Statistical Analysis Plan

Sponsor:	SPYRYX Biosciences, Inc.
Protocol No:	SPX-101-CF-201
PRA Project ID:	SPYPX101-SPX101
Protocol Version Date:	14-May-2018
Protocol Version No.:	4.0 (including Versions 4.0A and 4.0B)

Title:	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)
CRF Version No./Date:	3.0/17-Jul-2018
SAP Version Date:	July 9, 2019
SAP Version No.	2.1

1.0 Approvals

Sponsor	
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Table of Contents

2.0 Purpose

The statistical analysis plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under SPYRYX Biosciences Protocol SPX-101-CF-201.

3.0 Scope

This plan is a living document that will be created during the trial start-up. The Statistical Analysis Plan will be finalized prior to database lock. SAP will require sign off from the Project Manager and the sponsor.

The Statistical Analysis Plan outlines the following:

- Study objectives
- Study design
- Variables analyzed and analysis sets
- Applicable study definitions
- Statistical methods regarding important protocol deviations, study drug exposure, efficacy analysis, concomitant medications, adverse events handling, laboratory data and physical examinations

4.0 Introduction

The Statistical Analysis Plan (SAP) Version 1.0 was written and finalized prior to database lock of the first cohort. Version 2.1 of the SAP was revised and finalized prior to the database lock of Cohort 2.

This statistical analysis plan (SAP) describes the methods to be used during the reporting and analyses of data collected under SPYRYX Biosciences Protocol SPX-101-CF-201.

This SAP should be read in conjunction with the study protocol and CRF. For Cohort 1, Version 1.0 of the plan was developed using the protocol dated 14Nov2017 and CRF dated 11MAY2017. For Cohort 2, Version 2.1 of the plan was developed using the protocol dated 14May2018 and CRF dated 17Jul2018. Any further changes to the protocol or CRF may necessitate updates to the SAP.

4.1 Changes from Protocol

Cohort 1 was analyzed per Version 1 of this SAP which covered Protocol Versions 1.0, 2.0 and 3.0).

Version 2 of this SAP covers changes associated with Protocol Version 4.0. The primary changes associated with Protocol Version 4.0 are summarized in Section 6.0.

Protocol Version 4.0 lists “Incidence of antidrug antibodies (ADA)” as an exploratory variable, but this version of the SAP will not include this as an exploratory variable. Instead, ADA will be explored as a safety variable and presented as such.

5.0 Study Objectives

5.1 Primary Objective

- To evaluate the efficacy of SPX-101 in subjects with Cystic Fibrosis (CF)

5.2 Safety Objective

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

5.3 Pharmacokinetic Objective

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

6.0 Study Design

This randomized, double-blind, placebo-controlled, Phase II study is designed to evaluate the efficacy and safety of SPX-101 inhalation solution in a 28-day treatment period in approximately 90 adults with CF. Two sequential cohorts were planned to compare the study drug SPX-101 (2 groups) with placebo (1 group) in Cohort 1 (1:1:1 ratio). Cohort 2 compares the study drug SPX-101 (120 mg BID) to placebo in a 2:1 randomization ratio. Each cohort will have its own baseline and will be analyzed separately. To achieve balance between treatment groups, randomization will be 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%) in Cohort 1 and stratified by concomitant hypertonic saline use in Cohort 2.

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 SPX-101 daily doses were anticipated to not exceed those studied in Cohort 1 and may have included 240 mg once daily (QD), 120 mg QD, 60 mg QD, or 30 mg BID. The actual dose regimens used in the 2 active treatment groups in Cohort 2 were determined by the Sponsor in consultation with the Data Safety Monitoring Board (DSMB) based on the efficacy and safety results of Cohort 1.

Cohort 2 treatment groups are the following:

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

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 (± 3 days) of an inhaled antibiotic cycle. If subjects still meet the randomization criteria on Day 1 (Visit 2), they will be randomized to the treatment groups 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 post dose 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.

The duration of the study from first subject first visit to last subject last visit will be dependent on the ability of the sites to identify and enroll eligible subjects.

Figures 1 and 2 show the design for Cohort 1 and Cohort 2, respectively. Table 1 shows the schedule of events and assessments.

Figure 1 Study Diagram – Cohort 1

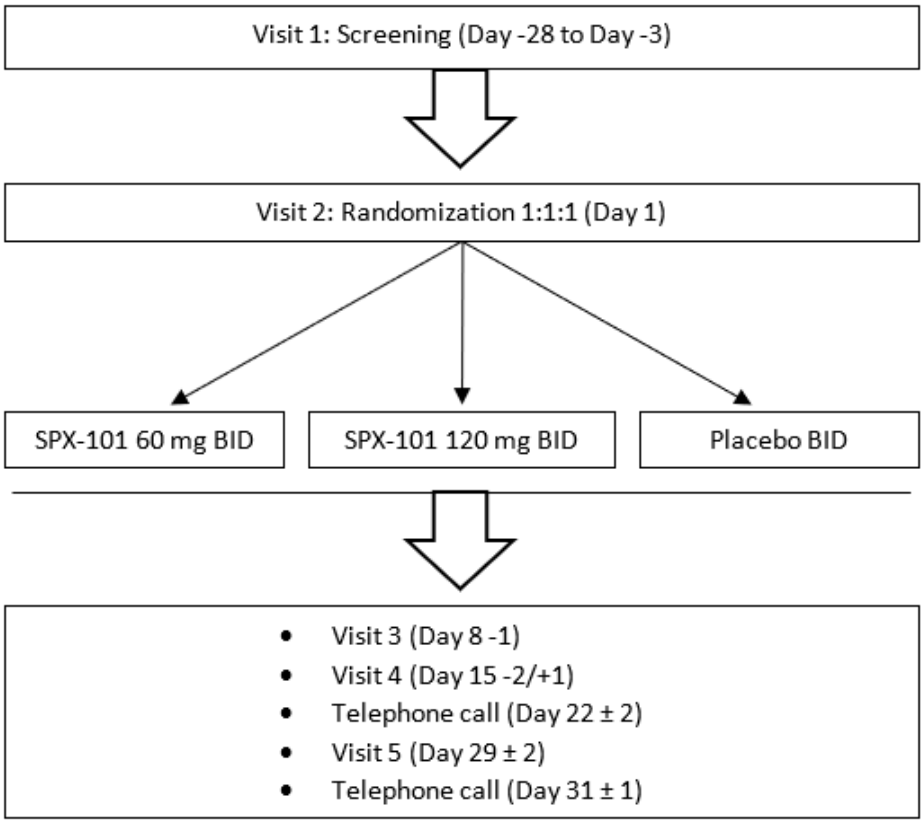

Figure 2 Study Diagram – Cohort 2

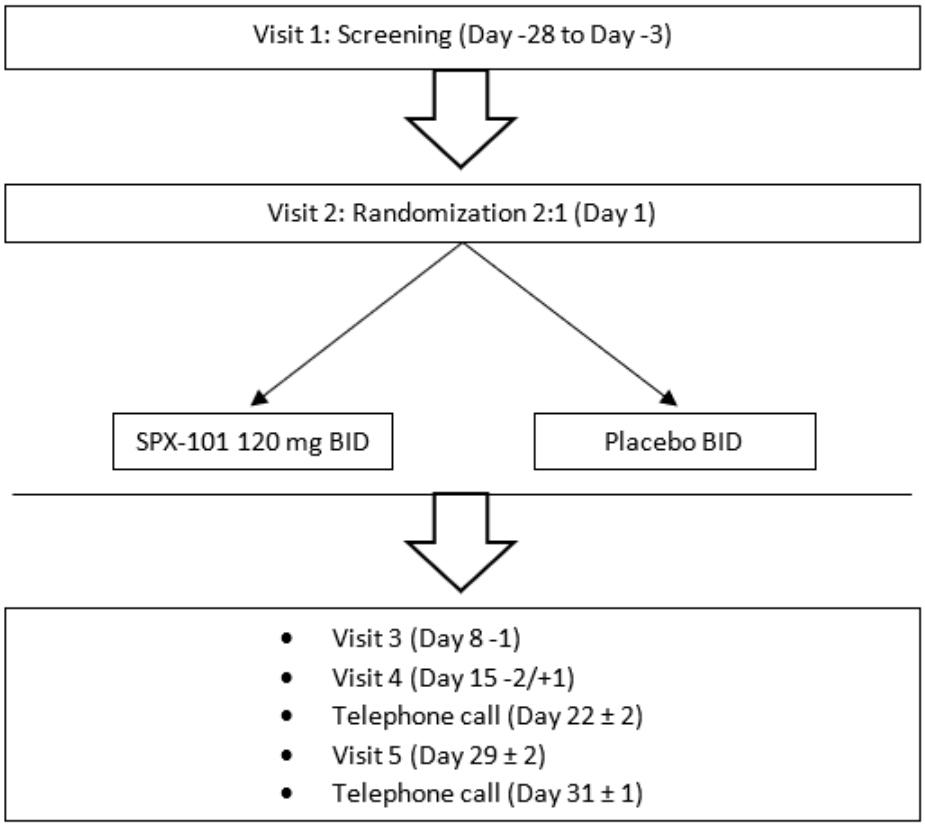

Table 1 Schedule of Events

	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 of the protocol).

[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 of the protocol 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 of the protocol).
[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±5 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.

6.1 Sample Size Considerations

In Cohort 1, the study was planned for a minimum of 39 subjects per cohort to be enrolled. The subjects will be randomized in a 1:1:1 ratio to one of the two treatment groups or placebo. The sample size of 39 subjects provided 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 two-sided alpha level of 0.15 assuming a common standard deviation of 7%. The attrition rate is expected to be minimal given the 4 week 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%. The estimated treatment difference and standard deviation are based on the Cohort 1 results from the 120 mg SPX-101 dose group and placebo, among subjects with baseline ppFEV$_1$ between 50.0% and 80.0%, since this reflects the subjects enrolled in Cohort 2.

6.2 Randomization

The Investigator or designee will use an Interactive Voice/Web Response System to randomize eligible subjects for each cohort. In Cohort 1, randomization was stratified by the Visit 2 pre-dose ppFEV$_1$ (between 40.0% and 55.0% vs between 55.1% and 80.0%). Only those subjects who met the randomization criteria were eligible for assignment to study treatment. Subjects who failed to meet the randomization criteria could have had Visit 2 repeated once, or they were considered screen failures. In Cohort 2, randomization was stratified by concomitant use of hypertonic saline. NOTE: The Cohort 2 FEV$_1$ entrance criterion was modified to require a screening ppFEV$_1$ between 50 – 80% of predicted normal thus no stratification by baseline ppFEV$_1$ was undertaken.

7.0 Study Variables and Covariates

7.1 Primary Efficacy Variables

- Change from baseline in ppFEV$_1$ at visit 5 (week 4)

7.2 Secondary Efficacy Variables

- Change from baseline in ppFEV$_1$ at visits 3 and 4 (weeks 1 and 2)
- Change from baseline in FEV$_1$ at visits 3, 4 and 5
- Change from baseline in forced vital capacity (FVC) and percent predicted FVC (ppFVC) at visits 3, 4 and 5
- Change from baseline in 25-75% forced expiratory Flow (FEF$_{25-75}$) at visits 3, 4 and 5
- Change from baseline in Cystic Fibrosis Questionnaire-Revised (CFQ-R) (respiratory and physical domains) at visit 5.

7.3 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

7.4 Pharmacokinetic Variable:

- Systemic exposure to SPX-101 (Cohort 1 only)

7.5 Exploratory Variables:

- Incidence of pulmonary exacerbation, as defined by Fuchs (1994)
- Incidence of concomitant hypertonic saline use

7.6 Predetermined Covariates and Prognostic Factors

In Cohort 1, baseline lung function ppFEV$_1$ categories (percent predicted forced expiratory volume in 1 second between 40.0% and 55.0% vs between 55.1% and 80.0%) will be included as a covariate in the model of the analysis of covariance (ANCOVA).

In Cohort 2, the use (yes/no) of concomitant hypertonic saline and baseline ppFEV$_1$ will be covariates in the ANCOVA model.

8.0 Definitions and Data Derivations

Baseline

For two sequential cohorts, each cohort will have its own baseline. Unless otherwise specified, baseline is defined as the last non-missing value before the first dose of study drug (SPX-101 or placebo). Baseline values will be assessed at Visit 2 (Day 1) in this study. If Visit 2 measures are missing, corresponding variables at screening visit will be used as baseline.

For spirometry endpoints, the baseline is defined as the last non-missing value before the first dose of study drug (SPX-101 or placebo) at Visit 2 (Day 1) for primary analysis. The average of screening and Visit 2 (Day 1) values will also be derived for exploratory purpose.

Change from Baseline

Change from baseline (CFB) will be calculated as (value at post-baseline visit – value at baseline). CFB will be calculated for subjects with both a baseline and post-baseline value as applicable. Percent CFB will be calculated as (CFB/baseline)*100, where applicable. If a baseline or post-baseline value has not been recorded for a parameter, then CFB will not be calculated for that parameter. Subjects with missing CFB values will be excluded from analyses in which CFB is the endpoint unless otherwise noted.

Study Day 1

The first day of study product administered.

Study Day

Study day is defined as the number of days from Study Day 1.

- Before Study Day 1: Study Day = (Date of Interest – Date of Study Day 1)
- On or After Study Day 1: Study Day = (Date of Interest – Date of Study Day 1) + 1

Therefore, the day prior to Study Day 1 is -1.

Study Completion

A subject is considered completed the study if a subject is not terminated early and completes Visit 5 (Day 29).

Prior and Concomitant Medications

Prior medications will be any medication with a start date before the first day of treatment that has been discontinued prior to the first dose of study treatment.

Concomitant medications are defined as any medications ongoing at the start of treatment or with a start date on or after the first dose date but on or prior to the last dose of treatment date.

Treatment-emergent Adverse Event (TEAE)

A TEAE is defined as an AE which begins or an aspect of medical history that increases in severity after the first dose of study drug until the final study visit phone call on Day 31. Adverse Events will be considered treatment-emergent if they have missing or partial start dates for which it cannot be determined whether the AE started before or after the first dose of study medication.

Adverse Events with Outcome of Death

Any AE with an outcome of "fatal" will be considered as AE with an outcome of death.

Imputation of AE (for determination of TEAE only) and concomitant medication start and stop dates:

Start Date:

If only 'day' is missing, and the month and year are not the same as the month of first dose, then impute day with '01'. Otherwise, if the month and year are the same as the first dose date, use the first dose date.

If 'day' and 'month' are missing, and 'year' is not missing, then impute month and day with month and day of the first dose date (assuming same 'year').

If 'day' and 'month' are missing and 'year' is not missing and is not the same year as first dose date, then impute with '01' for both 'day' and 'month'. If the start date is completely missing, it will be set to the first dose date.

Stop Date:

If only 'day' is missing, impute day with last day of the month.

If 'day' and 'month' are missing, and 'year' is not missing, then impute month with '12' and day with '31' (or date of study discontinuation/completion if earlier than 12-31 and year is the same as the year of discontinuation).

If the stop date is completely missing, it will be set to the date of study discontinuation/completion. A stop date will not be applied to ongoing AEs.

The imputed dates will be used to assess whether AEs should be considered as treatment-emergent and if medications should be included in the safety summaries as prior or concomitant, however the original, partial dates will be included in data listings. The duration of AEs will be derived as the AE end date (including imputation date for incomplete AE end date) –AE onset date (imputed date for incomplete AE onset date) +1.

Treatment-related Adverse Events

Any AE with a relationship to study treatment of "Possibly Related" or "Definitely Related" or missing will be considered a treatment-related AE as determined by the Investigator.

Analysis Visit window

Since the actual visit for a subject may not exactly coincide with the scheduled visit date, the actual visit date is mapped to the study analysis visit as follows:

Study Analysis Visit	Target Day	Study Day	Interval (days)
Visit 1, Screening	Varies	<= -1	NA
Visit 2, Baseline	1	1	NA
Visit 3, Week 1	7	2 – 11	10
Visit 4, Week 2	14	12 – 21	10
Visit 5, Week 4	28	≥22	NA

For lab assessment and spirometry assessment, if more than one actual visit falls within the same defined window, the visit closest to the target day with non-missing data will be considered for analysis. If two actual visit dates are at the same distance from the target day, the latest visit with non-missing data will be considered for analysis.

All summaries and analyses will utilize analysis visits, where appropriate. Data listings will present actual visit names as recorded in the clinical database. Tabulations will not include rows for unscheduled visits, however, unscheduled visits will be included in listings.

Cystic Fibrosis Questionnaire – Revised (CFQ-R) Domain Scores

The recall period for this instrument is within the past 2 weeks. The domain scores are calculated as follows, as per University of Miami's scoring guidelines:

First, item scores are assigned as values between 1 and 4. Next, items #6, #10, #13, #15, #17, #18, #23, #28, #30, #32, #34, #35, and #43 are reverse scored, where the new score is (5 – old score). If an item response is missing, the median value of scores for other items within that domain (e.g., #1 - #5, #13, #19, #20 for physical domain, and #40 – #42, #44 – #46 for respiratory domain) will be imputed, if no more than half of the responses for that domain are

missing. If more than half of the answers for a specific domain are missing, that domain score will be missing.
The respiratory domain score is calculated as (#40 + #41 + #42 + #44 + #45 + #46 – 6)/18 * 100.
The physical domain score is calculated as (#1 + #2 + #3 + #4 + #5 + #13 + #19 + #20 – 8)/24 * 100.

Concomitant Hypertonic Saline

For Cohort 1, concomitant hypertonic saline use will be identified by a review of all concomitant medication terms by ATC, preferred name, and verbatim term. The Medical Monitor or designee will review all concomitant medications (while blinded to treatment information) prior to database lock, and identify which medications are inhaled hypertonic saline. This information will be recorded on a spreadsheet, finalized prior to database lock, and incorporated into analysis datasets for use in summaries and analyses.

For Cohort 2, concomitant hypertonic saline use will be identified prior to randomization by the investigator or designee and will be entered into the Interactive Voice/Web Response System.

9.0 Analysis Sets

9.1 Intention-to-Treat Analysis Set

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

9.2 Per Protocol Analysis Set

The Per-Protocol (PP) Analysis set will include all subjects in the ITT population who did not have important protocol violations. Important 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 for the primary and secondary efficacy endpoints, to provide supportive evidence for efficacy.

9.3 Safety Analysis Set

The Safety Analysis Set will include all enrolled subjects who receive at least 1 dose of the study drug. This analysis set will be used for all safety analyses.

10.0 Interim Analyses

There was 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 which enabled selection of the Cohort 2 dosing regimens.

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: the approximate midpoint of Cohorts 1 and 2 (after approximately 19 subjects have been randomized or at the discretion of the DSMB) and at the completion of Cohort 1.

11.0 Data Review

11.1 Data Handling and Transfer

Data will be entered into the clinical database and exported as SAS® datasets. Subsequent datasets will be created using SAS and following standard Clinical Data Interchange Standards Consortium Standard Data Tabulation Model (CDISC SDTM, version 1.3, Implementation Guide version v3.1.3) conventions. Analysis datasets will be created using SAS and following CDISC Analysis Data Model (ADaM, version 2.1, Implementation Guide 1.0) standards.

Medical history and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 20.0 at the time of the analysis to assign a system organ class (SOC) and preferred term (PT) to each event. Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary WHODRUG 2017SEP01 DDE+HD B2 at the time of the analysis.

Additional details can be found in the PRA Data Management Plan and the Data Quality Plan for this study.

11.2 Data Screening

Beyond the data screening built into the PRA Data Management Plan, the PRA programming of analysis datasets, tables, figures, and listings (TFL) provides additional data screening. Presumed data issues will be output into SAS logs identified by the word “Problem” and extracted from the logs by a SAS macro and sent to Data Management.

The PRA statistician and the SPYRYX Biosciences signatory must approve database lock.

12.0 Statistical Methods

All statistical programming will use SAS® version 9.4 or higher.

All statistical analyses will be performed using a two-sided alpha = 0.05, unless otherwise specified.

For two sequential cohorts, each cohort will be analyzed separately based on its own baseline.

Unless otherwise specified, descriptive summaries will be tabulated by treatment groups and overall. Categorical data will be presented using counts and percentages, with the number of subjects in each category as the denominator for percentages. Percentages will be rounded to one decimal place except 100% will be displayed without any decimal places and percentages will not be displayed for zero counts.

All continuous data will be summarized using the number of observations (n), mean, standard deviation (SD), median, first quartile, third quartile, minimum, and maximum. Minimum and maximum will be rounded to the precision of the original value. Mean and median will be

rounded to one decimal place greater than the precision of the original value. The SD will be rounded to two decimal places greater than the precision of the original value.

12.1 Subject Disposition

The number of subjects screened will be tabulated. In addition, the number and percentage of enrolled (randomized), and the number of subjects in each defined analysis set (ITT, PP, safety) will be summarized by treatment group and total for all enrolled subjects.

Subject disposition will be summarized by treatment group and overall using the ITT analysis set. The table will present the number and percentage of subjects who completed study. The number and percentage of subjects who discontinued from the study will be summarized by the reason for study discontinuation collected in the CRF.

Disposition data will be listed by subject using the ITT analysis set.

12.2 Demographic and Baseline Characteristics

Demographic and baseline characteristics, including age (years and categories of <30, ≥30-<40, ≥40, sex, ethnicity, race, baseline height [cm], weight [kg], BMI and smoking tobacco history will be summarized descriptively by treatment group and total using the ITT analysis set.

Cystic Fibrosis (CF) history, including CF diagnosis, time from diagnosis to first dose date in years, the number of CF related hospitalization within past year and the number of pulmonary exacerbations within the past year will be summarized by treatment group and total using the ITT analysis set. In addition, the number of subjects who were concomitantly taking inhaled hypertonic saline and inhaled antibiotics during the treatment period will be summarized as above (Cohort 2 only). A listing of Cystic Fibrosis history will also be presented by treatment group and total using the ITT analysis set.

Baseline Spirometry, including absolute FEV_1, $ppFEV_1$, absolute FVC and ppFVC, will be summarized and listed by treatment group and total and by timepoints using the ITT analysis set.

12.3 Important Protocol Deviations

Per PRA processes, protocol deviations (PDV) data will be entered into our Clinical Trials Management System (CTMS). The study team and the sponsor will conduct on-going reviews of the deviation data from CTMS and the resulting set of evaluable subjects throughout the study, finalizing the PDV criteria as seems appropriate. The evaluable subjects set must be finalized at the post-freeze data review meeting (or earlier), prior to database lock.

Important protocol deviations (IPD) are a subset of protocol deviations that might significantly affect the completeness, accuracy, and/or reliability of the study data or that might significantly affect a subject's rights, safety, or well-being. IPDs will be summarized by category. The sponsor and study team will review and categorize IPDs. A list of subjects with IPDs will be presented.

12.4 Treatment Exposure

The investigational PARI eFlow nebulizer with PARI eTrack will be used for the dose administration. The 'Adherence Report' will be generated by the eTrack controller in the PARItrack web portal by the vendor.

The variables from PARI's 'adherence report' are the following:

- Start date and time
- Day of this subject´s therapy
- Status of each nebulization
- Device serial number

There will be no additional summary of drug exposure and drug compliance in this SAP. A data transfer from PARI to PRA is not intended. An "adherence report" in PDF-file format through the PARItrack web portal will be available for inclusion in the eTMF.

12.5 Prior and Concomitant Medications

Prior/concomitant medications will be coded by World Health Organization Drug Dictionary (as per Section 8) and will be summarized by Anatomic Therapeutic Classification (ATC) class and preferred name. The analysis will be performed using the ITT population.

The numbers and percentages of subjects using each medication will be displayed. Subjects taking more than one medication in the same ATC class or preferred name will be counted once per ATC and once per preferred name.

Prior and concomitant medications will be listed, including start and stop dates, dose, unit, frequency, route of administration, indication and reason for administration.

12.6 Efficacy Analyses

The primary analysis, secondary efficacy endpoints and exploratory efficacy endpoints will be performed on the ITT analysis set. The primary and secondary efficacy endpoints will be repeated on the PP analysis set, to provide supportive evidence for efficacy, only if there are substantial differences from ITT.

12.6.1 Primary Variable

Primary Efficacy Analysis

The primary efficacy endpoint is the change from baseline in $ppFEV_1$ at visit 5 (week 4). 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. No imputation of missing data will be done for this analysis.

The comparison between each treatment group and placebo will be evaluated by using an analysis of covariance (ANCOVA) model, which will include treatment as the factor, and the stratification factor as a covariate (i.e., baseline lung function categories [$ppFEV_1$ 40.0% to 55.0% or 55.1% to 80.0% for Cohort 1] and the concomitant use of hypertonic saline for Cohort 2). Also included in the ANCOVA model for Cohort 2 is baseline $ppFEV_1$. Descriptive statistics will include the number of observations, unadjusted mean, standard deviation, median, first quartile, third quartile, minimum and maximum, and least squares mean. Treatment differences from placebo will be presented and estimated by least squares means from the analysis model along with 95% confidence intervals and associated 2-sided p-values, which are presented for descriptive purposes.

As described in the power and sample size calculations in Section 6.1, Cohort 1 will be evaluated for success using a two-sided 0.15 significance level. Cohort 2 will be evaluated for success using a one-sided (upper) 0.10 significance level. For Cohort 2, both the one-sided p-value and the lower bound (i.e. one-sided 90% confidence interval) will be presented.

The assumption of homogeneity of variance will be evaluated using Levene's test, on an ANOVA model with treatment as a factor, since Levene's test cannot be performed with an additional covariate in the model (i.e., the ANCOVA model). The assumption of normality will be evaluated using the Shapiro-Wilk test, applied to the raw residuals from the ANCOVA model. Both will be tested at the 0.05 level of significance. If Levene's test has $p > 0.05$ but the Shapiro-Wilk test is significant, the ANOVA model with treatment as a factor will be evaluated for the assumption of normality. If that model meets criteria, it will be used as the primary efficacy analysis. If either Levene's test is significant, or the Shapiro-Wilk test is significant for both the ANCOVA and ANOVA models, a non-parametric Kruskal-Wallis test (for Cohort 1) or Wilcoxon-Mann-Whitney test (for Cohort 2) will be used to compare $ppFEV_1$ between treatment arms. Note that both the Kruskal-Wallis and Wilcoxon-Mann-Whitney tests do not allow for covariates, so baseline lung function category will be excluded from the analysis.

In Cohort 1 only, the primary efficacy analysis will also be repeated comparing the combined SPX-101 treatment groups with the placebo group.

Analyses of ppFEV1 at Additional Timepoints

The above approach will be repeated for testing treatment differences from placebo in the other timepoints, with a two-sided significance level of 0.05:

- Change from baseline in $ppFEV_1$ at visit 3 (week 1)
- Change from baseline in $ppFEV_1$ at visit 4 (week 2).

Mixed Model for Repeated Measures

The change from baseline in $ppFEV_1$ at each visit (week 1, 2, and 4) will also be evaluated using a mixed effect model for repeated measure (MMRM).

For Cohort 1, to evaluate treatment differences of $ppFEV_1$ change from baseline across time points (week 1, 2 and 4), a likelihood-based mixed effect model repeated measures (MMRM) analysis will be run on ITT analysis set. The model will include the change from baseline in $ppFEV_1$ as the dependent variable; the treatment, visit and treatment by visit interaction term will be treated as fixed effects, and covariates of baseline lung function category ($ppFEV_1$ 40.0% to 55.0% or 55.1% to 80.0% for Cohort 1). Subject will be treated as a random effect.

For Cohort 2, the MMRM analysis will be performed for the change from baseline in $ppFEV_1$ to estimate the treatment difference at Week 4. The model will include all participants in the ITT population, and the dependent variable will be the observed change from baseline in $ppFEV_1$ at each post-randomization visit. Covariates in the model will include the fixed categorical effects of treatment, concomitant use of hypertonic saline, visit and treatment by visit interaction, as well as the continuous fixed covariates of baseline $ppFEV_1$ and baseline $ppFEV_1$ by visit interaction.

Descriptive statistics will be presented for Visit 5 (week 4), and for all visits for Cohort 2, and will include number of observations, unadjusted mean, standard deviation, median, first quartile, third quartile, minimum and maximum, and adjusted mean from the mixed model. Treatment differences from placebo will be presented and estimated via least squares means from the analysis model along with 95% confidence intervals. The 2-sided p-value of the overall analysis at Week 4 will be presented. These models will be performed using an unstructured correlation matrix to model the within-subject errors and the Kenward-Rogers correction to degrees of freedom will be applied. If the unstructured covariance matrix does not converge when fitting the mixed model, further investigation into the most appropriate correlation matrix will be conducted. The final selection of the correlation matrix to be used in fitting the mixed model will be noted in the footnote and fully documented in the results section of the clinical study report.

ANCOVA with Multiple Imputation of $ppFEV_1$

Multiple imputation will be used to explore the effect of missing data on the primary endpoint in Cohort 2 only. All missing $ppFEV_1$ data at baseline and Visit 5 (week 4) will be imputed for all enrolled subjects, utilizing the full conditional specification method (FCS). Missing values will be imputed using treatment, hypertonic saline use, sex, age group, and the $ppFEV_1$ recorded at each time point (including baseline). For this analysis, CFB will be calculated for all subjects following imputation.

An ANCOVA model with treatment as a factor, and concomitant use of hypertonic saline and baseline $ppFEV_1$ as covariates, will be run on the imputed data from PROC MI and results will be combined using PROC MIANALYZE. Further details of the multiple imputation are described in section 12.6.5.

Descriptive statistics of the imputed data combined with observed data will include the number of observations, unadjusted mean, standard deviation, median, first quartile, third quartile, minimum and maximum, and least squares mean. Treatment differences from placebo will be presented and estimated by least squares means from the analysis model along with 95% confidence intervals and associated 2-sided p-value.

12.6.2 Secondary Variables

FEV_1,

Similar ANCOVA analyses as the primary analysis, using a two-sided significance level of 0.05, will be repeated for testing treatment differences from placebo in the endpoints:

- Change from baseline in FEV_1 at visit 3 (week 1)
- Change from baseline in FEV_1visit 4 (week 2)
- Change from baseline in FEV_1 visit 5 (week 4)

FVC and ppFVC

Similar ANCOVA analyses as the primary analysis, using a two-sided significance level of 0.05, will be repeated for testing group differences in the endpoints:

- Change from baseline in FVC at visit 3 (week 1)
- Change from baseline in FVC at visit 4 (week 2)
- Change from baseline in FVC at visit 5 (week 4)
- Change from baseline in ppFVC at visit 3 (week 1)
- Change from baseline in ppFVC at visit 4 (week 2)
- Change from baseline in ppFVC at visit 5 (week 4)

FEF_{25-75}

Similar ANCOVA analyses as the primary analysis, using a two-sided significance level of 0.05, will be repeated for testing group differences in the endpoints:

- Change from baseline in FEF_{25-75} at visit 3 (week 1)
- Change from baseline in FEF_{25-75} at visit 4 (week 2)
- Change from baseline in FEF_{25-75} at visit 5 (week 4)

Cystic Fibrosis Questionnaire – Revised (CFQ-R)

The respiratory and physical domain scores will be calculated as described in Section 8. The observed scores at Visit 1, Visit 5 (week 4), and change-from baseline at Visit 5 (week 4) will be summarized for each domain. Scores from the respiratory and physical domains will be listed.

12.6.3 Exploratory Analyses

Pulmonary Exacerbation

The number and percentage of subjects with pulmonary exacerbation will be tabulated using the ITT analysis set, by treatment groups and total and by visit. A subject listing will be provided. Further analysis may be explored.

Concomitant Hypertonic Saline

The number and percentage of subjects using concomitant hypertonic saline will be tabulated using the ITT analysis set, by treatment groups and total. A subject listing will be provided. Further analysis may be explored.

12.6.4 Additional Analyses

Others

The change from baseline in the $ppFEV_1$, FEV_1, FVC, and ppFVC (at Weeks 1, 2 and 4, as described for the primary efficacy analysis) will be summarized and analyzed separately by subgroup (using concomitant hypertonic saline vs no concomitant hypertonic saline usage), utilizing the ITT analysis set.

The change from baseline in ppFEV$_1$ by strata (baseline ppFEV1 <= 55%, baseline ppFEV1 >55%) will be explored by treatment groups and by visit, also by combined active group and placebo group (Cohort 1 only).

The change from baseline in ppFEV$_1$ by treatment groups, by visit and by category (≤ -10%, > -10% – ≤ -5%, > -5% - ≤ 0%, > 0% - ≤ 5%, > 5% - ≤ 10%, > 10% - ≤ 15%, > 15%) will be explored.

The change from baseline in the ppFEV$_1$, FEV$_1$, FVC, and ppFVC (at Weeks 1, 2 and 4, as described for the primary efficacy analysis) may be summarized and analyzed separately by subgroup (CFTR Modulator Response vs No Response) , utilizing the ITT analysis set. This will be done only if there is a sufficient sample size to warrant this analysis. This population cannot be defined programmatically, therefore the sponsor will review the genetic mutations for each subject, and will identify those that have class 1 or other CFTR modulator non-responsive mutations, and will provide this information to PRA.

The change from baseline in the ppFEV$_1$ (at Weeks 1, 2 and 4, as described for the primary efficacy analysis) will be summarized and analyzed separately by subgroup based on starting a new cycle of concomitant inhaled antibiotics within +/- 3 days of start of study drug vs not starting a new cycle of inhaled antibiotics within +/- 3 days of start of study drug use in the ITT analysis set. The first data set will include patients taking inhaled antibiotics defined as those who start a cycle of inhaled antibiotics with study drug (patients who are on an on-month of an alternating month on/month off cycle with a single inhaled antibiotic and those that alternate monthly between 2 inhaled antibiotics). The second data set will include all the patients that do not begin a new inhaled antibiotic with study drug (patients who are off-cycle of an alternating month on/month off cycle, patients who are taking continuous inhaled antibiotics, or patients who are not taking inhaled antibiotics. Due to data capture limitations, these populations cannot be defined programmatically with certainty. Therefore, the sponsor will review the inhaled antibiotic usage and will identify the patients to be included in each data set.

The change from baseline in the ppFEV$_1$ (at Weeks 1, 2 and 4, as described for the primary efficacy analysis) will be summarized and analyzed separately by subgroup (using dornase alfa and not using dornase alfa), utilizing the ITT analysis set.

12.6.5 Methods for Handling Dropouts and Missing Data

Missing data will not be imputed, with the exception of missing or partial dates, missing item responses on the CFQ-R, and missing baseline and Visit 4 (week 5) ppFEV$_1$.

Missing or partial dates will be imputed if dates are missing or incomplete for an AE (including death event) or concomitant medication as described in Section 8.0.

Partial dates for the date of CF diagnosis will be imputed. If only ‘day’ is missing, then impute day with ‘01’. If ‘day’ and ‘month’ are missing, and ‘year’ is not missing, then impute both month and day with ‘01’.

Missing item responses will be imputed using the median of item responses within that domain, when no more than half the item responses are missing, for CFQ-R domain scores. See Section 8 for more details.

Missing ppFEV$_1$ data will be imputed only for use in the sensitivity analysis described in section 12.6.1. The FCS multiple imputation method will be used. The multiple imputation will be carried out in SAS using PROC MI. Twenty sets of imputed data will be generated and the default maximum of 100 iterations will be used to attempt to impute values within the appropriate range for each variable, though this may be increased to impute appropriate values.

12.6.6 Multiplicity

There will be no multiplicity adjustments for this study.

12.6.7 Pooling of Sites

All sites will be pooled together for the analyses.

12.7 Pharmacokinetics (PK)

At Visit 2, blood samples for PK testing will be collected pre-dose and at 5±2 minutes and 15±5 minutes after completion of administration of the study drug, for Cohort 1 only.

There will be no additional summary of PK in this SAP. A data transfer from QPS to PRA is not intended. QPS will send a listing of PK results for inclusion in the eTMF

12.8 Safety Analyses

The Safety Analysis set will be used to summarize all safety endpoints.

12.8.1 Adverse Events

Any condition, event and/or signs and symptoms occurring after signing the informed consent form and before starting study drug treatment should be recorded as medical history in the eCRF. Medical conditions/diseases are considered AEs only if they start or worsen after starting study drug treatment (first application of study drug).

Therefore, an AE includes any condition (including a pre-existing condition) that: 1) was not present prior to study treatment, but appeared or reappeared following initiation of study treatment, or 2) was present prior to study treatment, but worsened during study treatment.

A Treatment Emergent Adverse Event (TEAE) is defined as per Section 8.

Subject incidence of adverse events (AEs) will be summarized by MedDRA system organ class (SOC) and preferred term (PT), severity and relationship to study drug for each treatment group and total. For incidence reporting, subjects will be counted only once within same SOC or PT. All AEs, TEAEs and pertinent subject information will be presented in data listings. Serious adverse events (SAEs), AEs leading to death, and AEs leading to withdrawal from study will be presented in separate listings respectively. An overall summary of AEs will be provided by treatment groups and total.

Missing values for severity will be assigned as 'severe' and missing values for relationship to treatment will be considered as 'related' in the summary.

A summary of AEs by SOC and PT will be tabulated in descending order of frequency by treatment groups and total for the following:

- AEs leading to study discontinuation
- AEs leading to death
- All serious adverse events (SAEs)
- SAEs related to study treatment
- All treatment-emergent AEs (TEAEs)
- Treatment-related TEAEs
- TEAEs by maximum severity. For each system organ class or preferred term, multiple occurrences of events within a subject are counted only once at the highest severity

12.8.1.1 Adverse Events of Special Interest (AESI)

For those adverse events considered as TEAEs of special interest (hyperkalemia/increased potassium) classification will be final before final database lock (DBL).

The total number of AESIs will be summarized for each treatment group and total.

12.8.2 Laboratory Data

All laboratory test results will be reported in International System of Units (SI).

Descriptive summaries of changes from baseline for each laboratory test listed below will be provided for numerical laboratory assessments by visit.

Each laboratory value will be assessed as low, normal or high based on the normal ranges provided by the laboratory. Laboratory shift tables from baseline to each post-baseline visit for lab values evaluation of 'High' 'Low' or 'Normal', when available, will be planned when the baseline results are available.

The categorical lab results will be summarized by frequency and percentage and by visit.

The clinical safety laboratory tests will include the following parameters:

- 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 and urine electrolytes: sodium, potassium, chloride, and bicarbonate
- Urinalysis: pH, specific gravity, protein, glucose, and ketones

Pregnancy test results for females of childbearing potential only will be listed by visit for each treatment group.

If multiple assessments per lab test are done on the same date, then the average value will be used. The denominator for the percentages will be the total number of subjects in the treatment group with a baseline and post-baseline value. Laboratory parameters with abnormal clinically significant results will be identified and listed, where available.

12.8.3 Vital Signs

Vital signs (systolic and diastolic blood pressure, heart rate, and body temperature) will be measured at each visit during screening and treatment period.

Observed values and changes from baseline in vital signs will be summarized by vital sign parameter and visit. Descriptive statistics will be shown for baseline, each post-baseline time point, and the change from baseline to each post-baseline time point.

12.8.4 Electrocardiograms (ECGs)

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.

The number and percentage of subjects for each category of ECG evaluation will be presented by treatment group and total. ECG shift tables from baseline to visit 5 for clinical interpretation of normal, abnormal, clinically significant abnormal will be presented.

A listing of subjects with ECG assessments, including the clinical evaluation results, will be provided.

12.8.5 Physical Examinations and Other Observations Related to Safety

Physical examination results for all body systems examined will be summarized and listed by visit for each treatment group.

12.8.6 Antidrug Antibodies (ADA)

Anti-Drug Antibody (ADA) response to the study drug will be listed. A summarization of ADA may be considered based on the frequency of ADA detected.

12.8.7 Post-Dose FEV1 (Day 1 only)

A summary of Day 1 change from baseline from pre-dose at each post-dose time point on Day 1 (ie, 30 min, 2 hrs and 4 hours post-dose). Only descriptive statistics will be presented.

13.0 Pooled Analyses Across Cohorts

The analyses specified in Sections 12 will be performed separately for each cohort, where applicable. In addition, the following summaries and analyses may be performed on the pooled cohorts for exploratory purposes. If performed, summaries and analyses will be presented by dose level (60 mg SPX-101 Cohort 1, 120 mg SPX-101 Cohort 1 and 2, and pooled placebo) and overall.

- Summary of subject disposition
- Summary of subject demographics
- Summary of CF history
- An ANCOVA as described in Section 12.6.1, using baseline ppFEV$_1$ use as a covariate.

14.0 Validation

PRA's goal is to ensure that each TFL delivery is submitted to the highest level of quality. Our quality control procedures will be documented separately in the study specific quality control plan.

15.0 References

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

CFQ-R Scoring guidelines: Handscoring instructions. University of Miami, copyright 2008. http://www.psy.miami.edu/cfq_QLab/scoring.html accessed 31Oct2018.

Appendix 1 Glossary of Abbreviations

Glossary of Abbreviations:

ADaM	Analysis Data Model
AE	Adverse event
AESI	Adverse event of special interest
AR1	First order autoregressive
ALT	Alanine aminotransferase test
ALP	Alkaline phosphatase test
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase test
BID	Twice daily
BMI	Body mass index
CF	Cystic Fibrosis
CFB	Change from baseline
CFQ-R	Cystic fibrosis questionnaire revised
CRF	Case Report Form
CS	Compound symmetry
CSR	Clinical Study Report
CTMS	Clinical trials management system
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ET	Early termination
FEF	Forced Expiratory Flow
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
FU	Follow up
HE	Health Economics
IP	Investigational product
IPD	Important protocol deviations
ITT	Intention-to-treat
IVRS	Interactive Voice Response System
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed effect model repeated measures
PDV	Protocol deviation
PK	Pharmacokinetic

PP	Per Protocol
$ppFEV_1$	Percent Predicted forced expiratory volume in 1 second
ppFVC	Percent Predicted forced vital capacity
QD	Once daily
QoL	Quality of Life
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SAS	Statistical analysis system
SDTM	Standard Data Tabulation Model
TC	Telephone call
TEAE	Treatment-emergent adverse event
TFL	Table, figures and listings
WHO	World Health Organization

Appendix 2 Tables, Figures, Listings, and Supportive SAS Output Appendices

The TFL shells and table of contents are provided in a separate document titled “SPYPX101 TFL Shells Version 2_0”.