# A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE II STUDY TO EVALUATE THE EFFICACY AND SAFETY OF SPX-101 INHALATION SOLUTION IN SUBJECTS WITH CYSTIC FIBROSIS (HOPE-1 STUDY: HYDRATION FOR OPTIMAL PULMONARY EFFECTIVENESS)

**Test Drug:** SPX-101 Inhalation Solution

**Protocol Number:** SPX-101-CF-201 **EudraCT Number:** 2016-005230-30
**Study Phase:** II
**Global Amendment 3**: **14 May 2018; Version 4.0**

Original Global Protocol: 09 March 2017; Version 1.0
Global Amendment 1: 19 April 2017; Version 2.0
Global Amendment 2: 14 November 2017; Version 3.0
Country-Specific Amendments: 14 June 2017; Version 2.0A (UK)
20 July 2017; Version 2.0B (Portugal)
14 November 2017; Version 3.0A (UK)

**Sponsor:**
Spyryx Biosciences, Inc.
801-10 Capitola Drive
Durham, NC 27713
(919) 899-9399

**Spyryx Chief Medical Officer:**
Alistair Wheeler, MD, MFPM

**Contract Research Organization:**
PRA Health Sciences
4130 ParkLake Avenue, Suite 400
Raleigh, NC
Phone: +1 (919) 786-8200
Fax: +1 (919) 786-8201

**PRA Medical Monitors:**
Kirill Rudinskiy, MD, PhD
Navreet Sindhwani, MD

**Safety Contacts (SAE reporting):**
Europe: MHGSafety@prahs.com
Fax: +44 1792 525 720
North America: CHOSafety@prahs.com
Fax: 888-772-6919 or 434-951-3482

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) as set forth in the International Council for Harmonisation (ICH) guidelines on GCP (ICH E6), and applicable local regulatory requirements.

**CONFIDENTIAL**

SPYRYX Biosciences

## 1. SIGNATURES

**Representatives of Sponsor and Clinical Research Organization**

I have read and agree to the protocol SPX-101-CF-201, entitled "A Randomized, Double-Blind, Placebo-Controlled Phase II Study to Evaluate the Efficacy and Safety of SPX-101 Inhalation Solution in Subjects with Cystic Fibrosis (HOPE-1 Study: Hydration for Optimal Pulmonary Effectiveness)." I am aware of my responsibilities under the guidelines of GCP, local regulations (as applicable) and the study protocol. I agree to conduct the study according to these responsibilities.

**Version 4.0 14 May 2018**

**Accepted for the Sponsor – Spyryx Biosciences, Inc.**

| | |
|---|---|
| Alistair Wheeler, MD, MFPM | Chief Medical Officer |
| Print Name | Title |
| [signature] | 17 MAY 2018 |
| Signature | Date |

**Accepted for the Contract Research Organization - PRA Health Sciences:**

| | |
|---|---|
| Kirill Rudinskiy | Medical Director |
| Print Name | Title |
| [signature] | 18 May 2018 |
| Signature | Date |

**Investigator**

I have read and agree to the protocol SPX-101-CF-201, entitled "A Randomized, Double-Blind, Placebo-Controlled Phase II Study to Evaluate the Efficacy and Safety of SPX-101 Inhalation Solution in Subjects with Cystic Fibrosis (HOPE-1 Study: Hydration for Optimal Pulmonary Effectiveness)." I have also read the Investigator's Brochure. I am aware of my responsibilities as an Investigator under the guidelines of GCP, local regulations (as applicable) and the study protocol. I agree to conduct the study according to these responsibilities and to appropriately direct and assist the staff under my control, who will be involved in the study.

**Version 4.0 14 May 2018**
**Clinical Site:** _____

**Site Number:** _____
**Site Principal Investigator:**

| | |
|---|---|
| Print Name | Title |
| Signature | Date |

## 2. SYNOPSIS

| | |
|---|---|
| **NAME OF SPONSOR:** Spyryx Biosciences, Inc. | **PROTOCOL No.:** SPX-101-CF-201 |
| **NAME OF STUDY TREATMENT:** SPX-101 Inhalation Solution | |
| **TITLE OF STUDY:** A Randomized, Double-Blind, Placebo-Controlled Phase II Study to Evaluate the Efficacy and Safety of SPX-101 Inhalation Solution in Subjects with Cystic Fibrosis (HOPE-1 Study: Hydration for Optimal Pulmonary Effectiveness) | |
| **STUDY CENTERS:** Approximately 25 centers are planned internationally | |
| **STUDY PERIOD:** After a 3- to 28-day screening period, enrolled subjects will be in the study for approximately 30 days. | **PHASE OF DEVELOPMENT:** Phase II |
| **PLANNED STUDY DATES:** The study is expected to start in Q3 2017 and conclude in 2019 | |

**OBJECTIVES:**

**Primary Objective:**

- To evaluate the efficacy of SPX-101 in subjects with cystic fibrosis (CF)

**Safety Objectives:**

- To evaluate the safety and tolerability of SPX-101 in subjects with CF

**Pharmacokinetic Objective:**

- To assess the extent of systemic exposure of SPX-101 in a subset of subjects

**STUDY DESIGN AND METHODOLOGY:**

This randomized, double-blind, placebo-controlled, Phase II study is designed to evaluate the efficacy and safety of SPX-101 inhalation solution, administered in different dose regimens, in a 28-day treatment period in approximately 90 adults with CF. Two sequential cohorts are planned to compare the study drug SPX-101 with placebo. Each cohort will have its own baseline and will be analyzed separately.

**Cohort 1**

In Cohort 1, 46 subjects were randomized in a 1:1:1 ratio to 3 groups (2 treatment groups and 1 placebo group); to achieve balance between treatment groups, randomization was stratified by baseline lung function (percent predicted forced expiratory volume in 1 second [ppFEV$_1$] 40.0% to 55.0% or 55.1% to 80.0%). Cohort 1 treatment groups were the following:

- Placebo twice daily (BID) x 28 days
- SPX-101 60 mg BID x 28 days
- SPX-101 120 mg BID x 28 days

**Cohort 2**

In Cohort 2, approximately 45 subjects will be randomized in a 2:1 ratio to either SPX-101 120 mg BID or placebo BID. Subjects will be stratified according to concomitant hypertonic saline use, so that approximately 50% will be using hypertonic saline (defined as having used a constant regimen for 28 days prior to screening and planning to continue their usual concentration without change for 28 days post-randomization). The decision to use 120 mg BID in Cohort 2 was based on the relatively greater improvements in the lung function at that dose in Cohort 1, and a need to define the variability in response within the population.

For each cohort, there will be 5 clinic visits and 2 telephone calls: Visit 1 (Screening; Day -28 to Day -3); Visit 2 (Randomization; Day 1); Visit 3 (Day 8 -1); Visit 4 (Day 15 -2/+1); telephone

call (Day 22 ± 3); Visit 5 (Day 29 ± 2; end-of-treatment); and a follow-up telephone call 2 days later (Day 31 ± 1; end-of-study). All study visits for an individual subject will be scheduled at approximately the same time of day to minimize the confounding effects of diurnal variation in lung function, background therapies, and daily chest physiotherapy regimens. Subjects will be screened at Visit 1 and then will enter a variable-length screening period of 3 to 28 days, to time the randomization so that the first dose of the investigational product will coincide, if applicable, to the first day of an inhaled antibiotic cycle. In Cohort 1, subjects who still met the randomization criteria on Day 1 (Visit 2) were randomized to 1 of the 3 treatment groups. In Cohort 2 subjects who still meet the randomization criteria on Day 1 (Visit 2) will be randomized either to the treatment or placebo group, as described.

At Visits 2, 3, and 4, after completing spirometry and other assessments, subjects will self-administer the study medication in the clinic. On Day 1 (Visit 2) only, subjects will be monitored for safety for the first 4 hours after completion of administration of the study drug. Monitoring will include spirometry, vital signs, and adverse event (AE) assessments as described in more detail in the protocol. After the 4-hour postdose assessments are complete, subjects who have tolerated the study medication will be discharged with a sufficient supply of medication to take BID on an outpatient basis until their next clinic visit.

Subjects who regularly use a short-acting β-agonist (SABA) should administer this medication at least 4 hours before spirometry on clinic days, and they should use the SABA consistently throughout the study.

**STUDY POPULATION AND MAIN CRITERIA FOR INCLUSION/EXCLUSION:**

Subjects cannot be enrolled or randomized before all inclusion criteria (including test results) are confirmed. A subject may be re-screened once if they fail to meet randomization criteria for any reason. If this is the case, a new consent form must be signed, and a new screening number will be used.

**Inclusion Criteria:**

1. Ability to provide written, personally signed, and dated informed consent to participate in the study, in accordance with the ICH GCP Guideline E6 and applicable regulations, before completing any study-related procedures
2. Ability and willingness (in the judgment of the Investigator) to attend study visits and fully comply with study procedures and restrictions; study visits will occur at approximately the same time of day throughout the study and under specified conditions with respect to daily respiratory clearance and medication regimens
3. Ability to self-administer the investigational product
4. Male or nonpregnant, nonlactating female subjects aged 18 to 50 years inclusive
5. Diagnosis of cystic fibrosis as determined by the 2008 Cystic Fibrosis Foundation Consensus Guidelines for Diagnosis [1] defined as the presence of signs and symptoms of CF in conjunction with at least 1 of the following:
    a. Documented sweat chloride test ≥ 60 mEq/L by quantitative pilocarpine iontophoresis
    b. Two well-characterized, disease-causing genetic mutations in the CF transmembrane conductance regulator (CFTR) gene; the original documentation of genetic testing is not required if the specific mutations are documented in the subject's medical record
6. $ppFEV_1$ as follows:

a. Cohort 1: between 40.0% and 80.0%
b. Cohort 2: between 50.0% and 80.0%

Note: spirometry must be performed in accordance with American Thoracic Society/European Respiratory Society end-of-test criteria and the Global Lung Initiative predicted values [2, 3]

7. Stable CF lung disease, defined as no pulmonary exacerbation or acute upper or lower respiratory illness other than CF within 28 days preceding screening, except for allergic or nonallergic rhinitis; in addition, subjects may not have had any of the following:
    a. Significant hemoptysis (at the discretion of the Investigator) within 28 days of screening
    b. Pneumothorax within 28 days of screening
    c. Diagnosis of active allergic bronchopulmonary aspergillosis (at the discretion of the Investigator)
    d. Documented colonization with *Burkholderia cenocepacia* or *Mycobacterium abscessus* within 6 months prior to screening; subjects with other infections in the *Burkholderia* or *Mycobacterium* genii are allowed at the discretion of the Investigator
8. No significant changes (removal or addition of medications) to inhaled or oral CF medication regimen within 28 days preceding screening, and no anticipation of need to change this regimen during study participation; short courses of medications used to address an ongoing medical problem are allowed, provided the last dose of such medication was taken > 28 days before screening; subjects for whom a significant change in medication is anticipated during the study will be excluded. Specific requirements for ongoing concomitant medications are below:
    a. Inhaled antibiotic use: stable antibiotic medication, defined as at least 2 complete 28-day cycles of intermittent monotherapy (if alternating month on and month off with a single inhaled antibiotic) or 2 full cycles if continuous alternating every 28 days between 2 inhaled antibiotics
    b. Dornase alfa: stable use of daily or intermittent dosing for at least 28 days before screening
    c. Inhaled bronchodilators: stable dose for at least 28 days before screening
    d. Inhaled hypertonic saline: stable dose for at least 28 days before screening
9. Other concomitant medications may be taken during the study, provided the dose and regimen have been stable for at least 14 days before screening, and the regimen is expected to remain stable during the study
10. Stable chest physiotherapy and clearance techniques for at least 28 days before screening, including chest percussion, use of vibrating vests, etc
11. All female subjects of childbearing potential must be using a highly effective method of contraception through the duration of the study as follows:
    a. Female subjects of childbearing potential must not be pregnant, breastfeeding, or attempting to become pregnant for 28 days before screening, throughout the duration of the study, and for 28 days after the last study visit. Subjects must have a negative serum pregnancy test at screening, a negative urine pregnancy test at randomization, and be willing to commit to using a consistent and

acceptable method of contraception for at least 28 days before screening and for the duration of the study, if they are (or become) sexually active. Female subjects of childbearing potential must meet at least 1 of the following:

i. Consistent use of systemic contraception, including birth control pills, transdermal patch, vaginal ring, implants, and injectables
ii. Consistent use of double barrier method, eg, condoms, cervical cap, diaphragm, vaginal contraceptive film with spermicide
iii. Use of intrauterine device with a low failure rate (defined as < 1% risk of pregnancy per year)
iv. Monogamous intercourse with a vasectomized man or has only same-sex partners
v. Female subjects who are not sexually active but become so during the study must agree to follow the contraceptive requirements above

b. Female subjects who are not of childbearing potential must meet at least 1 of the following criteria:

i. At least 1 year since the last menstrual period
ii. Surgically sterile (tubal ligation, bilateral oophorectomy, salpingectomy, or hysterectomy)
iii. Congenitally sterile
iv. Diagnosed as infertile and not undergoing treatment to reverse infertility

**Exclusion Criteria:**

1. BMI < 18 kg/$m^2$
2. Use of a CFTR corrector or potentiator during the study or within 60 days before screening
3. The presence of significant and unstable comorbidities (based on the Investigator's judgment) within 28 days before screening, including diabetes, significant renal or hepatic disease, significant respiratory disease other than CF, cardiac, neurological, or gastrointestinal disease other than those associated with CF. Subjects with stable co-morbidities associated with CF (such as diabetes) may participate per the Investigator's judgment unless specifically prohibited elsewhere.
4. Subjects with either of the following:
   a. Hepatic impairment defined by elevation of bilirubin > 2 times the upper limit of normal (ULN) or aspartate aminotransferase, alanine aminotransferase, or alkaline phosphatase > 5 times the ULN
   b. Chronic hepatitis B and/or C
5. History of malignancy in the previous 5 years; subjects who have been successfully treated for non-melanoma skin cancer, cervical cancer, or prostate cancer within the previous 5 years are allowed to enroll with the approval of the Medical Monitor
6. Administration of another investigational drug within 28 days before screening
7. Administration of a vaccination (including influenza) within 7 days before screening
8. Requirement for supplemental oxygen of more than 2 L/min, and/or noninvasive ventilation for the management of respiratory failure; subjects must have been on a stable regimen for at least 60 days before screening (patients who have been using a stable regimen of oxygen ≤ 2 L/min for at least 60 days before screening are eligible to enroll)
9. Current use of tobacco or tetrahydrocannabinol-containing products, delivered orally, by

vaporizing, or by smoking; or a greater than 10 pack-year history of tobacco use

10. Actively listed for lung transplantation
11. Any history of organ transplantation
12. Hospitalization for a respiratory event or CF-related condition within 28 days before screening or any plans for hospitalization for any reason during the study
13. Current use of > 10 mg/day of prednisone/prednisolone (use of ≤ 10 mg/day is acceptable)
14. Use of any of the following antihypertensive medications or combinations containing these medications: diuretics (eg, thiazides, loop diuretics, spironolactone, amiloride), angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or any other drugs known to affect the serum potassium concentration
15. Hypersensitivity to the study drug or any of its excipients
16. Subjects with significant electrocardiogram (ECG) abnormalities at screening or before the first dose on Day 1, including a QT interval corrected by the Fridericia correction formula (≥ 440 msec in men and ≥ 460 msec in women)

**Randomization Criteria:**

1. Continue to meet entrance criteria
2. Negative serum (Visit 1) and urine (Visit 2, pre-randomization) pregnancy test result for all female subjects of childbearing potential
3. Stable $FEV_1$ at Visit 2, defined as $FEV_1$ within ± 15% of the relative change between the Visit 1 and Visit 2 best $FEV_1$. Relative change is defined as follows:

$$\frac{FEV_1 \text{ (Screening; Visit 1)} - FEV_1 \text{ (Visit 2)}}{FEV_1 \text{ (Screening; Visit 1)}} \times 100$$

4. No new intervening illnesses since Screening (Visit 1) and the subject's CF is still stable in the judgment of the Investigator
5. No clinically significant laboratory or ECG abnormalities that pose a significant risk to the subject, per the judgment of the Investigator

Note: A subject may be re-screened once if they fail to meet randomization criteria for any reason. If this is the case, a new consent form must be signed, and a new screening number will be used.

**NUMBER OF SUBJECTS:**

In Cohort 1, 46 subjects were randomized in a 1:1:1 ratio to 1 of the 2 treatment groups or placebo. The sample size of 39 subjects was chosen to provide 70% power for the Analysis of Variance (ANOVA) to detect a difference range of 4.5% to 6% in the change from baseline in $ppFEV_1$ and $FEV_1$ between each of the 2 treatment groups versus placebo, with the alpha level of 0.15 assuming a common standard deviation of 7%. The attrition rate was expected to be minimal given the 4 weeks' treatment duration.

In Cohort 2, a total of 45 subjects will be randomized in a 2:1 ratio to either SPX-101 120 mg BID or placebo BID. The sample size of 45 subjects will provide 88% to 97% power to detect a difference range of 6% to 8% in the change from baseline in $ppFEV_1$ between the treatment group versus placebo, with the 1-sided alpha level of 0.1 assuming a common standard deviation of 7.58%.

**STUDY TREATMENT(S):**

**Test Product, Dose and Mode of Administration:** SPX-101 is a clear, sterile solution for inhalation consisting of 60 mg/mL SPX-101 drug substance in 0.9% sodium chloride adjusted to pH 7, intended for delivery as a nebulized aerosol by the investigational PARI eFlow nebulizer with PARI eTrack.

**Reference Therapy, Dose and Mode of Administration:**
The placebo for this study is sterile 0.9% saline adjusted to pH 7, free of preservatives and other excipients, suitable for inhalation via a nebulized aerosol by the investigational PARI eFlow nebulizer with PARI eTrack.

All drug product manufacturing and labeling will be managed by Spyryx Biosciences, Inc., and shipment and management of the investigational product will be the responsibility of PRA.

**DURATION OF TREATMENT:** 28 days

**STUDY EVALUATIONS:**

**Efficacy Variables:**

Primary Efficacy Variable:

- Change from baseline in ppFEV$_1$ at Week 4

Secondary Efficacy Variables:

- Change from baseline in ppFEV$_1$ at other study weeks
- Change from baseline in FEV$_1$
- Change from baseline in forced vital capacity (FVC) and percent predicted FVC (ppFVC)
- Change from baseline in Cystic Fibrosis Questionnaire-Revised (CFQ-R)

**Safety Variables:**

- Incidence of treatment-emergent AEs (TEAEs)
- Changes in vital signs
- Changes in clinical laboratory tests, including urine and blood electrolytes
- Changes in electrocardiograms

**Pharmacokinetic Variable (for Cohort 1 Only):**

- Systemic exposure to SPX-101 (subset of subjects)

**Exploratory Variables**

- Incidence of pulmonary exacerbation, as defined by Fuchs [4] (See Appendix 3, Section 18.3 for details)

**STATISTICAL METHODS:**

**Efficacy Analysis**

The primary endpoint is the change from baseline in ppFEV$_1$ at Week 4 (Day 29).

The change from baseline in ppFEV$_1$ at Week 4 will be summarized and compared between each treatment group and placebo. The primary analysis will be based on an analysis of covariance (ANCOVA), incorporating the categorical stratification variable of baseline lung function in Cohort 1, and the stratification variable of concomitant hypertonic saline use in Cohort 2.

Similar analysis will be performed for the change from baseline in ppFEV$_1$ (Weeks 1 and 2), FEV$_1$, ppFVC, and FVC at each visit (Week 1, 2, and 4).

The change from baseline in ppFEV$_1$ at each visit (week 1, 2, and 4) will also be considered based on a mixed effect model for repeated measure (MMRM).

The change from baseline in CFQ-R respiratory domain score and physical domain score at Week 4 will be summarized and compared between each treatment group and placebo.

The number and percentage of subjects with pulmonary exacerbation will be summarized.

The effect of regular use of concomitant hypertonic saline will be explored across the primary and secondary endpoints.

**Safety Analysis**

Safety evaluations will include TEAEs, vital signs, clinical laboratory tests, ECGs, and physical examinations.

Descriptive statistics will be provided. Continuous variables will be summarized using the number of observations, mean, standard deviation, median, the first quartile (Q1), the third quartile (Q3), minimum, and maximum. Categorical variables will be summarized using frequency and percentages. The changes from baseline for the continuous variables will be similarly summarized.

**Pharmacokinetic Analysis (for Cohort 1 only)**

The pharmacokinetic results will be summarized to assess systemic exposure to SPX-101.

**Data Safety Monitoring Board**

An independent DSMB will periodically review the accrued safety data in an unblinded manner to monitor the safety of subjects. Three DSMB meetings were scheduled at the following time points: midpoint of Cohorts 1 and 2 (after approximately 19 subjects have been randomized) and at the completion of Cohort 1. Additional meetings may be scheduled at the discretion of the DSMB members. The DSMB will be empowered to make any recommendations relevant to safety, but specifically will make recommendations to revise study procedures to increase subject safety or to stop the study based on periodic data reviews, and make recommendations on the dosage selection in Cohort 2.

The DSMB charter, which defines the membership and organization of the DSMB, was finalized before the first subject in Cohort 1 was randomized to study drug. In addition, the DSMB charter defines the safety information that will be reviewed, the reporting obligations and responsibilities of the DSMB, and specifies what information, if any, may be shared with the Sponsor or investigators.

**DATE AND VERSION:** 14 May 2018 (Version 4.0)

# 3. TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF FIGURES

# 4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

| Term | Definition |
|---|---|
| ADA | Antidrug antibody |
| AE | Adverse event |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| ANCOVA | Analysis of Covariance |
| ANOVA | Analysis of Variance |
| AST | Aspartate aminotransferase |
| BID | Twice daily |
| BMI | Body mass index |
| CF | Cystic fibrosis |
| CFR | Code of Federal Regulations |
| CFTR | Cystic fibrosis transmembrane conductance regulator |
| CFQ-R | Cystic Fibrosis Questionnaire-Revised |
| CRO | Contract research organization |
| DSMB | Data Safety Monitoring Board |
| ECG | Electrocardiogram |
| eCRF | Electronic case report form |
| EDC | Electronic data capture |
| ENaC | Epithelial sodium channel |
| ET | Early termination |
| FDA | Food and Drug Administration |
| $FEV_1$ | Forced expiratory volume in one second |
| FVC | Forced Vital Capacity |
| GCP | Good Clinical Practice |
| GLP | Good Laboratory Practice |
| HBE | Human bronchial epithelial |
| IB | Investigator's Brochure |
| ICF | Informed consent form |
| ICH | International Council for Harmonisation |
| IEC | Independent Ethics Committee |
| IP | Investigational product |
| IRB | Institutional Review Board |
| ITT | Intent-to-treat |
| IXRS | Interactive voice/web response system |

| Term | Definition |
|---|---|
| LABA | Long-acting β-agonist |
| MAD | Multiple ascending dose |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MMRM | Mixed Effect Model for Repeated Measures |
| PK | Pharmacokinetic(s) |
| PP | Per protocol |
| $ppFEV_1$ | Percent predicted forced expiratory volume in 1 second |
| ppFVC | Percent predicted forced vital capacity |
| QD | Once daily |
| QTcF | QT Interval corrected by the Fridericia correction formula |
| SABA | Short-acting β-agonist |
| SAD | Single ascending dose |
| SAE | Serious adverse event |
| SAP | Statistical Analysis Plan |
| SPLUNC1 | Short palate, lung, and nasal epithelial clone 1 |
| TEAE | Treatment-emergent adverse event |
| TC | Telephone call |
| TMV | Tracheal mucus velocity |
| ULN | Upper limit of normal |
| US/USA | United States/United Sates of America |
| WHO | World Health Organization |
| WMA | World Medical Association |

# 5. ETHICS

## 5.1 Ethics Committee

This study will be conducted in compliance with independent ethics committee (IEC) and International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines, in accordance with applicable regulations regarding clinical safety data management (E2A, E2B(R3)), European Community directives 2001/20, 2001/83, 2003/94 and 2005/28 as enacted into local law, and with ICH guidelines regarding scientific integrity (E4, E8, E9 and E10). In addition, this study will adhere to all local regulatory requirements, and requirements for data protection.

Before initiating a trial/study, the Investigator/institution must have written and dated approval/favorable opinion from the IEC for the study protocol/amendment(s), written informed consent form, any consent form updates, subject recruitment procedures (eg, advertisements), and any written information to be provided to subjects and a statement from the IEC that they comply with GCP requirements. The IEC approval must identify the protocol version as well as the documents reviewed.

## 5.2 Ethical Conduct of the Study

This study will be conducted in accordance with the Note for Guidance on GCP (ICH Harmonized Tripartite Guideline E6 (R1); Food and Drug Administration (FDA) Code of Federal Regulations (CFR) (21 CFR § 50, 56, 312)), Declaration of Helsinki (Seoul 2008) (Appendix 1, Section 18.1) and all applicable regulatory requirements.

## 5.3 Subject Information and Consent

The Investigator will explain the benefits and risks of participation in the study to each subject, the subject's legally acceptable representative or impartial witness and obtain written informed consent. Written informed consent must be obtained prior to the subject entering the study and before initiation of any study related procedure (including administration of study drug).

The Sponsor will provide a sample informed consent form, based on the elements of informed consent in Appendix 2, Section 18.2. The final, version dated, form must be agreed to by the Sponsor and the IRB/IEC and will contain all elements in the sample form, in language readily understood by the subject. Each subject's original consent form, personally signed and dated by the subject or by the subject's legally acceptable representative, and by the person who conducted the informed consent discussion, will be retained by the Investigator. The Investigator will supply all enrolled subjects with a copy of their signed informed consent.

The consent form may need to be revised during the study should important new information become available that may be relevant to the safety of the subject. In this instance approval should always be given by the IRB/IEC and existing subjects informed of the changes and reconsented. This is documented in the same way as previously described.

The Investigator will, with the consent of the subject, inform the subject's primary physician about participation in the clinical study.

# 6. STUDY ADMINISTRATIVE STRUCTURE AND VENDORS

**Clinical Laboratories:**
Central Laboratory

Eurofins Central Laboratory (North American sites)
US:
2430 New Holland Pike
Lancaster, PA 17601, USA

Eurofins Central Laboratory, B.V. (European sites)
Bergschot 71
4817 PA
Breda
The Netherlands

Specialty Laboratory for Antidrug Antibody and PK Analyses

QPS, LLC
3 Innovation Way, Suite 240
Newark, DE 19711, USA

**Contract Research Organization**
PRA Health Sciences
4130 ParkLake Avenue, Suite 400
Raleigh, NC, USA 27612
Tel: +1 (919) 786-8200
Fax: +1 (919) 786-8201

**PRA Study Medical Monitor/ Medical Expert**
Kirill Rudinskiy, MD, PhD (Europe)
Navreet Sindhwani, MD (Canada)

**Central Spirometry Testing**
Biomedical Systems (BMS)
77 Progress Parkway
St. Louis, MO 63043

**Interactive Voice/Web Response System**
Bioclinica
800 Adams Ave.
Audubon, PA 19403, USA

# 7. INTRODUCTION

## 7.1 Disease Review

Cystic fibrosis is the most common life-threatening disease among Caucasians, affecting 1 in every 2,000 to 3,500 newborn infants worldwide, depending on the region, and is severely underdiagnosed in Asia. Current therapies for CF focus on antibiotic treatment of airway infections or through targeted reduction of airway inflammation with correctors and potentiators of the mutated chloride and bicarbonate secretory channel, cystic fibrosis transmembrane conductance regulator (CFTR). Despite these advances as well as supportive therapies, lung function decline with respiratory failure continues to be a major cause of death in this patient population.

Cystic fibrosis patients' lack of functional CFTR leads to chronic lung disease. The loss of CFTR function also triggers abnormal upregulation of ENaC, which leads to $Na^+$ and fluid hyperabsorption that is directly associated with dehydrated airway surface liquid/mucus [5;6;7]. The dehydration of airway surface liquid causes resident mucus to adhere to airway surfaces, preventing its clearance, and allowing concentrated mucus plaques to accumulate until the airways become occluded and colonized by bacteria [8;9;10]. In CF, there is also an abundance of neutrophil elastase, which has been shown to hyperactivate ENaC and further contribute to mucus dehydration [11].

Although ENaC inhibition has been proven to help rehydrate CF mucus in preclinical models by retarding the hyperabsorption noted above, clinical trials of small molecule ENaC inhibitors for the treatment of CF have largely failed because these compounds are systemically absorbed and induce hyperkalemia from inhibition of ENaC in the kidney. For instance, amiloride is an ENaC inhibitor that is approved for human use as a diuretic. However, amiloride and its analogues are rapidly cleared from the lungs (half-life = 9 minutes) and failed to have any impact on CF lung disease at doses that did not adversely affect the kidney [12;13].

Recently, a secreted protein, short palate lung and nasal epithelial clone 1 (SPLUNC1), was found to be a natural, allosteric inhibitor of ENaC that binds extracellularly to the ENaC subunits and reduces their concentration on the cell surface. Reduced ENaC protein in the plasma membrane diminishes the related channel activity and increases mucus transport. Importantly, the effects of SPLUNC1 are pH-dependent and are lost in the acidic CF airway. To address this deficiency, Spyryx has developed SPX-101, a pH-independent mimetic of SPLUNC1's ENaC regulatory function, which acts via a novel mechanism of action to reduce the effects of ENaC in the airways that is fundamentally distinct from the mechanism of action of previously investigated ENaC receptor inhibitors.

To translate these findings for clinical use, Spyryx has leveraged SPX-101's novel mechanism of action, which durably reduces ENaC activity by promoting channel internalization, thereby reducing the density of ENaC on the cell surface in human bronchial epithelial (HBE) cultures. This unique and innovative approach of using a small peptide to reduce ENaC membrane concentration has several key advantages. Most importantly, the compound can be effectively delivered to the CF lung, a major site of disease in CF.

Additionally, because a combination of low absorption and rapid clearance of small peptides from the circulation limits systemic exposure, there is reduced potential for systemic effects previously seen with small molecule ENaC inhibitors. Further, because the mechanism acts through ENaC instead of CFTR, SPX-101 may be effective for all patients with CF, independent of genetic mutation.

## 7.2 Compound Review

### 7.2.1 Description of SPX-101

SPX-101 is a novel peptide being developed by Spyryx Biosciences, Inc for the treatment of lung associated pathologies in patients with CF. Specifically, SPX-101 will be developed as a genotype-independent treatment of the muco-obstructive pathology of the airways of pediatric and adult patients with CF. SPX-101 will be delivered via a high efficiency vibrating membrane nebulizer device.

The SPX-101 drug product is an aqueous, sterile filled inhalation solution prepared by dissolving either 20 mg or 60 mg peptide in 1 mL of 0.9% sodium chloride, adjusting to pH 7, and sterile filtering into sterile glass vials that are then stoppered, sealed, and labeled. SPX-101 contains no excipients. Drug product purity and nebulized droplet size distribution studies have been conducted at multiple concentrations and pH levels. Each vial of drug product will have a 1-mL fill in a 2-mL vial.

The drug substance in SPX-101 is a linear, dodecapeptide containing 6 natural amino acids and 1 unnatural amino acid with a carboxamide group at the C-terminus. Additional information is provided in the Investigator's Brochure (IB).

### 7.2.2 Structural Formula and Physical Characteristics

See the IB for details.

### 7.2.3 Nonclinical and Pharmacology Studies

SPX-101 acts as a mimetic of the ENaC regulatory domain of the endogenous protein, SPLUNC1. It reduces ENaC activity by internalization of the channel. This novel mechanism of action leads to a durable decrease in amiloride-sensitive current in cells from healthy and CF lungs. By restoring mucus transport to normal or near normal levels in animal models of CF, this activity provides a rationale for its use in the treatment of patients with CF, because airway dehydration leads to profound muco-obstructive pulmonary disease, morbidity, and mortality.

Spyryx has completed a series of preclinical investigations with SPX-101, which include nonclinical pharmacology, PK, and toxicology studies. As part of these studies, assessments included primary and secondary pharmacodynamics, respiratory, cardiovascular, and central nervous system (CNS) safety pharmacology, absorption following inhalation exposure, inhalation toxicology of up to 28 days in duration, and in vitro and in vivo genotoxicity assessments.

The effects of SPX-101 in vivo have been observed in a mouse model that develops CF-like lung disease manifested by goblet cell metaplasia, leukocyte infiltration, and mucus plugging of the airways. If untreated, approximately 50% of these mice die by 2 weeks of age.

However, once daily intranasal administration of SPX-101 in these animals resulted in increased survival to > 90%.

The ability of SPX-101 to increase mucus transport in large animals was investigated in a tracheal mucus velocity (TMV) sheep model. In this approach, both nebulized isotonic saline and an alphabetized control peptide (with the same molecular weight and amino acid composition as SPX-101 but in a pharmacologically inactive sequence) failed to demonstrate an increase in TMV. However, increasing doses of SPX-101 show a dose dependent increase in TMV sufficient to restore approximately 90% of TMV at a dose of 2 mg/kg and 100% recovery of TMV at a dose of 4 mg/kg. This increase was sustained over the course of the experiment (8 hours postdose). Notably, SPX-101 formulated in hypertonic saline showed a greater increase in TMV than the same dose in isotonic saline. In contrast, amiloride partially increased TMV, and this effect was short-lived (half-life of 4 hours), whether amiloride was formulated in isotonic or hypertonic saline. This important finding suggests that SPX-101 can robustly and in a sustained manner, rescue the reduced inhibition of mucus movement caused by inhibition of CFTR function in the complex lung of this large animal.

SPX-101 has not exhibited any test article-related respiratory, cardiovascular, or CNS effects, was well tolerated in two animal species (rats and dogs) when treated for 7-days and 28-days with inhaled doses up to 9.4 and 12.4-times the starting human dose in the completed Phase I clinical study, and showed no evidence of mutagenic activity.

Additional details on the nonclinical and pharmacology studies can be found in the IB.

### 7.2.4 Clinical Studies

The first human clinical study was a single-center, randomized, double-blind, placebo-controlled Phase 1 trial to evaluate the safety and PK profile of inhaled SPX-101 administered via nebulizer to 64 healthy adults in a domiciled clinic setting to ensure continuous monitoring of subject safety. The study was conducted in two parts, as a single ascending dose (SAD) phase comprising 4 escalating dose cohorts (Part 1) and as a multiple ascending dose (MAD) phase comprising 4 escalating dose cohorts (Part 2). In each part of the study, 32 subjects were randomized in a 3:1 ratio to SPX-101 or placebo. In the MAD portion of the study, subjects took SPX-101 or matching placebo twice daily for 14 days.

The SAD portion of the trial demonstrated that SPX-101 at all doses tested (20 mg to 240 mg) were safe and well-tolerated. All subjects completed the study, and there was no evidence of significant or adverse findings in any parameter. Observed plasma concentrations of SPX-101 were limited to the highest dose (240 mg) and detected in 4/6 subjects and only at the 5-minute postdose timepoint.

The MAD portion of the trial has also demonstrated that the range of doses taken, from 10 mg twice daily (BID) for 14 days to 120 mg BID for 14 days, were safe and well tolerated. All 32 subjects completed the study as planned (no treatment withdrawals) and there have been no severe adverse events (AEs), serious adverse events (SAEs), or significant or notable changes in any safety parameters. There were no measurable plasma concentrations in any subject at any time point.

Additional details of this study are provided in the IB.

## 7.3 Clinical Study Rationale

The unmet medical needs of patients with CF remain substantial with no available treatments that prevent the irreversible pulmonary damage associated with recurrent infections. Consequently, life expectancy remains limited to 30 to 40 years of age and quality of life decreases rapidly with advancing age. Although new treatments can be made available only through clinical trial data, the small population of patients with CF results in under-enrollment and delayed development of new treatments [14] Preclinical pharmacology data supported by the absence of dose-limiting toxicity in animal studies and good safety and tolerability in healthy adults indicate a high probability that SPX-101 will have an important impact on patients with CF, with the potential for disease-modifying effects on the lung. Therefore, this study has been designed to maximize scarce clinical research resources and optimize the availability of patients for clinical trials of other products that are currently in clinical development. Moreover, this study will avoid wasteful assessments of doses that are unlikely to be clinically important. This goal will be achieved by initially assessing dosing regimens in the middle of the dose range that has been shown to be safe and well tolerated in adults, as well as a higher dose. A cohort with lower doses will then be studied only if deemed appropriate to address regulatory requirements to describe the dose-response relationship prior to initiation of pivotal efficacy and safety studies.

### 7.3.1 Rationale for Cohort 1 Dosages

Specifically, the initial dose of 60 mg BID is at the midpoint of the dose range previously studied (20 to 120 mg BID) in adults. An additional dose of 120 mg BID was included in the first cohort to ensure that a scientifically sound decision could be made, driven by scientific data with regard to the future development of the drug. To optimize the opportunity to make this potential treatment available to patients, this protocol allows flexibility to adjust the dosing regimens in the second cohort to reflect the experience of the first cohort, but was not to exceed the total daily dose range approved for the first cohort (see Table 4).

### 7.3.2 Rationale for Cohort 2 Dosage

In Cohort 1, a total of 45 adult subjects with cystic fibrosis were treated with one of two doses of SPX-101 (60 mg BID and 120 mg BID) or matching placebo, and 40 subjects completed the study.

The SPX-101 120 mg BID group demonstrated an improvement in the primary endpoint, trough ppFEV$_1$ at day 28, (least squares mean difference from placebo ± standard error) of 3.08 ± 2.411; p = 0.21 for the SPX-101 60 mg BID group and 5.19 ± 2.606; p = 0.05 for the SPX-101 120 mg BID group (source: Table 14.2.1.1).

SPX-101 was well tolerated in both dose groups with no dose-limiting toxicity observed. There were no deaths in the study. Two subjects had serious adverse events (SAEs); one subject had tachycardia and one subject had cough and dyspnea, and both subjects were in the SPX-101 120 mg BID group. Treatment-emergent adverse events (TEAEs) were reported for 71.4%, 66.7%, and 75.0% of subjects in the placebo, SPX-101 60 mg BID, and SPX-101 120 mg BID treatment groups, respectively. TEAEs that coded to the Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class of Respiratory, Thoracic, and

Mediastinal Disorders were reported for 35.7%, 53.3%, and 43.8% of subjects in the placebo, SPX-101 60 mg BID, and SPX-101 120 mg BID treatment groups, respectively. Although more respiratory TEAEs were reported for subjects on active treatment than placebo, the most commonly reported respiratory TEAEs were associated with the therapeutic effect of SPX-101: sputum increased was reported for 7.1%, 20%, and 12.5% of subjects in the placebo, SPX-101 60 mg BID, and SPX-101 120 mg BID treatment groups, respectively; and cough was reported for 0%, 26.7%, and 6.3% of subjects in the placebo, SPX-101 60 mg BID and SPX-101 120 mg BID treatment groups, respectively. The only pre-defined adverse event of special interest was hyperkalemia, which was not reported for subjects in any treatment group. Table 1 shows these results.

**Table 1 Selected Adverse Events from Cohort 1**

| | Placebo BID | SPX-101 60 mg BID | SPX-101 120 mg BID |
|---|---|---|---|
| All TEAEs | 71.4% | 66.7% | 75.0% |
| SOC: Respiratory, Thoracic, and Mediastinal Disorders | 35.7% | 53.3% | 43.8% |
| PT: Sputum Increased | 7.1% | 20% | 12.5% |
| PT: Cough | 0 | 26.7% | 6.3% |
| AESI (hyperkalemia) (n) | 0 | 0 | 0 |
| SAEs (n) | 0 | 0 | 2 |
| Fatal SAEs (n) | 0 | 0 | 0 |

AESI = adverse event of special interest; BID = twice daily; PT = preferred term; SAE = serious adverse event; SOC = System Organ Class; TEAE = treatment-emergent adverse event

Sources: Table 14.3.2.1, Table 14.3.2.8, Table 14.3.2.10

In conclusion, the results of Cohort 1 showed evidence that the SPX-101 120 mg dose was associated with greater efficacy than the 60 mg BID. However, in view of the variability observed in the relatively small sample, it was decided that the second cohort should include additional subjects at the 120 mg BID dose level only. This decision is supported by the absence of dose-limiting safety concerns.

# 8. STUDY OBJECTIVES

## 8.1 Primary Objective

- To evaluate the efficacy of SPX-101 in subjects with CF

## 8.2 Safety Objectives

- To evaluate the safety and tolerability of SPX-101 in subjects with CF

## 8.3 Pharmacokinetic Objective (Cohort 1 Only)

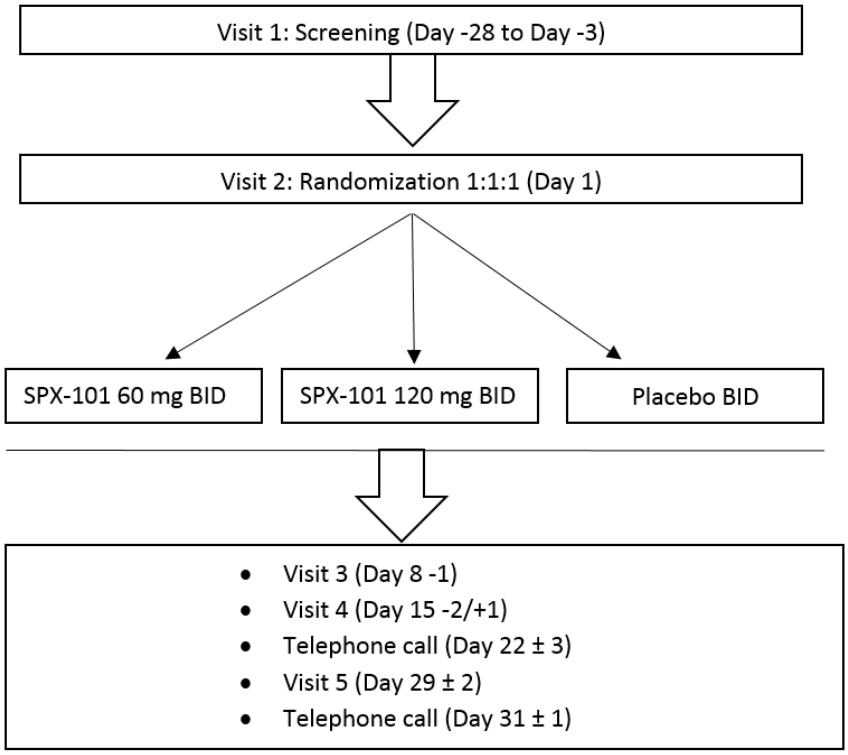
- To assess the extent of systemic exposure to SPX-101 in a subset of subjects

# 9. INVESTIGATIONAL PLAN

## 9.1 Overall Study Design and Plan

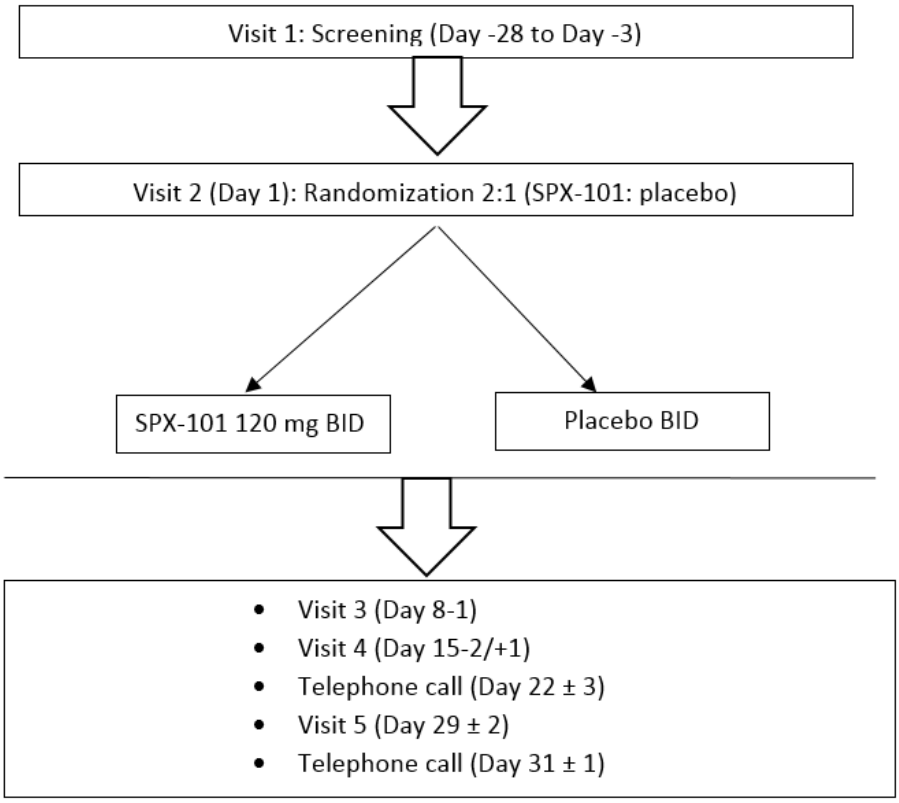
Figure 1 shows the Cohort 1 design and Table 2 shows the schedule of events and assessments for both cohorts. Figure 2 shows Cohort 2, which is also a double-blind, placebo-controlled design to assess the safety and efficacy of SPX-101 120 mg BID as a regimen (see Table 4).

**Figure 1 Study Diagram – Cohort 1**




BID = twice daily

**Figure 2 Study Diagram – Cohort 2**




BID = twice daily

**Table 2 Schedule of Events**

| | Screening | Treatment Period | | | | | FU |
|---|---|---|---|---|---|---|---|
| **Visit (Day)** | **1 (-28 to -3)** | **2 (1)** | **3 (8-1)** | **4 (15 -2/+1)** | **TC (22±3)** | **5 (29±2/ET)** | **TC (31 ± 1)** |
| Informed consent | X | | | | | | |
| Eligibility | X | | | | | | |
| Randomization[a] | | X | | | | | |
| Medical history and prior medications | X | | | | | | |
| Concomitant medications | X | X | X | X | X | X | X |
| Vital signs[b] | X | X | X | X | | X | |
| Weight, height[c], BMI | X | X | | | | X | |
| Physical examination[d] | X | X | X | X | | X | |
| AE assessment | | X | X | X | X | X | X |
| Spirometry[e] | X | X | X | X | | X | |
| 12-lead ECG | X | X | | | | X | |
| CFQ-R | | X | | | | X[f] | |
| Hematology and serum chemistry including electrolytes[g] | X | X | | | | X | |
| ADA | | | | | | X | |
| Urinalysis | | X | | | | X | |
| Urine and blood electrolytes | | | X | X | | X | |
| Pregnancy testing[h] | X | X | | | | X | |
| Issue study nebulizer | | X | | | | | |
| IP administration in clinic | | X | X | X | | | |
| 4-hr postdose monitoring[i] | | X | | | | | |
| Dispense IP | | X | X | X | | | |
| Cohort 1 only: Plasma PK[j] | | X | | | | | |
| Collect unused IP | | | X | X | | X | |
| Collect study nebulizer | | | | | | X | |

ADA = antidrug antibodies; AE = adverse event; BMI = body mass index; CFQ-R = cystic fibrosis questionnaire revised; ECG = electrocardiogram; ET = early termination; FU = follow up; IP = investigational product; PK = pharmacokinetics; TC = telephone call

[a] Only subjects who meet the randomization criteria at Visit 2 will be randomized (see Section 9.4.3).

Footnotes continued on next page

[b] Vital signs will include systolic and diastolic blood pressure, temperature, and heart rate.
[c] Height will be measured only at the screening visit.
[d] At Visit 1, a full physical examination of all body systems will be performed. At subsequent visits, an abbreviated physical examination (head, ears, eyes, nose, and throat; neck, respiratory, cardiovascular, and skin) may be performed.
[e] At Visit 2, spirometry including forced expiratory volume in 1 second ($FEV_1$), percent predicted $FEV_1$, forced vital capacity (FVC) and percent predicted FVC will be evaluated pre-dose, and postdose at 30 minutes, 2 hours, and 4 hours. At all other visits, only pre-dose spirometry will be performed.
[f] The CFQ-R will not be performed for subjects who are terminated early from the study
[g] See Section 11.8 for specific laboratory tests to be performed.
[h] At Screening (Visit 1), a serum pregnancy test will be performed; at Visits 2 and 5, a urine pregnancy test will be performed.
[i] At Visit 2 only, the subject will be observed and monitored for at least 4 hours after completion of administration of the study drug. The subject may be discharged after 4 hours at the discretion of the Investigator (See Section 10.2).
[j] For Cohort 1 only, at Visit 2, blood samples for PK testing will be collected pre-dose and at 5±2 minutes and 15±3 minutes after completion of administration of the study drug.
Note: A subject may be re-screened once if they fail to meet randomization criteria for any reason. If this is the case, a new consent form must be signed, and a new screening number will be used.

Table 3 shows the schedule and timing of events at Visit 2 (Randomization Visit; Day 1). The order of procedures in the table is the suggested approximate order in which the procedures should be carried out.

**Table 3 Visit 2 (Randomization Visit) Schedule of Events**

| | Pre-Dose | Dosing | After Completion of Administration of Study Drug | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | IP Dosing | 5 min (±2 m) | 15 min (±3 m) | 30 min (±15 m) | 1 hr (±15 m) | 2 hrs (±15 m) | 4 hours (±15 m) |
| **CFQ-R** | X | | | | | | | |
| **Confirm randomization criteria[a] are met, including the following:** | X | | | | | | | |
| -AE assessment[b] | X | | | | X | X | X | X |
| -Concomitant medications | X | | | | | | | |
| -Spirometry[c] | X | | | | X | | X | X |
| -Urine collected for electrolytes and pregnancy test | X | | | | | | | |
| -Urine pregnancy test | X | | | | | | | |
| **Vital signs[d]** | X | | | | X | X | X | X |
| **Brief physical exam[e], weight and BMI** | X | | | | | | | |
| **12-lead ECG** | X | | | | | | | |
| **Blood collected for hematology and serum chemistry including electrolytes** | X | | | | | | | |
| **Randomization** | X | | | | | | | |
| **Issue nebulizer** | X | | | | | | | |
| **Administration of IP[f]** | | X | | | | | | |
| **Cohort 1 only: Blood collected for PK[g]** | X | | X | X | | | | |
| **Confirm subject may be discharged from clinic** | | | | | | | | X |
| **Dispense IP[h]** | | | | | | | | X |

AE = adverse event; BMI = body mass index; CFQ-R = Cystic Fibrosis Questionnaire – Revised; ECG = electrocardiogram; IP = investigational product; min/m = minutes; PK = pharmacokinetics

[a] See Section 9.4.3 for a list of randomization criteria.

[b] The Investigator or designee will ask the subject about any symptoms that could indicate an AE during the time vital signs are being collected postdose. Additional assessments are not required for the study but may be done at the discretion of the Investigator.

Footnotes continued on next page

[c] Spirometric parameters to be recorded include forced expiratory volume in 1 second ($FEV_1$), percent predicted $FEV_1$, forced vital capacity (FVC), and percent predicted FVC.
[d] Vital signs include systolic and diastolic blood pressure, heart rate, and temperature.
[e] The abbreviated physical examination should include at a minimum, the following organs or systems: head, ears, eyes, nose, and throat; respiratory; cardiovascular; and skin. Additional systems may be evaluated at the discretion of the Investigator.
[f] The time of completion of administration of the study drug nebulizer treatment is defined as Time 0, and this time will be the reference for the timing of all postdose assessments and procedures.
[g] PK analysis applies only to Cohort 1.
[h] The Investigator or designee should issue a enough study drug to allow for BID administration until the date of the subject's next clinic visit.

Note: The Investigator or designee should provide education and instructions to the subject throughout the visit regarding study details, subject responsibilities, nebulizer use and care, timing of study drug and concomitant medications, study drug storage, procedures to be followed in case of a pulmonary exacerbation or emergency, and any other pertinent information

### 9.2 Discussion of Study Design

This randomized, double-blind, placebo-controlled study is designed to evaluate the efficacy and safety of inhaled SPX-101 at a range of doses in adult subjects with CF in 2 cohorts.

Randomization in the first cohort was stratified by the Baseline (Visit 2) ppFEV$_1$ (40.0% to 55.0% versus 55.1% to 80.0%).

Randomization in the second cohort will be stratified according to the use of concomitant hypertonic saline (defined as having used a constant regimen for 28 days prior to screening and planning to continue their usual concentration without change for 28 days post-randomization) so that approximately 50% of subjects will be using concomitant hypertonic saline.

In Cohort 1, subjects were randomized to receive either placebo BID, SPX-101 60 mg BID, or SPX-101 120 mg BID for 28 days. After the Cohort 1 data were complete, unblinded, and analyzed, the DSMB evaluated the safety results, and Spyryx, in conjunction with the DSMB, reviewed the efficacy results to determine the appropriate dosage for Cohort 2.

Cohort 2 will have the same design as Cohort 1; subjects will be randomized in a 2:1 ratio to either SPX-101 120 mg BID or placebo BID.

### 9.3 Study Duration

Subjects will enter a screening period of up to 28 days before randomization. Randomized subjects will be in the study for 28 days, with a follow-up phone call approximately 2 days later. The end of the study is defined as the date of the last data recorded, eg, the follow-up phone call at Day 31 or an unscheduled phone call, whichever is later.

### 9.4 Study Population

Subjects who satisfy eligibility criteria at the Screening Visit (Visit 1), and meet all the randomization criteria at Visit 2 will be eligible to enroll in the study. A subject may be re-screened once if they fail to meet the screening or randomization criteria. If this occurs, a new consent form must be signed, and a new screening number will be used.

#### 9.4.1 Inclusion Criteria

Subjects must satisfy all of the following inclusion criteria before they will be allowed to participate in the study:

1. Ability to provide written, personally signed, and dated informed consent to participate in the study, in accordance with the ICH GCP Guideline E6 and applicable regulations, before completing any study-related procedures
2. Ability and willingness (in the judgment of the Investigator) to attend study visits and fully comply with study procedures and restrictions; each subject's study visits will occur at approximately the same time of day throughout the study and under specified conditions with respect to daily respiratory clearance and medication regimens
3. Ability to self-administer the investigational product
4. Male or nonpregnant, nonlactating female subjects aged 18 to 50 years inclusive

5. Diagnosis of cystic fibrosis as determined by the 2008 Cystic Fibrosis Foundation Consensus Guidelines for Diagnosis [1], defined as the presence of clinical signs and symptoms of CF in conjunction with at least 1 of the following:
    a. Documented sweat chloride test result ≥ 60 mEq/L by quantitative pilocarpine iontophoresis
    b. Two well-characterized, disease-causing genetic mutations in the CFTR gene; the original documentation of genetic testing is not required if the specific mutations are documented in the subject's medical record.
6. ppFEV$_1$ as follows:
    a. Cohort 1: between 40.0% and 80.0%
    b. Cohort 2: between 50.0% and 80.0%

    Note: spirometry must be performed in accordance with American Thoracic Society (ATS)/European Respiratory Society (ERS) end-of-test criteria and the Global Lung Initiative (GLI) predicted values [2; 3]
7. Stable CF lung disease, defined as no pulmonary exacerbation or acute upper or lower respiratory illness other than CF within 28 days preceding screening, except for allergic or nonallergic rhinitis; in addition, subject must not have had any of the following:
    a. Significant hemoptysis (at the discretion of the Investigator) within 28 days of screening
    b. Pneumothorax within 28 days of screening
    c. Diagnosis of active allergic bronchopulmonary aspergillosis (at the discretion of the Investigator)
    d. Documented colonization with *Burkholderia cenocepacia* or *Mycobacterium abscessus* within 6 months prior to screening; subjects with other infections in the *Burkholderia* or *Mycobacterium* genii are allowed at the discretion of the Investigator.
8. No significant changes (removal or addition of medications) to inhaled or oral CF medication regimen within 28 days preceding screening, and no anticipation of need to change this regimen during study participation; short courses of medications used to address an ongoing medical problem are allowed, provided the last dose of such medication was taken > 28 days before screening; subjects for whom a significant change in medication is anticipated during the study will be excluded. Specific requirements for ongoing concomitant medications are below:
    a. Inhaled antibiotic use: stable antibiotic medication, defined as at least 2 complete 28-day cycles of intermittent monotherapy (if alternating month on and month off with a single inhaled antibiotic) or 2 full cycles if continuous alternating every 28 days between 2 inhaled antibiotics
    b. Dornase alfa: stable use of daily or intermittent dosing for at least 28 days before screening
    c. Inhaled bronchodilators: stable dose for at least 28 days before screening
    d. Inhaled hypertonic saline: stable dose for at least 28 days before screening

9. Other concomitant medications may be taken during the study, provided the dose and regimen have been stable for at least 14 days before screening, and the regimen is expected to remain stable during the study.
10. Stable chest physiotherapy and clearance techniques for at least 28 days before screening, including chest percussion, use of vibrating vests, etc
11. All female subjects of childbearing potential must be using a highly effective method of contraception through the duration of the study as follows:
    a. Female subjects with childbearing potential must not be pregnant, breastfeeding, or attempting to become pregnant for 28 days before screening, throughout the duration of the study, and for 28 days after the last study visit. Subjects must have a negative serum pregnancy test at screening, a negative urine pregnancy test at randomization, and be willing to commit to using a consistent and acceptable method of contraception for at least 28 days before screening and for the duration of the study, if they are (or become) sexually active. Female subjects of childbearing potential must meet at least 1 of the following:
        i. Consistent use of systemic contraception, including birth control pills, transdermal patch, vaginal ring, implants, and injectables
        ii. Consistent use of double barrier method, eg, condoms, cervical cap, diaphragm, vaginal contraceptive film with spermicide
        iii. Use of intrauterine device with the low failure rate (defined as < 1% risk of pregnancy per year)
        iv. Monogamous intercourse with a vasectomized man or has only same-sex partners
        v. Female subjects who are not sexually active but become so during the study must agree to follow the contraceptive requirements above
    b. Female subjects who are not of childbearing potential must meet at least 1 of the following criteria:
        i. At least 1 year since the last menstrual period
        ii. Surgically sterile (tubal ligation, bilateral oophorectomy, salpingectomy, or hysterectomy)
        iii. Congenitally sterile
        iv. Diagnosed as infertile and not undergoing treatment to reverse infertility

### 9.4.2 Exclusion Criteria

If any of the following apply, the subject must not enter the study:

1. BMI < 18 kg/m$^2$
2. Use of a CFTR corrector or potentiator during the study or within 60 days before screening
3. The presence of significant and unstable comorbidities (based on the Investigator's judgment) within 28 days before screening, including diabetes, significant renal or

hepatic disease, significant respiratory disease other than CF, cardiac, neurological, or gastrointestinal disease other than those associated with CF. Subjects with stable co-morbidities associated with CF (such as diabetes) may participate per the Investigator's judgment unless specifically prohibited elsewhere.

4. Subjects with either of the following:
    a. Hepatic impairment defined by elevation of bilirubin > 2 times the upper limit of normal (ULN) or aspartate aminotransferase, alanine aminotransferase, or alkaline phosphatase results > 5 times the ULN
    b. Chronic hepatitis B and/or C
5. History of malignancy in the previous 5 years; subjects who have been successfully treated for non-melanoma skin cancer, cervical cancer, or prostate cancer within the previous 5 years are allowed to enroll with the approval of the Medical Monitor
6. Administration of another investigational drug within 28 days before screening
7. Administration of a vaccination (including influenza) within 7 days before screening
8. Requirement for supplemental oxygen more than 2 L/min and/or noninvasive ventilation for the management of respiratory failure; subjects must have been on a stable regimen for at least 60 days before screening; (patients who have been using a stable regimen of oxygen ≤ 2 L/min for at least 60 days before screening are eligible to enroll)
9. Current use of tobacco or tetrahydrocannabinol-containing products, delivered orally, by vaporizing, or by smoking; or a greater than 10 pack-year history of tobacco use
10. Actively listed for lung transplantation
11. Any history of organ transplantation
12. Hospitalization for a respiratory event or CF-related condition within 28 days before screening or any plans for hospitalization for any reason during the study
13. Current use of > 10 mg/day of prednisone/prednisolone (≤ 10 mg/day is acceptable)
14. Use of any of the following antihypertensive medications or combinations containing these medications: diuretics (eg, thiazides, loop diuretics, spironolactone, and amiloride), angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or any other drugs known to affect the serum potassium concentration
15. Hypersensitivity to the study drug or any of its excipients
16. Subjects with significant electrocardiogram (ECG) abnormalities at screening or before the first dose on Day 1, including a prolonged QT interval corrected by the Fridericia correction formula (QTcF; ≥ 440 msec in men and ≥ 460 msec in women)

### 9.4.3 Randomization Criteria (Assessed at Visit 2)

On Day 1 of the study, after completion of the screening period, the following criteria will be assessed to determine subject eligibility for randomization:

1. Continue to meet the entrance criteria
2. Negative serum (Visit 1) and urine (Visit 2 pre-randomization) pregnancy test result for all female subjects of childbearing potential

3. Stable $FEV_1$ at Visit 2 defined as $FEV_1$ within ± 15% of the relative change between the Visit 1 and Visit 2 best $FEV_1$. Relative change is defined as follows:

$$\frac{FEV_1 \text{ (Screening; Visit 1)} - FEV_1 \text{ (Visit 2)}}{FEV_1 \text{ (Screening; Visit 1)}} \times 100$$

4. No new intervening illnesses since Screening (Visit 1), and the subject's CF is still stable in the judgment of the Investigator
5. No clinically significant laboratory or ECG abnormalities that pose a significant risk to the subject, per the judgment of the Investigator

Note: A subject may be re-screened once if they fail to meet randomization criteria for any reason. If this is the case, a new consent form must be signed, and a new screening number will be used.

### 9.4.4 Withdrawal and Replacement of Subjects

*9.4.4.1 Criteria for Subject Withdrawal*

In accordance with the Declaration of Helsinki (see Appendix 1, Section 18.1) and other applicable regulations, a subject has the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care by the physician or at the institution.

Subjects may also be withdrawn from the study for any of the following reasons:

- A female subject becomes pregnant (See Section 12.6 for details on pregnancy reporting requirements)
- The Investigator may decide that the subject should be withdrawn. If this decision is made because of an intolerable AE or a clinically significant abnormality of a laboratory value, the study medication is to be discontinued and appropriate measures are to be taken. The Sponsor or Sponsor designee is to be notified immediately.
- The subject is unwilling to continue in the study.
- The Investigator stops participating in the study or the Sponsor, for any reason, stops the study.
- The subject or the Investigator decides it in the best interest of the subject to seek another treatment.
- The subject fails to return to the clinic for scheduled visits and does not respond to attempts at communication.

The reason for withdrawal will be recorded in the clinical records and the electronic case report form (eCRF). All subjects who prematurely discontinue the study treatment will undergo an early termination visit (see Section 10.6). Subjects who are withdrawn from the study will not be replaced. All subjects who are withdrawn or discontinue should be offered other approved medical care.

*9.4.4.2 Evaluations at Withdrawal*

For any subject who is withdrawn before completing all study visits, the Investigator should:

- Perform the procedures included in the early termination visit (see Section 10.6) These assessments should be performed as soon as possible after the last administration of the study drug, but in any case, will be performed no later than 14 days after withdrawal or discontinuation of the study treatment.
- Complete all appropriate eCRF pages, providing the date of and explanation for the subject's withdrawal/discontinuation.
- When indicated, arrange for appropriate medical follow-up care for the discontinued subject.

If the subject fails to attend the scheduled early termination visit, the Investigator or designee will make at least 2 attempts to contact the subject by telephone. If the subject does not respond, s/he will be considered lost to follow up.

*9.4.4.3 Termination of the Study by the Sponsor*

The study may be prematurely terminated at any time at the discretion of Spyryx. Should premature termination be considered necessary, written notification documenting the reason for study termination will be provided to the appropriate Regulatory Authorities and IEC/IRBs, and procedures for termination of the study will be arranged. Circumstances that may warrant premature study termination include, but are not limited to, the following examples:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Failure to accrue subjects at an acceptable rate
- Insufficient adherence to the requirements of the protocol
- Insufficient provision of complete and evaluable data
- Plans to modify, suspend, or discontinue development of the study drug

If the study is terminated prematurely, all study materials must be returned to Spyryx or its designee.

*9.4.4.4 Replacement of Subjects*

Subjects who are withdrawn will not be replaced. However, a sufficient number of subjects will be included to ensure the minimum sample size defined (see Section 13.2).

## 9.5 Treatment

### 9.5.1 Treatments Administered

The Investigator must ensure that the study drug and investigational PARI eFlow nebulizer with PARI eTrack will be used in accordance with the protocol, and must instruct subjects that the study drug must be administered only with the study nebulizer and that all other inhaled medications must be administered with the subject's regular nebulizer.

Table 4 shows the planned dispensing of study medication for Cohort 1 and Cohort 2 .

**Table 4 Planned Study Medication Dosing for Cohort 1 and Cohort 2**

| Cohort | Placebo Group | | | SPX-101 – Dose Group 1 | | | SPX-101 – Dose Group 2 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Dosage** | **How each dose is supplied** | **Total volume** | **Dosage** | **How each dose is supplied** | **Total volume** | **Dosage** | **How each dose is supplied** | **Total volume** |
| **1** | Placebo BID | 2 vials placebo in the a.m. and the p.m. | 2 mL | 60 mg BID | 1 vial 60 mg/mL and 1 vial placebo in the a.m. and the p.m. | 2 mL | 120 mg BID | 2 vials 60 mg/mL in the a.m. and the p.m. | 2 mL |
| **2** | Placebo BID | 2 vials placebo in the a.m. and the p.m. | 2 mL | 120 mg BID | 2 vials 60 mg/mL in the a.m. and the p.m. | 2 mL | | | |

BID = twice daily

### 9.5.2 Study Treatment Formulation

The SPX-101 drug product is an aqueous, aseptically filled inhalation solution prepared by dissolving 20 mg or 60 mg peptide in 1 mL 0.9% sodium chloride, adjusting to pH 7, and sterile filtering into sterile glass vials that are then stoppered, sealed, and labeled. SPX-101 contains no excipients. Drug product purity and nebulized droplet size distribution studies have been conducted at multiple concentrations and pH levels. Each vial of drug product will have a 1-mL fill in a 2-mL vial. The maximum anticipated dose is 240 mg/day, which could be achieved as a single 4-mL inhalation QD or a 2-mL inhalation BID.

The placebo is 0.9% sodium chloride, pH adjusted to 7 and filtered for sterility into sterile glass vials that are then stoppered, sealed, and labeled, with a 1-mL fill in a 2-mL vial. Table 5 shows some details about the study drug and placebo.

**Table 5 Investigational Medicinal Products**

| **Product name** | **SPX-101** | **Placebo** |
|---|---|---|
| **Dosage Form** | Solution for oral inhalation | Solution for oral inhalation |
| **Unit Dose** | 60 mg/mL<br>(1 mL in a 2-mL vial) | --- |
| **Route of Administration** | Oral inhalation by Investigational eTrack nebulization device | Oral inhalation by Investigational eTrack nebulization device |
| **Physical Description** | Clear, sterile solution for inhalation consisting of 60 mg/mL of SPX-101 peptide in 0.9% sodium chloride adjusted to pH 7 | Clear, sterile solution for inhalation consisting of 0.9% sodium chloride adjusted to pH 7 |
| **Storage Conditions** | Vials should be refrigerated at 2 -8º C | |
| **Manufacturer of Drug Substance** | PolyPeptide Laboratories<br>9395 Cabot Drive<br>San Diego, CA 92126 | |
| **Manufacturer of Drug Product** | Ajinomoto Althea<br>11040 Roselle Street<br>San Diego, CA 92121 | |

### 9.5.3 Study Treatment Labeling and Packaging

Both the investigational product and placebo will be supplied and labeled in accordance with the applicable regulatory requirements. The labeling will include the following information in the official language of the country in which the study drug is to be used:

The primary packaging label (vials) will include at least the following information except as mandated by individual country requirements:

- Pharmaceutical dosage form, route of administration, and quantity of dosage units
- A blinded batch and/or code number that will link the contents and packaging operation
- Protocol number
- Kit number
- An expiration or “use by” date in a non-ambiguous format

- Subject ID number (write in)
- Site/Investigator name (write in)

The secondary packaging label (container for the vials) should contain all the same information as on the primary packaging label plus at least the following information:

- Directions for use or reference to separate instructions to be provided to the subject
- "Investigational Product - For clinical trial use only" or similar wording
- Storage conditions (2° to 8° C or 35° to 45°F)
- An expiration or "use by" date in a non-ambiguous format
- "Keep out of reach of children"
- Name and address of the Sponsor
- EudraCT number (EU sites only)

For Cohort 1, to reduce the risk of inadvertent unblinding, half the vials issued to each subject were labeled to indicate the morning dose, and half were labeled to indicate the evening dose. This strategy helped ensure that subjects, the Sponsor, Investigators, site staff members, and other study participants remained blinded to the study medication.

### 9.5.4 Blinding of Study Medication

This is a randomized, double-blind, placebo-controlled study. The investigational medicinal product and placebo will be identical in physical appearance. The treatment each subject will receive will not be disclosed to the Investigator, study center personnel, subject, or Sponsor. The treatment codes will be held according to the interactive web response system (IXRS). Bioclinica will provide the IXRS services and further instructions such emergency unblinding procedures will be provided in a separate IXRS manual. By logging into the IXRS system, Investigators will be able to unblind the treatment of any subject, should that be warranted. The Investigator is encouraged but not required to consult with the PRA Medical Monitor or the Sponsor prior to unblinding.

### 9.5.5 Study Treatment Storage and Accountability

The investigational drug and study supplies must not be used for purposes other than as defined in this protocol.

*9.5.5.1 Study Treatment Storage*

SPX-101 and the placebo are to be stored at 2° to 8°C. Investigators must store the study drug in a secure location, and must implement a temperature monitoring method for the refrigerator in which the study drugs are stored. The product remains stable with no significant increase in impurities for up to three months at 25°C, indicating that temporary excursions to room temperature will not alter the purity.

*9.5.5.2 Study Treatment Accountability*

All supplies of study medication and placebo will be accounted for in accordance with GCP. There will be an individual study drug accountability record for each subject and the Investigator will maintain accurate records of the disposition of all study medication supplies received during the study. These records will include the amounts and dates clinical drug supplies were received and then dispensed to the subject, and unused supplies returned by the

subject to the Sponsor. If errors or damages in the clinical drug supply shipments occur, the Investigator will contact the study monitor and the Sponsor immediately. Copies of the study medication accountability records will be provided by each Investigator for inclusion in the Trial Master File after database lock. The study monitor will periodically check the supplies of study medication held by the Investigator or pharmacist to verify accountability of all medication used.

The Investigator will dispense the medication only to the identified subjects in this study, according to the procedures described in this study protocol. After the end of the study, all unused medication and all medication containers will be returned to Spyryx Biosciences, Inc. or its designee for destruction. The Sponsor will verify that a final report of drug accountability is prepared and maintained in the Investigator's Study Center File.

### 9.5.6 Dose Adjustments and Dose Escalation

Not applicable to this study

### 9.5.7 Prior and Concomitant Therapy

Any treatment not explicitly excluded and considered necessary for the subject's welfare may be given during the study at the discretion of the Investigator. Administration of concomitant medications must be reported in the appropriate eCRF with dosage information, dates of administration, and reasons for use. Generic names for concomitant medication should be used if possible. The total daily dose should be filled in whenever possible.

Randomization (Day 1) and initiation of study drug should be scheduled to coincide with the first day of a cycle of inhaled antibiotics, if applicable. If simultaneous initiation of the study drug and inhaled antibiotics cannot be accommodated, the subject may be randomized, provided they start their 28-day cycle within ± 3 days of Visit 2.

In general, the subject's usual routine of medication and chest physiotherapy are to be continued unchanged throughout the study. The study drug should be added into the subject's treatment regimen as the last mucolytic therapy inhaled (ie, after inhalation of dornase alfa, mannitol, and/or hypertonic saline) but before the inhalation of antibiotics or corticosteroids. The only exception to this rule is on the mornings of all clinic visits. The following procedures should be followed on clinic days only:

- If a long-acting β-agonist (LABA) is taken regularly in the morning, instruct the subject to take it the evening prior to all clinic visits and not take it the morning until after all clinic assessments have been completed.
- If a short-acting β-agonist (SABA) is taken regularly, the subject should ensure that it is taken at least 4 hours before the clinic visit.
- The subject should be instructed not to take the morning dose of the study drug, but they should complete the regular routine of medications and chest physiotherapy at home (including inhaled antibiotics); the study drug will be administered during the clinic visit.

*9.5.7.1 Prohibited Medications*

Use of the following antihypertensive medications or combinations containing them is

prohibited within 28 days before screening and during the study:

- Diuretics (eg, thiazides, loop diuretics, spironolactone, and amiloride)
- Angiotensin-converting enzyme inhibitors
- Angiotensin receptor blockers
- Any other drugs known to affect the serum potassium concentration

### 9.5.8 Treatment Compliance

It is the Investigator's responsibility to ensure that subjects are correctly instructed about how to take their study medication and use the PARI eFlow nebulizer with PARI eTrack, and understand the importance of being compliant with their assigned dose regimen. Records of study medication used and intervals between visits will be kept during the study. Drug accountability will be noted by the study monitor during site visits and at the completion of the study. Subjects will be asked to return all unused medication at the end of the study. Subjects should be instructed to discard used medication vials at home as the vials are used, to reduce the risk of infection. The study treatment should be dispensed by the Investigator, or by a qualified individual under the Investigator's supervision. The Investigator must maintain an up-to-date treatment inventory and dispensing record (see Section 9.5.5.2).

At each visit, the Investigator will collect previously dispensed, unused study medication and assess compliance. Subjects exhibiting poor compliance should be counseled on the importance of complying with study procedures. Subjects who are persistently noncompliant may be withdrawn from the study but only after consultation with the Medical Monitor.

Where available, study drug compliance will be assessed electronically via a Bluetooth accessory called eTrack on the PARI eFlow investigational nebulizer. This monitor will capture the date and time of use of the investigational nebulizer. Subjects will be made aware of this feature and provided with the equipment and instructions to set this up for use in their home. Study coordinators will be expected to monitor study drug usage and intervene via phone call, etc. if there are any obvious problems (such as using the nebulizer too much, too little, etc).

### 9.5.9 Assignment to Treatment

The Investigator or designee will use IXRS to randomize eligible subjects. Randomization for Cohort 1 was stratified by the Visit 2 pre-dose ppFEV$_1$ (between 40.0% and 55.0% vs between 55.1% and 80.0%). Randomization for Cohort 2 will be stratified by use of concomitant hypertonic saline (defined as having used a constant regimen for 28 days prior to screening and planning to continue with no change through 28 days post-randomization), so that approximately 50% of subjects will be using hypertonic saline. Only those subjects who have met the randomization criteria (Section 9.4.3) will be eligible for assignment to study treatment. Subjects who fail to meet the randomization criteria may have Visit 2 repeated once, or they will be considered a screen failures.

### 9.5.10 Unblinding Procedures

Within the IXRS system, the Investigator may break the blind by clicking on the "Break Blind" button. The Investigator will then be prompted to enter either the Kit ID number or

Subject ID number and select "next." The Investigator will then be prompted to confirm the blind should be broken. After selecting "Yes," the Investigator will be prompted to enter a reason for breaking the blind. Once a reason has been properly entered, a warning will appear and once again the Investigator will select "confirm" and then will be prompted to enter the appropriate IXRS user password. The Investigator will then confirm this selection and the subject information with the unblinding data will be displayed. Additional procedures for unblinding in case of emergency will be detailed in the IXRS manual. See Appendix 5 (Section 18.5) for more details.

## 9.6 Efficacy and Safety Variables

### 9.6.1 Efficacy and Safety Measurements Assessed

*9.6.1.1 Efficacy Variables*

- Primary
  - Change from baseline in ppFEV$_1$ at Week 4
- Secondary
  - Change from baseline in ppFEV$_1$ at other visits
  - Change from baseline in FEV$_1$
  - Change from baseline in FVC and ppFVC
  - Change from baseline in CFQ-R

*9.6.1.2 Safety Variables*

- Incidence of treatment-emergent AEs
- Changes in vital signs
- Changes in clinical laboratory tests, including urine and blood electrolytes
- Changes in ECGs

*9.6.1.3 Pharmacokinetic Variable (Cohort 1 Only)*

- Systemic exposure to SPX-101 (subset of subjects)

*9.6.1.4 Exploratory Variables*

- Incidence of pulmonary exacerbation, as defined by Fuchs [4] (See Appendix 3, Section 18.3 for details)
- Incidence of antidrug antibodies (ADA)

# 10. STUDY EVALUATIONS BY VISIT

## 10.1 Visit 1: Screening (Day -28 to -3)

There is a variable-length screening period of 3 to 28 days to the time of randomization so that the first dose of the study drug coincides, if applicable, to the first day of an inhaled antibiotic cycle. Additional information can be found in Section 9.5.7.

At the Screening Visit, the following assessments/procedures will be performed in approximately the following general order:

- Informed consent
- Medical history
- Previous and concomitant medications
- Vital signs
- Height, weight, BMI
- Complete physical examination
- Spirometry
- 12-lead ECG
- Blood samples collected for hematology and serum chemistry tests and serum pregnancy test
- Assessment of eligibility

Note: Subjects may be re-tested once within 7 days to complete their eligibility assessments.

## 10.2 Visit 2: Day 1 (Randomization)

### 10.2.1 Pre-Randomization Assessment

- CFQ-R
- AE assessment
- Concomitant medications
- Pre-dose spirometry
- Urine collected for urinalysis, urine electrolytes, and urine pregnancy
- Vital signs
- Abbreviated physical examination
- Weight and BMI
- 12-lead ECG
- Blood collected for hematology and serum chemistry tests, including electrolytes and pre-dose sample for PK (Cohort 1 only)
- Review randomization criteria to ensure eligibility

Note: A subject may be re-screened once if they fail to meet the Randomization criteria for any reason. If this is the case, a new consent form must be signed, and a new screening number will be used.

### 10.2.2 Randomization and Study Procedures

- Randomization using IXRS

- The Investigator or designee will issue the eTrack study nebulizer and will train subjects on its use and all study procedures, and will review all instructional materials and ensure subjects understand all procedures and instructions
- Study medication administration and postdose monitoring for at least 4 hours after completion of administration
    - Blood for PK analysis will be collected at 5 ± 2 minutes and 15 ± 3 minutes after completion of administration of the study drug (Cohort 1 only)
    - Post-dose spirometry at 30 (± 15) minutes, 2 hours (± 15 minutes), and 4 hours (± 15 minutes) after completion of administration of the study drug
    - Vital signs at 30 (± 15) minutes, 1 hour (± 15 minutes), 2 hours (± 15 minutes), and 4 hours (± 15 minutes) after completion of administration of the study drug
- Dispense study medication, nebulizer, and baby bottle sterilizer
- Schedule the subject's next visit and review instructions (See Appendix 4, Section 18.4)

If the subject experiences symptoms of acute bronchospasm following the first dose of study medication, the subject should be treated with SABAs or other treatment at the discretion of the Investigator and continued to be observed for at least 2 hours post-SABA or the 4-hour postdose timepoint, whichever is longer. If the subject's bronchospasm has been relieved and the subject is stable for at least 30 minutes at the 2-hour post-SABA or 4-hour postdose timepoint, the subject may continue in the trial at the discretion of the Investigator. If medically essential, the Investigator may elect to require that the subject be pre-treated with a SABA or other medication before every subsequent dose, or the PI may withdraw the subject from the study. If the subject requires pre-treatment with a SABA or other medication for subsequent doses, this drug will be recorded as a concomitant medication, and the bronchospasm will be reported as an AE. Additional follow-up procedures may be instituted at the discretion of the Investigator**.**

### 10.2.3 Post-Randomization Visit Schedule

Randomized subjects will return to the clinic after approximately 1, 2, and 4 weeks of treatment. Study drug consisting of an 8-day supply will be dispensed weekly to cover the period from Visit 2 to Visit 3 and again from Visit 3 to Visit 4. At Visit 4, a 2-week supply (2 boxes with an 8-day supply in each) will be dispensed to cover the period from Visit 4 to Visit 5. Sites must schedule subjects for Visit 3, 4, and 5 within the provided visit windows taking into account drug supply.

## 10.3 Visit 3: Day 8 (-1 Day)

- Concomitant medications
- AE assessment
- Vital signs
- Abbreviated physical examination
- Pre-dose spirometry
- Urine and blood samples collected for electrolytes

- Study drug administration
- Dispense study drug
- Collect unused study drug from previous visit
- Schedule the subject's next clinic visit and review instructions (Appendix 4, Section 18.4)

### 10.4 Visit 4: Day 15 (-2/+1 Days)

- Concomitant medications
- AE assessment
- Vital signs
- Abbreviated physical examination
- Pre-dose spirometry
- Urine and blood samples collected for electrolytes
- Study drug administration
- Dispense study drug
- Collect unused study drug from previous visit
- Schedule the subject's Week 3 telephone call and review instructions if needed (Appendix 4, Section 18.4)

### 10.5 Day 22 (± 3 Days): Telephone Contact

- Concomitant medications
- AE assessment
- Schedule the subject's next clinic visit and review instructions if needed (Appendix 4, Section 18.4)

### 10.6 Visit 5: Day 29 (± 2 Days) (Also the Early Termination Visit, If Applicable)

- CFQ-R (not to be performed for subjects who discontinue early)
- Concomitant medications
- AE assessment
- Vital signs
- Weight and BMI
- Abbreviated physical examination
- Spirometry (the study drug will not be administered)
- 12-lead ECG
- Blood samples collected for hematology and serum chemistry tests, including electrolytes
- Blood sample collected for ADA testing
- Urine samples collected for electrolytes, urinalysis, and urine pregnancy test
- Urine pregnancy test performed
- Collect study nebulizer and unused study drug from previous visit
- Schedule the subject's follow-up telephone call

### 10.7 Follow-up Telephone Call: Day 31 (± 1 Day)

- Concomitant medications
- AE assessment

# 11. METHODS OF ASSESSMENT

## 11.1 Cystic Fibrosis Questionnaire - Revised

At the Randomization Visit and at Week 4 (Visit 5) subjects will complete the respiratory and physical domains of the CFQ-R, and the Investigator or designee will record the data in the appropriate eCRF.

## 11.2 Pregnancy Testing

Subjects who are pregnant will be excluded from the study. At the screening visit (Visit 1), blood will be collected for a serum pregnancy test. At the randomization visit (Visit 2) and at Visit 5, a urine pregnancy test will be performed. For more information about pregnancy-related procedures and reporting, see Section 12.6.

## 11.3 Pulmonary Function Tests

Spirometry will be performed at all clinic visits using equipment provided by the centralized spirometry vendor, Biomedical Systems (BMS). Spirometry will be performed in accordance with ATS/ERS guidelines and the GLI predictors of spirometric lung volume normal ranges [2; 3]. At Visit 2, spirometry will be performed pre-dose, and postdose at 30 minutes, 2 hours, and 4 hours postdose after completion of administration of the study drug. At all other visits, only pre-dose spirometry will be performed. The following parameters will be recorded:

- Absolute $FEV_1$ and $ppFEV_1$
- Absolute FVC and ppFVC

## 11.4 Vital Signs and Weight

Systolic blood pressure and diastolic blood pressure will be measured on the same arm (preferentially on the left arm) after the subject has been in a supine/sitting position for 5 minutes. The heart rate will be recorded simultaneously with blood pressure measurements. The body temperature will also be recorded. On the first day of dosing, vital signs will be measured pre-dose, at 30 minutes and 1, 2, and 4 hours postdose.

Body weight (kg) will be measured without shoes or jacket. Height will be measured at the Screening Visit to assess BMI.

During the study, the measurement of vital signs may be repeated at the discretion of the Investigator for safety reasons. Clinically relevant abnormal findings will be reported as AEs.

## 11.5 Physical Examination

Physical examinations will be performed by a physician or qualified designee and will include examination of the following: general appearance, head, ears, eyes, nose, throat (HEENT), neck, skin, cardiovascular system, respiratory system, abdomen, and nervous system (including a neurological examination; with an assessment of the reflexes, motor and sensory nerve assessment, sensory checks [extremities] and mental status assessment). At all visits after the Screening visit, the Investigator or qualified designee may perform an abbreviated physical examination, defined as a review of HEENT, neck, respiratory system, cardiovascular system, and the skin.

For each body system, the Investigator will document any abnormalities and will provide an assessment of normal or abnormal on the eCRF.

### 11.6 Electrocardiogram

Computerized 12-lead ECG recordings will be obtained, after the subject has been supine for 5 minutes. Each lead will be recorded for at least 3-5 beats at a speed of 25 mm/sec paper speed and 10 mm/mV amplitude. At a minimum, heart rate and the P, PR, QRS, QT, and QTcF intervals will be obtained and entered into the eCRF. The Investigator will also evaluate whether the ECG is normal or abnormal, and if abnormal, whether the abnormality is clinically significant. A copy of all ECGs will be retained on site and may be collected for central review if required (ie, in case emerging ECG data necessitate expert evaluation).

An ECG may be repeated for quality reasons and additional ECGs may be collected by the Investigator for safety reasons. Any clinically relevant abnormal findings noted at Screening will be exclusionary. Any clinically relevant abnormal findings after the first dose of the study drug will reported as AEs. Subjects with clinically relevant cardiovascular AEs (or clinically relevant ECG changes) must be withdrawn from the study (see Section 9.4.4).

### 11.7 Adverse Events and Pulmonary Exacerbation

At each clinic visit, the Investigator or designee will ask the subject about any symptoms experienced since the previous contact to assess for AEs. The Investigator will also assess whether the subject had a pulmonary exacerbation since the previous visit, and if so, record it in the eCRF, complete the pulmonary exacerbation eCRF, and follow all procedures for reporting an AE or SAE. See Appendix 3, Section 18.3 for the Fuchs 1996 criteria for pulmonary exacerbation.

On the first day of dosing, AEs will be assessed pre-dose, at 30 minutes, and 1, 2, and 4 hours postdose.

### 11.8 Clinical Laboratory Testing

Venous blood samples and urine samples will be collected for hematology and chemistry testing at study visits as outlined in Section 10.

- **Hematology:** White blood cell count, red blood cell count, red blood cell indices, hemoglobin, hematocrit, platelet count, and a 5-part white blood cell differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils)
- **Clinical chemistry:** Liver function tests (ALT, ALP, AST, and gamma glutamyltranspeptidase), electrolytes (sodium, potassium, chloride, and bicarbonate), total bilirubin, blood urea nitrogen, calcium, C-reactive protein, creatinine, glucose, lactate dehydrogenase, total protein, and albumin
- **Blood electrolytes:** sodium, potassium, chloride, and bicarbonate
- **Urine electrolytes:** sodium, potassium, and chloride
- **Urinalysis:** pH, specific gravity, protein, glucose, and ketones

Routine laboratory tests will be performed by Eurofins Central Laboratories. Details about the procedures to be followed for sample collection, storage, and shipment will be provided in a Laboratory Manual.

Additional and repeat laboratory testing may be performed at the discretion of the Investigator.

### 11.9 Blood Samples for Pharmacokinetic Testing (Cohort 1 Only)

At Visit 2 only, venous blood samples were collected pre-dose and at 5 ± 2 minutes and 15 ± 3 minutes after completion of administration of the study drug. Pharmacokinetic testing will be performed by QPS. Details about the procedures to be followed for sample collection, handling, storage, and shipment will be provided in a Laboratory Manual.

### 11.10 Blood Samples for Antidrug Antibody Testing

At Visit 5 only, a venous blood sample will be collected for ADA testing, which will be performed by QPS. Details about the procedures to be followed for sample collection, handling, storage, and shipment will be provided in a Laboratory Manual.

# 12. SAFETY MEASUREMENTS AND VARIABLES

## 12.1 Adverse Events

The definitions and reporting procedures provided in this protocol comply with the current CFR 21 Part 312. An AE is any untoward medical occurrence or worsening of a pre-existing condition, regardless of its relationship to the investigational medicinal product. An AE can therefore be any unfavorable or unintended sign, including an abnormal laboratory finding, symptom, or disease temporally associated with the use of an investigational medicinal product whether or not considered related to the product. Adverse events that are reported after signing the informed consent but prior to randomization, will be recorded in medical history. Adverse events that are reported after initiation of treatment up until the final study visit phone call on Day 31 will be considered TEAEs. Serious adverse events occurring at any time from informed consent to 28 days after the last dose of treatment with study drug will be reported. Subjects will be monitored for AEs throughout the entire study. Investigators will ask the subject at each visit if they have experienced any untoward effects since the last study visit. The Investigator or designee will record the following information about AEs on the eCRFs: an AE term or description of the event, severity, event start date, event end date (if known), any action (eg, treatment and follow-up tests), the outcome of the event, and the Investigator's assessment of the relationship to the study treatment.

Even if the Investigator feels there is no relationship to the study drug, all AEs must be recorded in the eCRF.

## 12.2 Serious Adverse Events

An SAE is any untoward medical occurrence or effect that meets any of the following criteria:

- results in death;
- is life-threatening (refers to events during which a subject was actually at risk of death, and not to events that might have become life-threatening if they had occurred in a more severe form or if the subject had not received treatment);
- requires inpatient hospitalization (> 24 hours) or causes prolongation of existing inpatient hospitalization;
- results in a persistent or significant disability or incapacity;
- is a congenital abnormality/birth defect in the offspring of a study subject;
- is an important medical event that does not meet any of the above criteria but may require medical intervention to prevent one of the outcomes listed above, or is considered medically important by the Investigator; examples of important medical events include:
    - Bronchospasm requiring treatment in the emergency department or at home
    - Development of a blood dyscrasia
    - Diagnosis of a substance use disorder

## 12.3 Reporting of Serious Adverse Events

On behalf of Spyryx Biosciences, Inc, PRA Drug Safety will be managing SAE processing and reporting to regulatory authorities, as required by local regulations.

In addition to completing all eCRFs related to SAEs, Investigators must report all SAEs to PRA Drug Safety within 24 hours after learning about the event. PRA Drug Safety contact information is provided below:

**PRA Drug Safety Contact Information:**

European Investigative Sites:

Drug Safety Helpline: +49 621 878 2154
Fax: +44 1792 525 720
E-mail: MHGSafety@prahs.com

North American Investigative Sites:

Drug Safety Helpline: 1-800-772-2215 or 1-434-951-3482
Fax: 1-888-772-6919 or 1-434-951-3482
E-mail: CHOSafety@prahs.com

To be valid, the initial SAE report must include the following minimal data:

- A reporting source (the Investigator's name and/or site number, and contact information)
- A subject identifier
- The identity of the investigational product
- A SAE term, description, or outcome

The following additional data should be provided as soon as possible, but are not required for the initial report if they are not immediately available:

- Event start date
- Event end date if known
- Severity: mild, moderate, severe, life-threatening, or fatal
- The Investigator's assessment of causality: unrelated, unlikely related, possibly related, probably related, or definitely related
- Any serious criteria that apply to the event (see Section 12.2)

Investigators should submit follow-up reports for SAEs whenever additional pertinent information becomes available, and should provide copies of all pertinent documents such as hospital records, admission and discharge summaries, surgical records, laboratory reports, autopsy reports, and other documents when requested and applicable.

## 12.4 Monitoring of Subjects for Adverse Events

Each subject must be carefully monitored for AEs, including clinically significant abnormal laboratory test results, physical examination findings, and ECG parameters. At each study visit or communication, the Investigator or designee will ask subjects about any new symptoms or worsening of baseline symptoms that could indicate an AE.

### 12.4.1 Abnormal Laboratory Test Results

Changes or abnormalities in clinical laboratory results judged to be clinically significant by the Investigator will be recorded as AEs. If an unexplained abnormal laboratory test result occurs, the test should be repeated immediately, and the subject should be monitored until the result has returned to the normal range or an adequate explanation of the abnormality is found.

### 12.4.2 Abnormal Physical Examination Findings or ECG Changes

Changes or abnormalities in physical examination findings or ECGs judged to be clinically significant by the Investigator will be recorded as AEs.

## 12.5 Overdose of Study Medication

No overdoses have occurred to date in any clinical studies with SPX-101. Any overdose of the study medication, with or without associated AEs, must be reported to PRA Drug Safety. Overdose will be reported in the eCRF. All reports of overdoses must be filed in the Study Center File. Any AEs associated with the overdose should be reported on relevant AE/SAE eCRFs.

Investigators will instruct subjects regarding the management of their study medication, other medications including those for diabetes, and procedures to be followed if an overdose occurs. Subjects who experience an overdose of study medication should notify the Investigator as soon as feasible and seek appropriate supportive management if needed.

## 12.6 Pregnancy

Pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication. However, any pregnancy during the study and the outcome of the pregnancy (eg, spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be reported to PRA.

If a subject becomes pregnant after exposure to the study drug, she must stop the investigative drug and report the pregnancy to the Investigator immediately. The Investigator must then submit a completed Pregnancy Report Form to PRA Drug Safety and follow all procedures for early withdrawal from the study (Section 9.4.4.2). In the event of pregnancy, the Investigator should continue to monitor the subject through either childbirth or the end of pregnancy, and must submit a pregnancy follow-up form to PRA Drug Safety. Any congenital anomaly or birth defect in the offspring of the subject should be reported as an SAE. Investigators should contact the Medical Monitor through the Medical Monitoring Support Center if questions arise.

# 13. DATA MANAGEMENT AND STATISTICAL ANALYSIS

## 13.1 Data Management

An eCRF will be used for the current study and a Data Management Plan will be prepared by PRA Health Sciences.

Previous medications taken within 28 days of screening and all concomitant medications will be coded using the latest available WHO Drug Dictionary. Coexistent diseases and AEs will be coded using MedDRA.

When the database for a given cohort has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time can be made only by written agreement between Spyryx Biosciences, Inc, and the PRA project team. For further details, see the Data Management Plan.

## 13.2 Sample Size Estimation

The study was planned for a minimum of 39 subjects per cohort to be enrolled. In Cohort 1, 46 subjects were randomized in a 1:1:1 ratio to one of the two treatment groups or placebo. The sample size of 39 subjects was chosen to provide 70% power for the Analysis of Variance (ANOVA) to detect a difference range of 4.5% to 6% in the change from baseline in $ppFEV_1$ and $FEV_1$ between the 2 treatment groups versus placebo, with the alpha level of 0.15 assuming a common standard deviation of 7%. The attrition rate is expected to be minimal given the 4 weeks' treatment duration.

In Cohort 2, a total of 45 subjects will be randomized in a 2:1 ratio to SPX-101 120 mg BID or placebo. The sample size of 45 subjects will provide 88% to 97% power to detect a difference range of 6% to 8% in the change from baseline in $ppFEV_1$ between the treatment group versus placebo, with the 1-sided alpha level of 0.1 assuming a common standard deviation of 7.58%.

## 13.3 Statistical Analysis Plan

The Statistical Analysis Plan (SAP) will be written and finalized prior to lock of the first cohort database. The SAP will provide a detailed description of the statistical methods and expand on the details provided in the protocol. Additional analyses may be added. A shell of the tables, listings, and figures will also be provided.

## 13.4 Randomization

Two sequential, double-blind cohorts are planned to test the study drug SPX-101 at different dosages compared to placebo. Each cohort will have its own baseline. Cohort 1 had 3 groups: 2 treatment groups with different dosages of SPX-101 and a placebo group, and Cohort 2 will have 1 treatment group and a placebo group. Each cohort will be analyzed separately. In Cohort 1, randomization was stratified by whether the baseline (Visit 2) $ppFEV_1$ was between 40.0% and 55.0% or between 55.1% and 80.0%. In Cohort 2, randomization will be stratified according to use of concomitant hypertonic saline so that approximately 50% of subjects will be using hypertonic saline (defined as having used a constant regimen of hypertonic saline for 28 days prior to screening, and planning to use their same concentration

without change through 28 days post-randomization. All randomized subjects must satisfy the randomization criteria in order to be eligible for study medication.

## 13.5 Analysis Populations

### 13.5.1 Safety Population

The Safety population will include all enrolled subjects who receive at least 1 dose of the study drug.

### 13.5.2 Intent-to-Treat Population

The Intent-to-Treat (ITT) population will include all randomized subjects. Subjects will be grouped according to their randomized study group assignment regardless of the actual treatment received.

### 13.5.3 Per-Protocol Population

The Per-Protocol (PP) population will include all subjects in the ITT population who did not have major protocol violations. Major protocol violations are defined as those that may have a substantial impact on efficacy assessments. The criteria to be used for excluding subjects from the PP population will be determined before database lock and will be documented. The PP analysis may be performed only for the primary and secondary efficacy endpoints, to provide supportive evidence for efficacy.

## 13.6 Statistical Methods

### 13.6.1 Missing Data

Missing data will not be imputed, with the exception of missing or partial dates. The imputation rules of missing or partial dates will be specified in the SAP.

### 13.6.2 Demographic, Cystic Fibrosis History, and Baseline Data

The demographic and baseline data, including CF history, will be summarized descriptively by study group. The continuous variables will be summarized including the number of observations, mean, standard deviation, median, the first quartile (Q1), the third quartile (Q3), and minimum and maximum values. The categorical variables will be summarized using frequency and percentage. All demographic and baseline data will be listed. Baseline will be defined as the last observation of an assessment prior to the first dose of study drug.

### 13.6.3 Subject Disposition

The number and percentage of subjects who complete the study treatment, who discontinue early, and the reasons for early discontinuation will be summarized.

### 13.6.4 Efficacy

#### *13.6.4.1 Primary Efficacy Analysis*

The primary endpoint is the change from baseline in ppFEV$_1$ at Week 4 (Day 29). The change from baseline in ppFEV$_1$ at each visit (Weeks 1, 2, and 4) will be summarized and compared between each treatment group and placebo.

The primary analysis for the change from baseline in $ppFEV_1$ at Week 4 will be based on an analysis of covariance (ANCOVA). For Cohort 1, the model will also include the stratification variable baseline lung function categories ($ppFEV_1$ 40.0% to 55.0% or 55.1% to 80.0%). For Cohort 2, the model will include the stratification variable of concomitant use of hypertonic saline.

*13.6.4.2 Secondary Efficacy Analysis*

$ppFEV_1$

Similar analyses will be performed for the change from baseline in $ppFEV_1$ at each of the remaining visits (Weeks 1, and 2).

To support these analyses, the change from baseline in $ppFEV_1$ at each visit (Weeks 1, 2, and 4) will be considered based on a mixed effect model for repeated measure (MMRM). The model will include the change from baseline in $ppFEV_1$ as the dependent variable; treatment, visit and treatment by visit interaction as fixed effects; and subject as a random effect. In this model, visits will be treated as a class variable. The structure of the covariance matrix will be investigated and the most appropriate will be assumed. This will be documented in the final Statistical Analysis Plan prior to unblinding.

$FEV_1$

To investigate the robustness of the results, similar ANCOVA analyses will be performed for the change from baseline in $FEV_1$ at each visit (Weeks 1, 2, and 4).

FVC and Percent Predicted FVC

Similar to the $FEV_1$ analysis, FVC and ppFVC will be analyzed.

CFQ-R

The change from baseline in CFQ-R respiratory domain and physical domain at Week 4 will be summarized and compared between the treatment groups and the placebo.

*13.6.4.3 Exploratory Analyses*

Pulmonary Exacerbation

The number and percentage of subjects with pulmonary exacerbation (See Appendix 3, Section 18.3 for specific criteria) will be summarized.

Concomitant Hypertonic Saline

The effect of concomitant use of hypertonic saline will be explored. Each of the primary and secondary endpoints will be analyzed accounting for the use of hypertonic saline.

### 13.6.5 Pharmacokinetics – Cohort 1 Only

At Visit 2 only, blood samples were collected pre-dose and at 5 ± 2 minutes and 15 ± 5 minutes after completion of administration of the study nebulizer treatment. The pharmacokinetic results will be listed.

### 13.6.6 Safety

Safety data will be summarized for the safety population.

Exposure to the study drug will be summarized. Summaries will include the number of doses received and dose compliance.

All AEs reported after initiation of treatment, and pre-existing conditions that worsen or become more frequent after initiation of treatment will be considered TEAEs. Adverse events will be coded by system order class and preferred term using MedDRA. All recorded AEs will be included in the data listings.

The number and percentage of subjects with TEAEs, SAEs, AEs leading to death, and AEs leading to discontinuation of the study drug will be summarized by preferred term, and where appropriate, system organ class. Deaths, SAEs, and AEs leading to discontinuation of the study drug will also be listed, and narratives will be written.

Summary statistics for actual values and for change from baseline will be tabulated as appropriate for vital signs and clinical laboratory test results. Results of ECGs will be summarized and results of physical examinations will be listed.

All non-study medications received during the treatment period will be considered concomitant medications. Previous and concomitant medications will be coded using the WHO Drug Dictionary and will be summarized.

### 13.6.7 Additional Data

Additional summaries and data listings may be defined in the SAP as appropriate.

### 13.6.8 Interim Analysis

There will be no interim analysis in this study. At the end of the Cohort 1 part of the study, all data was locked prior to review by the Sponsor to select the Cohort 2 dosing regimens.

### 13.6.9 Data Safety Monitoring Board

An independent DSMB will periodically review the accrued safety data in an unblinded manner to monitor the safety of subjects. There will be 3 scheduled DSMB meetings at the following time points: midpoint of Cohorts 1 and 2 (after approximately 19 subjects have been randomized) and at the completion of Cohort 1. Additional meetings may be scheduled at the discretion of the DSMB members. The DSMB will be empowered to make any recommendations relevant to safety, and specifically will make recommendations to revise study procedures to increase subject safety or to stop the study based on periodic data reviews. The DSMB may also make recommendations on the dosage selection in Cohort 2.

The DSMB charter will be finalized before the first subject has been randomized to the study drug, and will define the membership and organization of the DSMB. In addition, the DSMB charter will define the safety information that will be reviewed, the reporting obligations and responsibilities of the DSMB, and will specify what information, if any, may be shared with the Sponsor or investigators.

# 14. MONITORING PROCEDURES (QUALITY ASSURANCE)

The Sponsor has ethical, legal, and scientific obligations to conduct this study in accordance with established research principles and ICH GCP guidelines. As such, to fulfill these obligations and to maintain current of study progress, the Sponsor's monitors or representatives will visit the investigative sites during study conduct, in addition to maintaining telephone and written communication. On-site visits, telephone calls, and regular inspection of the CRFs will be conducted to assess subject enrollment, compliance with protocol procedures, completeness and accuracy of data entered on the CRFs, verification of CRF data against original source documents, and occurrence of AEs. The Investigator must provide the monitor with full access to all source and study documents.

## 14.1 Routine Monitoring

PRA-assigned study monitors will conduct regular site visits to the investigational facilities to monitor various aspects of the study. The Investigator must agree to provide direct access for Sponsor- and PRA-authorized personnel to the clinical (or associated) files and clinical study supplies (dispensing and storage areas) for all study subjects considered for study entry, to verify entries made in the CRF, and they must assist the study monitors with their activities, if requested. Adequate time and space for monitoring visits should be made available by the Investigator.

The site must complete the CRFs in a timely manner and on an ongoing basis to allow regular review by the study monitor.

## 14.2 Inspections and Auditing Procedures

The Sponsor or its representative may conduct audits at the investigative sites including, but not limited to, drug supply, presence of required documents, the informed consent process, and comparison of eCRFs with source documents. All medical records (progress notes) must be available for audit. The Investigator agrees to participate with audits conducted at a convenient time in a reasonable manner.

Government regulatory authorities may also inspect the Investigator during or after the study. The Investigator or designee should contact the Sponsor and PRA immediately if this occurs. The Investigator must cooperate fully with regulatory authorities or other audits conducted at a convenient time in a reasonable manner.

The purpose of an audit is to assess whether ethics, regulatory, and quality requirements are fulfilled.

# 15. STUDY MANAGEMENT AND MATERIALS

## 15.1 Electronic Case Report Forms

Electronic CRFs will be used to store and transmit subject information. The file structure and format for the eCRF will be provided by the Sponsor or their representative and should be handled in accordance with the instructions provided.

The eCRF must be reviewed and electronically signed and dated by the Investigator.

Access to the eCRF will be strictly password protected and limited to personnel directly participating in the study. Data should be entered into the eCRF completely by examining personnel or the study coordinator. The eCRF must be completed as soon as possible after any subject evaluation or communication. If data is to be changed due to erroneous input or other reason, an electronic audit trail will track these changes. The eCRFs and computers that store them must be accessible to study monitors and other regulatory auditors.

## 15.2 Data Collection

During each study visit, a physician participating in the study will maintain progress notes, in ink, in the subject's medical records to document all significant observations. At a minimum, these notes will contain:

- The date of the visit and the corresponding day or visit in the study schedule (e.g., screening, Day 1, Day 29, etc.)
- General condition and status remarks by the subject and any significant medical findings, including the severity, frequency, duration, and resolution of any reported AE, and the Investigator's assessment regarding whether or not the reported AE is study drug-related
- Changes in concomitant medications or dosages
- A general reference to the procedures completed
- The signature or initials of all physicians making an entry in the medical record (progress notes)

In addition, any contact with the subject via telephone or other means that provides significant clinical information will also be documented in the medical record (progress notes), as described above.

Information from the medical records (progress notes) and other source documents will be promptly transcribed to the appropriate section of the eCRF.

Changes to information in the medical record (progress notes), eCRFs, and other source documents will be initialed and dated on the day the change is made by the Investigator or designee. If the reason for the change is not apparent, the Investigator or designee will write a brief explanation for the change.

## 15.3 Source Documents Maintenance

Source documents contain the results of original observations and activities of a clinical investigation. Source documents include, but are not limited to, medical records (progress notes), computer printouts, screening logs and recorded data from automated instruments.

All source documents from this study will be maintained by the Investigator and made available for inspection by authorized persons. The original signed informed consent for each subject shall be filed with records kept by the Investigator and a copy shall be given to the subject.

## 15.4 Record Maintenance

All data derived from the study will remain the property of Spyryx Biosciences, Inc.

Records must be retained in accordance with the current ICH Guidelines on GCP. All essential study documents including records of subjects, source documents, CRFs and study drug inventory must be kept on file.

Essential documents should be retained until at least two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or until at least two years have elapsed since the formal discontinuation of clinical development of the investigational products. However, essential documents may be retained for a longer period if required by the applicable regulatory requirements or by agreement with the Sponsor. The Sponsor is responsible for informing the Investigator when these documents need no longer be retained.

The Investigator will not dispose of any records relevant to this study without written permission from the Sponsor, and will provide the Sponsor the opportunity to collect such records. The Investigator shall take responsibility for maintaining adequate and accurate hard copy source documents of all observations and data generated during this study. Such documentation is subject to inspection by the Sponsor, its representatives and regulatory authorities.

If an Investigator moves, withdraws from an investigation, or retires, the responsibility for maintaining the records may be transferred to another person who will accept responsibility. Notice of transfer must be made to and agreed by the Sponsor.

## 15.5 Confidentiality

All information obtained during the conduct of the study with respect to the subject's state of health will be regarded as confidential. For disclosure of any such information, an agreement will be obtained in writing.

The Investigator must ensure that each subject's anonymity is maintained. On CRFs and other documents submitted to the Sponsor or the CRO, subjects must not be identified by name. Instead, subjects will be known only by the unique subject number allocated to them to ensure confidentiality on all study documentation. Subjects will retain this unique number throughout the study. The Investigator will keep a separate log of these codes.
To comply with government regulatory guidelines and to ensure subject safety, it may be necessary for the Sponsor and its representative, PRA personnel, the local research review

board, or regulatory authority to review subjects' medical records as they relate to this study. Only the subject's unique number on the eCRFs will identify him/her, but their full names may be made known to a drug regulatory authority or other authorized government or health care officials, if necessary, and to personnel designated by the Sponsor.

Documents that are not for submission to the Sponsor or PRA (eg, consent forms) will be maintained by the Investigator in strict confidence, except to the extent necessary to allow monitoring by the Sponsor and PRA, and auditing by regulatory authorities. No documents identifying subjects by name will leave the investigative site, and subject identity will remain confidential in all publications related to the study.

# 16. ADMINISTRATION PROCEDURES

## 16.1 Regulatory Approval

Spyryx Biosciences, Inc. or their appointed agents will be responsible for ensuring that appropriate regulatory authority approvals are obtained, according to local country requirements.

No subject may enter the study until this approval has been obtained. A copy of the approval (where one is provided, according to local country requirements) will be provided to the Investigator and to the IRB(s)/IEC(s).

## 16.2 Protocol Amendments

In accordance with ICH Topic E 6 (R1) Guideline for GCP the Investigator should not implement any deviation from, or changes to the protocol without agreement by the Sponsor and documented approval from the IRB/IECs of a protocol amendment, except where necessary to eliminate an immediate hazard(s) to study subjects, or when the change involves only logistical or administrative aspects of the study, eg, change in monitor or change of telephone numbers.

Substantial amendments will be submitted to the IRB/IEC for written approval and where applicable, to National Competent Authorities. Written approval must be obtained before implementation of the amended version occurs unless the amendment is implemented to increase safety measures for the patients in the study. The written signed approval from the IRB/IEC should specifically reference the Principal Investigator's name, protocol number, study title, and amendment number(s) that is/are applicable. The Investigator must await IRB/IEC approval of protocol amendments before implementing the changes, except where necessary to eliminate apparent immediate hazard to subjects. In these cases, the IRB/IEC must be notified within 5 days of the change.

Once approved, the protocol amendment will be distributed to all recipients of the original protocol, with instructions to append the amendment to the protocol.

If, in the judgment of the local IRB/IEC, the Investigator and/or Sponsor, the protocol amendment alters the study design, procedures and/or increases the potential risk to the subject, the currently approved written informed consent form will require modification. The modified informed consent form must also be reviewed and approved by the IEC and where applicable, National Competent Authorities as described above for protocol amendments. In such cases, repeat informed consent must be obtained from subjects enrolled in the study before participation continues.

## 16.3 Protocol Adherence and Deviations

The protocol must be read thoroughly and the instructions must be followed. However, exceptions will be made in emergency situations when the protection, safety, and well-being of the subject requires immediate intervention based on the judgment of the Investigator or a responsible, appropriately trained, and credentialed professional(s) designated by the Investigator as a sub-investigator.

In the event of a significant protocol deviation due to an emergency, accident, or error, the Investigator or designee must contact the Medical Monitor as soon as possible by telephone. An early joint decision will be made regarding whether or not the subject should continue in the study. The Investigator, the Sponsor, and the Medical Monitor will document this decision.

### 16.4 Publication Policy

After completion of the study, the Investigator(s) may prepare a joint publication with the Sponsor. The Investigator(s) must not undertake to submit any part of the data from this protocol for publication without the prior consent of Spyryx Biosciences, Inc.

### 16.5 Clinical Study Report

A final clinical study report will be prepared according to the ICH guideline on Structure and Contents of Clinical Study Reports. A final clinical study report will be prepared regardless of whether the study is completed or prematurely terminated.

### 16.6 Contractual and Financial Details

The Investigator (and/or, as appropriate, the hospital administrative representative) and the Sponsor will sign a clinical study agreement prior to the start of the study, outlining overall Sponsor and Investigator responsibilities in relation to the study. The contract should describe whether costs for pharmacy, laboratory and other protocol-required services are being paid directly or indirectly. Financial Disclosure Statements will need to be completed, as requested by FDA CFR 21 part 54.

### 16.7 Insurance, Indemnity, and Compensation

Spyryx Biosciences, Inc. undertakes to maintain an appropriate clinical study insurance policy.

Deviations from the study protocol - especially the prescription of a dose other than that scheduled in the study protocol, other modes of administration, other indications, and longer treatment periods - are not permitted and shall not be covered by the statutory subject insurance scheme.

### 16.8 Discontinuation of the Study

This study may be terminated by the Sponsor. The study may also be terminated prematurely at any time when agreed to by both the Investigators and the Sponsor as being in the best interests of subjects, and justified on either medical or ethical grounds. In terminating the study, Spyryx Biosciences, Inc, PRA Health Sciences, and the Investigator will ensure that adequate consideration is given to the protection of the subjects' interests.

### 16.9 Study Center File Management

The Investigator is responsible for ensuring that the Study Center File is maintained in accordance with GCPs.

## 17. REFERENCE LIST

1. Farrell PM, Rosenstein BJ, White TB, Accurso FJ, Castellani C, Cutting GR, et al. Guidelines for Diagnosis of Cystic Fibrosis in Newborns Through Older Adults: Cystic Fibrosis Foundation Consensus Report. *J Pediatr.*2008 August; 153(2):S4-S14.

2. Quanjer PH, Stanojevic S, Cole TJ, Baur, X, Hall GL, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J.* 2012;40:1324-1343.

3. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, et al. Series "ATS/ERS Task Force: Standardisation of Lung Function Testing" – Standardisation of spirometry. *Eur Respir J.* 2005;26:319-338.

4. Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsay BW, et al. Effect of aerosolized recombinant human DNAase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. *N Engl J Med.* 1994;331:637-642

5. Knowles MR, Boucher RC. Mucus clearance as a primary innate defense mechanism for mammalian airways. *J Clin Invest*. 2002;109(5):571-577.

6. Gaillard EA, Kota P, Gentzsch M, Dokholyan NV, Stutts MJ, Tarran R. Regulation of the epithelial Na+ channel and airway surface liquid volume by serine proteases. *Pflugers Arch*. 2010;460(1):1-17Caldwell RA, Boucher RC, Stutts MJ. Serine protease activation of near-silent epithelial Na+ channels. *Am J Physiol Cell Physiol*. 2004;286(1):C190-194.

7. Mall M, Grubb BR, Harkema JR, O'Neal WK, Boucher RC. Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. *Nat Med.* 2004;10(5):487-493.

8. Chmiel JF, Davis PB. State of the art: why do the lungs of patients with cystic fibrosis become infected and why can't they clear the infection? *Respir Res.* 2003;4:8.

9. Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med*. 2003;168(8):918-951

10. .Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, et al. Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. *J Clin Invest*. 2002;109(3):317-325.

11. Caldwell RA, Boucher RC, Stutts MJ. Serine protease activation of near-silent epithelial Na+ channels. *Am J Physiol Cell Physiol*. 2004;286(1):C190-194.

12. Knowles MR, Church NL, Waltner WE, Yankaskas JR, Gilligan P, King M, et al. A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. *N Engl J Med.* 1990 Apr 26;322(17):1189-1194.

13. Tarran R, Grubb BR, Parsons D, Picher M, Hirsch AJ, Davis CW, et al. The CF salt controversy: in vivo observations and therapeutic approaches. *Mol Cell.* 2001;Jul;8(1):149-158.

14. Why Participate in a Clinical Trial? Cystic Fibrosis Foundation website. https://www.cff.org/Our-Research/Clinical-Trials/Clinical-Trials-101/Why-Participate-in-a-Clinical-Trial/. Accessed January 5, 2017

# 18. APPENDICES

## 18.1 Appendix 1: Declaration of Helsinki

**WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI**
**Ethical Principles for Medical Research Involving Human Subjects**

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

### A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects**,** including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

4. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

**B. PRINCIPLES FOR ALL MEDICAL RESEARCH**

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy

volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where

consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

### C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the

physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

- The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
- Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

## 18.2 Appendix 2: Elements of Informed Consent

**ELEMENTS OF INFORMED CONSENT**

Both the informed consent discussion and the written informed consent form and any other written information to be provided to subjects should include explanations of the following:

- That the study involves research.
- The purpose of the study
- The study treatment(s) and the probability for random assignment to each treatment
- The study procedures to be followed including all invasive procedures
- The subject's responsibilities
- Those aspects of the study that are experimental
- The reasonably foreseeable risks or inconveniences to the subject and, when applicable, to an embryo, fetus, or nursing infant
- The reasonably expected benefits; when there is no intended clinical benefit to the subject, the subject should be made aware of this.
- The other approved procedure(s) or course(s) of treatment that may be available to the subject, and their important potential benefits and risks
- The compensation and/or treatment available to the subject in the event of study-related injury
- The anticipated prorated payment, if any, to the subject for participating in the study
- The anticipated expenses, if any, to the subject for participating in the study
- That the subject's participation in the study is voluntary and that the subject may refuse to participate or withdraw from the study, at any time, without penalty or loss of benefits to which the subject is otherwise entitled
- That the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the subject or the subject's legally acceptable representative is authorizing such access.
- That records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the study are published, the subject's identity will remain confidential.
- That the subject or the subject's legally acceptable representative will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the study.

- The person(s) to contact for further information regarding the study and the rights of study subjects, and whom to contact in the event of study-related injury
- The foreseeable circumstances and/or reasons under which the subject's participation in the study may be terminated
- The expected duration of the subject's participation in the study
- The approximate number of subjects involved in the study

## 18.3 Appendix 3: Criteria for Pulmonary Exacerbation

For the purpose of this study, the following criteria will be used to define pulmonary exacerbation:

Treatment with parenteral antibiotics for at least 4 of the following 12 signs or symptoms:

- Change in sputum
- New or increased hemoptysis
- Increased cough
- Increased dyspnea
- Malaise, fatigue, or lethargy
- Temperature higher than 38°C
- Anorexia or weight loss
- Sinus pain or tenderness
- Change in sinus discharge
- Change in physical examination of the chest
- Decrease in pulmonary function by 10% or more from a previously recorded value
- Radiographic changes indicative of pulmonary infection

Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsay BW, et al. Effect of aerosolized recombinant human DNAase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. *N Engl J Med.* 1994;331:637-642.

## 18.4 Appendix 4: Information for Subjects Enrolled in Study SPX-101-CF201

**General**

- Call your doctor if you have any questions about the study, the study medication or nebulizer, or anything else related to the study.
- During the study, call your doctor as soon as possible if you notice any of the following symptoms or worsening of symptoms:
    - Shortness of breath
    - Wheezing
    - Cough
    - Fever

**eFlow Nebulizer with eTrack**

- Remember that the eFlow study nebulizer with eTrack must be used **only** with the study drug, and you must use your regular nebulizer for all other medications.
- Always bring your eTrack study nebulizer to clinic visits.
- The study nebulizer should be cleaned after each use and sterilized once per day. Follow the instructions given to you for this process.

**Study Medication**

- Keep the study drug vials in the original container in the refrigerator between 2° and 8°C. The study drug is stable at room temperature for short periods of time, but should be refrigerated whenever possible.
- The study medication should be taken as instructed (ie, twice each day, approximately 12 hours apart. Study medication, should be inhaled only with the study nebulizer.
- You should take the last dose of study medication as instructed. For Cohorts 1 and 2, this will be the evening before your final clinic visit (Visit 5 [Day 29]).
- Discard used study drug vials at home after use.

**Order of Medications and Other Treatments**

- <u>In general:</u>
    - You should follow your usual routine of medication and chest physiotherapy, unchanged, throughout the study.
    - The study drug is added into your usual treatment regimen as the last mucolytic therapy inhaled:
        - The study drug should be used after inhalation of dornase alfa, mannitol, and/or hypertonic saline

        - The study drug should be used before inhalation of antibiotics or corticosteroids

- Long-acting β-agonist (LABA) like salmeterol or formoterol (if used):
    - If normally used in the morning, use the evening before the day of a clinic visit, and do not take the morning of the clinic visit.
- Short-acting β-agonist (SABA) like albuterol (if used):
    - On clinic days: take this at home, at least 4 hours before your clinic visit.
    - On non-clinic days: take this at the normal time.
- Other medications: If you have any questions about other medications and when or how to take them during this study, please ask your doctor.
- Preparing for Clinic Visit Days:
    - If you use a LABA, use it the evening before, and not on the morning of a clinic visit.
    - If you use a SABA, use it at least 4 hours before your clinic visit.
    - Do not take the study drug the morning before a clinic visit.
    - Take your routine medications, including oral antibiotics, and perform chest physiotherapy in the morning.
    - Remember to bring your study nebulizer and all unused study medication vials to the clinic.

## 18.5 Appendix 5: Bioclinica Trident User Requirement Specifications for Unblinding

| Visit Step | # | Visit Details |
|---|---|---|
| Visit Button Label | 8.8.1 | Break Blind |
| Transaction Eligibility | 8.8.2 | Site Is Active. |
| Subject Starting Status | 8.8.3 | Subject Status is RANDOMIZED, DROPPED |
| Visit Step | 8.8.4 | Select the BREAK BLIND button from the Home screen or subject data page |
| | 8.8.5 | Enter Kit ID<br>*Or*<br>Enter Subject ID |
| | 8.8.6 | Kit ID or Subject Number entered must exist in study and be valid for site. |
| | 8.8.7 | Next - Continue at next step |
| | 8.8.8 | Cancel - Return to home screen. No data is saved |
| Visit Step | 8.8.9 | If a subject number was entered or kit entered has been dispensed to a subject, continue to Step 8.8.10<br>If non-dispensed kit was entered, please go to 8.8.20 |
| Visit Step | 8.8.10 | Please confirm that you wish to break blind on subject (Subject ID) |
| Break Blind Reasons (required) | 8.8.11 | Blind Broken |
| Text Box Entry | 8.8.12 | ☐ Yes. "Other" option will display<br>☒ No. |
| Warning Message | 8.8.13 | WARNING! Subject will not be withdrawn from the study and will be able to continue in the visit schedule. |
| | 8.8.14 | Confirm – Continue |
| | 8.8.15 | Cancel - Return back to subject details. No data is saved |
| Visit Step | 8.8.16 | Please confirm that you wish to break blind on subject (Subject ID)<br>Reason: Break Blind<br><br>Enter Password: |
| Warning Message | 8.8.17 | WARNING! Subject will not be withdrawn from the study and will be able to continue in the visit schedule. |
| | 8.8.18 | Confirm – Continue |
| | 8.8.19 | Cancel - Return back to subject details. No data is saved |
| Subject Withdrawn after Break Blind<br>☐Yes ☒No | 8.8.20 | Subject is unblinded<br>Note: Subject status remains the same |
| Visit Result (Subject Unblinding) | 8.8.21 | The unblinded treatment group and kit type information is displayed |
| | 8.8.22 | Confirmation is sent to site based on role subscriptions |
| Break Blind Reasons (required) | 8.8.23 | Please confirm that you wish to unblind this kit:<br>Text box entry (required) |
| Visit Step | 8.8.24 | Confirm – Continue |
| | 8.8.25 | Cancel – Return to supply page. No data is saved |
| Visit Result (Kit Unblinding) | 8.8.26 | Kit ID, Kit Type ID and Description will be displayed |
| | 8.8.27 | Kit Status will be updated to UNBLINDED if status was currently AVAILABLE |
| | 8.8.28 | If Kit was Dispensed: Confirmation is sent to site based on role subscriptions for subject unblinded. |
| | 8.8.29 | If Kit not Dispensed: No document is generated for unblinding an undispensed kit. |

Source: Bioclinica Trident User Requirement Specifications, v3.0, pp 35-36