had over the past 4 weeks.

Please checkmark (✓) *one box for each questio*n:

		Most days a week	Several days a week	A few days a month	Only with chest infections	Not at all
1.	Over the past 4 weeks, I have coughed:	☐	☐	☐	☐	☐
2.	Over the past 4 weeks, I have brought up phlegm (sputum):	☐	☐	☐	☐	☐
3.	Over the past 4 weeks, I have had shortness of breath:	☐	☐	☐	☐	☐
4.	Over the past 4 weeks, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5. During the past 4 weeks, how many severe or very unpleasant attacks of chest problem have you had?

Please checkmark (✓) *one box only*:

- more than 3 attacks ☐
- 3 attacks ☐
- 2 attacks ☐
- 1 attack ☐
- no attacks ☐

6. How long did the worst attack of chest problem last:
(Go to question 7 if you had no severe attacks)

Please checkmark (✓) *one box only*:

- a week or more ☐
- 3 days or more ☐
- 1 or 2 days ☐
- Less than a day ☐

7. Over the past 4 weeks, in an average week, how many good days (with little chest problem) have you had:

Please checkmark (✓) *one box only*:

- No good days ☐
- 1 or 2 good days ☐
- 3 or 4 good days ☐
- Nearly every day was good ☐
- Every day was good ☐

8. If you have a wheeze, is it worse in the morning:

Please checkmark (✓) *one box only*:

- No ☐
- Yes ☐

St. George's Respiratory Questionnaire
PART 2

Section 1

How would you describe your chest condition?

Please checkmark (✓) *one box only*:

- The most important problem I have ☐
- Causes me quite a lot of problems ☐
- Causes me a few problems ☐
- Causes me no problem ☐

If you have ever had paid employment.

Please checkmark (✓) *one box only*:

- My chest problem made me stop work altogether ☐
- My chest problem interferes with my work or made me change my work ☐
- My chest problem does not affect my work ☐

Section 2

Questions about what activities usually make you feel breathless <u>these days</u>.

	For ***each item***, please checkmark (✓) the box as it applies to you ***these days***:	
	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around at home	☐	☐
Walking outside on the level	☐	☐
Climbing up a flight of stairs	☐	☐
Climbing hills	☐	☐
Playing sports or games	☐	☐

St. George's Respiratory Questionnaire
PART 2

Section 3

Some more questions about your cough and breathlessness <u>these days</u>.

	For ***each item***, please checkmark (✓) the box as it applies to you ***these days***: True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I talk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4

Questions about other effects that your chest problem may have on you <u>these days</u>.

	For ***each item***, please checkmark (✓) the box as it applies to you ***these days***: True	False
My cough or breathing is embarrassing in public	☐	☐
My chest problem is a nuisance to my family, friends or neighbours	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐
I do not expect my chest to get any better	☐	☐
I have become frail or an invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about your medication. If you are taking no medication go straight to Section 6.

	For ***each item***, please checkmark (✓) the box as it applies to you ***these days***: True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

St. George's Respiratory Questionnaire
PART 2

Section 6

These are questions about how your activities might be affected by your breathing.

For ***each item***, please checkmark (✓) the box as it applies to you ***because of your breathing***:

	True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐
My breathing makes it difficult to do things such as climbing up hills, carrying things up stairs, light gardening such as weeding, dancing, playing bowls or golf	☐	☐
My breathing makes it difficult to do things such as carrying heavy loads, digging the garden or shovelling snow, jogging or walking at 8 kilometres per hour, playing tennis or swimming	☐	☐
My breathing makes it difficult to do things such as very heavy manual work, running, cycling, swimming fast or playing competitive sports	☐	☐

Section 7

We would like to know how your chest problem <u>usually</u> affects your daily life.

For ***each item***, please checkmark (✓) the box as it applies to you ***because of your chest problem***:

	True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the groceries	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

St. George's Respiratory Questionnaire

Here is a list of other activities that your chest problem may prevent you doing (you do not have to checkmark these, they are just to remind you of ways in which your breathlessness may affect you):

Going for walks or walking the dog

Doing things at home or in the garden

Sexual intercourse

Going out to church or place of entertainment

Going out in bad weather or into smoky rooms

Visiting family or friends or playing with children

Please write in any other important activities that your chest problem may stop you doing:

. . . . . .

. . . . . .

. . . . . .

. . . . . .

Now, would you checkmark the box (one only) which you think best describes how your chest affects you:

It does not stop me doing anything I would like to do ☐

It stops me doing one or two things I would like to do ☐

It stops me doing most of the things I would like to do ☐

It stops me doing everything I would like to do ☐

Thank you for filling in this questionnaire. Before you finish, would you check to see that you have answered all the questions.

Signature: _____Date: _____

E-6

APPENDIX F

BODE Index for COPD

The BODE index is a composite marker of disease taking into consideration the systemic nature of COPD (Celli et al., 2004).

Scoring the BODE index

	0	1	2	3
FEV_1% pred	≥65	50-64	36-49	≤35
6MWD (m)	≥350	250-349	150-249	≤149
MMRC	0-1	2	3	4
BMI ($kg.m^{-2}$)	>21	≤21		

Total BODE index score = 0 to 10 units

(FEV1% pred = predicted amount as a percentage of the forced expiratory lung volume in one second; 6MWD = six minute walking distance; MMRC = modified medical research council dyspnea scale; BMI = body mass index)

Modified MRC Dyspnoea Scale	
0	Breathless only with strenuous exercise
1	Short of breath when hurrying on the level or walking up a slight hill
2	Slower than most people of the same age on the level because of breathlessness or have to stop for breath when walking at my own pace on the level
3	Stop for breath after walking about 100 meters or after a few minutes at my own pace on the level
4	Too breathless to leave the house or I am breathless when dressing

APPENDIX G

LIFE BRONCHOSCOPY PATIENT COMMUNICATION LOG
Source Document

Patient Name: _____ **Patient Phone #:** _____

Pre-Case Communication:
Date of Consent: _____
(The patient verbalized understanding of the consent and a copy of the signed consent was provided to the patient.)

_____
_____
_____
_____
_____
_____

Signature: _____ Date: _____

Procedure Date:

Date: _____ Time: _____
Physician: _____ Notified: _____

24-Hour Follow-up:

Date: _____

Comments:
_____
_____
_____

Signature: _____ Date: _____

72-Hour Follow-up:

Date: _____

Comments:

_____

_____

_____

The patient was instructed to call within 30 days if febrile or with increased sputum production, increasing shortness of breath, or any perceived deterioration of respiratory condition:
Yes: _____ No: _____

Signature: _____ Date: _____

G-2