

STATMIND

Statistical Analysis Plan

**A PHASE IIa, RANDOMISED, DOUBLE-BLIND, PLACEBO
CONTROLLED, THREE WAY CROSS-OVER STUDY TO
ASSESS THE PHARMACOKINETICS OF RPL554
ADMINISTERED TO ADULT PATIENTS WITH CYSTIC
FIBROSIS**

**Sponsor: Verona Pharma plc
Study No: RPL554-010-2015**

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Contents

ABBREVIATIONS	3
1 INTRODUCTION.....	5
2 STUDY OBJECTIVES.....	5
2.1 Primary Objective.....	5
2.2 Secondary Objectives	5
2.3 Exploratory Objectives	5
3 OVERALL STUDY DESIGN.....	5
3.1 Overview of Study Design	5
3.2 Withdrawals	6
3.3 Replacements	7
4 STUDY MEASUREMENTS AND VARIABLES.....	7
4.1 Endpoints	7
4.1.1 Primary Endpoint	7
4.1.2 Secondary Endpoints.....	7
4.1.3 Exploratory Endpoints.....	7
4.2 Screening and Demographic Measurements	7
4.3 Pharmacokinetic Measurements and Variables	8
4.4 Pharmacodynamic Measurements and Variables	8
4.4.1 Spirometry	8
4.4.2 Sputum Inflammatory Mediators.....	8
4.4.3 Exhaled Breath pH.....	9
4.5 Safety Measurements and Variables.....	9
4.5.1 Adverse Events.....	9
4.5.2 Laboratory Safety Measurements and Variables	10
4.5.3 Vital Signs	11
4.5.4 Physical Examination.....	11
4.5.5 12-Lead ECG.....	11
5 DATA MANAGEMENT	11
6 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE.....	11
6.1 Statistical Evaluation.....	11
6.2 Description of Analysis Sets	11
6.3 Summary Statistics	12
6.4 Methods of Statistical Analysis	12
6.4.1 Disposition	12
6.4.2 Demographic and Other Baseline Characteristics	12
6.4.3 Extent of Exposure and Treatment Compliance.....	12
6.4.4 Protocol Deviations.....	13
6.4.5 Pharmacokinetic Analysis.....	13
6.4.6 Pharmacodynamic Analysis.....	13
6.4.7 Safety Analysis	14
6.5 Determination of Sample Size	16
6.6 Statistical Issues	16
6.7 Interim Analyses	16
6.8 Changes in the Conduct of the Study or Planned Analyses.....	16
7 REFERENCES.....	16
8 DATA PRESENTATION PLAN	17
8.1 Tables for Section 14.1-3.....	17
8.2 Graphs for Section 14.2-3.....	18
8.3 Listings for Section 16.2	19
APPENDICES	20
Appendix 1 Inclusion and Exclusion Criteria.....	20
Appendix 2 Allowed and Disallowed Concomitant Medication	22

ABBREVIATIONS

Abbreviation	Term
AE	Adverse Event
ANCOVA	Analysis of Covariance
ATC	Anatomic Therapeutic Chemical
ATS	American Thoracic Society
AUC	Area Under the Curve
BMI	Body Mass Index
CAS	Completer Analysis Set
CF	Cystic Fibrosis
CRF	Case Report Form
CS	Clinically Significant
CV	Coefficient of Variation
EBC	Exhaled Breath Content
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
eCRF	Electronic Case Report Form
FAS	Full Analysis Set
FEV ₁	Forced Expiratory Volume in 1 Second
FSH	Follicle Stimulating Hormone
FVC	Forced Vital Capacity
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation (of Technical Requirements for Registration of Pharmaceuticals for Human Use)
ICS	Inhaled Corticosteroid
IL-8	Interleukin 8
LABA	Long-Acting β_2 -Agonist
LAMA	Long-Acting Muscarinic Antagonist
MedDRA	Medical Dictionary for Regulatory Activities
MPO	Myeloperoxidase
NCS	Not Clinically Significant
PKS	PharmacoKinetic data Set
PT	Preferred Term
QTcF	Heart-rate corrected QT interval (Fridericia's method)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SD	Standard Deviation
SOC	System Organ Class

SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Event
TFL	Tables, Figures and Listings
TNF- α	Tumour Necrosis Factor Alpha

1 INTRODUCTION

This is the Statistical Analysis Plan (SAP) for study RPL554-010-2015. It is based on the Clinical Study Protocol version 4.0 dated 23 August 2017.

2 STUDY OBJECTIVES

2.1 Primary Objective

To investigate pharmacokinetics of single nebulised doses of RPL554 in patients with cystic fibrosis (CF).

2.2 Secondary Objectives

Secondary objectives are:

- To investigate the bronchodilator effect on peak Forced Expiratory Volume in one second (FEV₁) after single nebulised doses of RPL554 as compared to placebo
- To investigate the bronchodilator effect on AUC FEV₁ over 4, 6 and 8 hours of single nebulised doses of RPL554, as compared to placebo
- To assess the tolerability and safety of single nebulised doses of RPL554 in patients with CF.

2.3 Exploratory Objectives

To examine the anti-inflammatory effects of RPL554 after a single dose in patients with CF.

3 OVERALL STUDY DESIGN

3.1 Overview of Study Design

This is a Phase IIa, randomised, double blind, placebo controlled, complete block three way crossover study to investigate the pharmacokinetics of nebulised RPL554 (1.5 mg and 6 mg) in adult patients with CF. It is planned to enrol sufficient patients to assure that 10 patients complete all three treatment periods at one centre in the UK. The study comprises five visits: screening (Visit 1), three treatment visits (Visit 2 to Visit 4) and an end of study visit (Visit 5). Patients will be screened for eligibility (Visit 1), including a reversibility test with salbutamol, between 3 and 14 days prior to the first dose of study treatment.

Eligible patients will then attend for three separate 1 day treatment visits (Visits 2 to Visit 4) each separated by a at least a 3-day washout period. Patients will be randomised pre-dose at Visit 2. Patients will receive a single nebulised dose of 1.5 mg RPL554 or 6 mg RPL554 or placebo at each visit. The pre-dose FEV₁ at Visit 3 and Visit 4 must be within $\pm 20\%$ of the pre-dose FEV₁ at Visit 2 in order to ensure consistent baseline FEV₁ for each study treatment. At each visit, patients will be resident at the study centre from the morning until at least 8 hours after dosing.

Patients will be discharged after 8 hours, but return the following morning for final assessments 24 hours after dosing.

The following will be performed at each treatment visit:

- Measurements of lung function (FEV₁ and forced vital capacity [FVC]) pre-dose and up to 24 hours post-dose
- Blood sampling pre-dose and up to 24 hours post-dose for pharmacokinetic analysis
- Sputum sampling pre-dose and at 8 and 24 hours post-dose for rheology and measurement of inflammatory mediators (interleukin 8 [IL-8], tumour necrosis factor alpha [TNF- α], myeloperoxidase [MPO])
- Exhaled breath pH measurement pre-dose and at 8 and 24 hours post-dose
- Urine pregnancy test pre-dose (female patients only)
- Vital signs and 12-lead electrocardiogram (ECG)s pre-dose and up to 8 hours post-dose

An end of study visit (Visit 5) will be performed 3 to 10 days after the last dose of study treatment. Adverse events will be recorded throughout the study and laboratory safety tests, a physical examination and urine pregnancy tests (female patients only) will be performed at screening (Visit 1) and at the end of study visit (Visit 5).

Inclusion/exclusion criteria are given in Appendix 1. Allowed/disallowed medications are given in Appendix 2.

3.2 Withdrawals

Investigators have the authority to ask for the withdrawal of a patient at any time for medical or non-compliance reasons. Should the Investigator decide it is necessary to withdraw any patient for specific reasons, this should be recorded in writing and discussed with the patient in question. Such reasons for withdrawal are expected to be medical or related to lack of co-operation by the patient.

If a patient withdraws following randomisation, every attempt should be made to contact the patient to determine the reason for withdrawal and to complete the recording of any available pharmacodynamic data and all adverse event data. If a patient agreed to enter the study and signed a consent form but withdrew from the study, or was withdrawn from the study, without receiving any study treatment, no further follow-up is necessary.

End of study visits will occur 3 to 10 days after the last dose of study treatment. All withdrawn patients will follow this routine unless it is considered by the Investigator that they require greater medical supervision and/or investigations and in which case an unscheduled visit prior to and in addition to the scheduled follow up visit may be performed.

If a patient decides to withdraw voluntarily, or is withdrawn by the Investigator responsible at any time, the reasons for withdrawal and results of all relevant tests will be recorded in the case report form (CRF). Patients who withdraw may be replaced.

3.3 Replacements

A total of 10 patients must complete all three treatment visits (Visit 2 to Visit 4) up to the 24 hour assessments in the study. Any patients who do not complete will be replaced. This should be discussed with the Sponsor on a case by case basis. Replacement patients will be allocated the next randomisation number in the randomisation list.

4 STUDY MEASUREMENTS AND VARIABLES

4.1 Endpoints

4.1.1 Primary Endpoint

Plasma RPL554 pharmacokinetic parameters (AUC_{0-24} , C_{max} , t_{max}).

4.1.2 Secondary Endpoints

Secondary endpoints are:

- Peak and AUC FEV₁ over 4, 6 and 8 hours
- Determination of onset of action
- Determination of duration of action
- Safety and tolerability
 - Continuous monitoring of adverse events
 - Laboratory safety tests (haematology, biochemistry and urinalysis)
 - 12-lead ECG (including QTcF and heart rate), supine vital signs (blood pressure and pulse) over 8 hours

4.1.3 Exploratory Endpoints

Levels of inflammatory mediators in sputum and sputum rheology.

4.2 Screening and Demographic Measurements

Written informed consent will be obtained by the Investigator prior to any study related procedures being performed.

Demographic variables, including date of birth, sex (plus details confirming non-childbearing status for females), height, weight, BMI (weight [kg]/height [m]²), race, ethnic origin and smoking status will be collected at screening (Visit 1). The date of diagnosis of CF and the specific CF mutations will also be recorded.

Active medical conditions and all surgeries will be recorded at screening (Visit 1), including disease history which includes date of diagnosis.

A reversibility test in response to salbutamol will be performed at screening (Visit 1). Spirometry (FEV₁ and FVC) will be performed before and after (at 30 minutes and 1 hour) two puffs of salbutamol administered using a pressurised metered dose inhaler. The pre-bronchodilator FEV₁ must be $\geq 40\%$ and $\leq 80\%$ of predicted normal for inclusion. The reversibility response is not an eligibility measure.

At all three time points, three technically acceptable measurements should be made and recorded in the CRF. Spirometry assessments may be repeated up to eight times to obtain three acceptable readings according to American Thoracic Society [ATS] guidelines (Miller, 2005). The highest reading from each assessment will be used for calculation of predicted values and increase from baseline. European Community of Coal and Steel reference equations (Quanjer, 1993) will be used as a reference for predicted normal values.

Laboratory safety assessments will be performed to check eligibility. Unscheduled and/or repeat testing may be performed at the discretion of the Investigator.

Prior respiratory therapies and medications will be recorded at screening (Visit 1) as well as concomitant use during the study. Other medications used prior or as concomitant use during the study will be recorded separately.

Patients will be confirmed as eligible according to the inclusion and exclusion criteria from assessments made at screening (Visit 1) with a final check of all results pre-dose at Visit 2.

4.3 Pharmacokinetic Measurements and Variables

Pharmacokinetic analysis will be performed on samples taken from all patients. Blood samples (4 mL) will be collected at the following time points at Visit 2 to Visit 4: pre-dose, 15 and 30 minutes and 1, 2, 4, 6, 8 and 24 hours post-dose. Samples will be collected by venepuncture or via indwelling cannula in the forearm into lithium heparin tubes and will be immediately chilled (ice bath). The blood will be centrifuged within 15 minutes of collection. The plasma will be separated in a refrigerated centrifuge (about 4°C) at 1100 g for 15 minutes and transferred into polypropylene tubes. After each blood collection, the plasma will be dispensed into two aliquots. After appropriate labelling, the plasma samples will be stored at, or below -20°C. The plasma samples will then be transported in dry ice to the central laboratory where they will be stored at or below -20°C until they are analysed using a validated method.

4.4 Pharmacodynamic Measurements and Variables

4.4.1 Spirometry

Spirometry assessments (FEV₁ and FVC) will be made at the following time points at Visit 2 to Visit 4: pre-dose (at -15 minutes) and 5, 15 and 30 minutes and 1, 2, 4, 6, 8 and 24 hours post-dose in accordance with ATS guidelines (Miller, 2005). At all time points, three technically acceptable measurements should be made and recorded in the CRF. Spirometry assessments may be repeated up to eight times to obtain three acceptable readings. The highest FEV₁ and FVC readings from each assessment will be used for analysis even if the FEV₁ and FVC values come from two different forced exhalations.

4.4.2 Sputum Inflammatory Mediators

Sputum samples will be taken pre-dose and at 8 and 24 hours post-dose at Visit 2 to Visit 4 by spontaneous expectoration over no more than 5 minutes and transported on ice for rapid processing (collection to storage within 30 minutes). Whole sputum will be immediately frozen at -20°C and then transferred to -80°C for storage prior to analysis (Horsley et al, 2014).

Rheological analysis will be performed before and after incubation at 37°C for 60 minutes (Horsley et al, 2014). Inflammatory mediators (IL-8, TNF- α and MPO) will be measured according to the published methodology (Downey et al, 2007).

4.4.3 Exhaled Breath pH

Exhaled breath pH will be measured pre-dose and at 8 and 24 hours post dose at Visit 2 to Visit 4. Exhaled breath pH measurement will be performed in accordance with the method described by MacNee et al, 2011. In brief, exhaled breath condensate (EBC) samples will be collected during tidal breathing for 10 minutes, without nose clips, using the RTube™ (Respiratory Research Inc., Charlottesville, Virginia). A 200 μ L aliquot of EBC will be used for the pH assay. Measurement of pH will be performed after deaeration by bubbling argon through the sample at 2 L/min while monitoring pH until the reading stabilised, usually after 8 minutes of deaeration with argon.

4.5 Safety Measurements and Variables

4.5.1 Adverse Events

Adverse event is defined as any undesirable experience occurring to a patient, or worsening in a patient, during a clinical study, whether or not considered related to the study treatment. An adverse event may be any of the following:

- A new illness
- An exacerbation of a sign or symptom of the underlying condition under treatment or of a concomitant illness
- Unrelated to participation in the clinical study or an effect of the study drug
- A combination of one or more of the above factors

No causal relationship with the study treatment is implied by the use of the term “adverse event.” An exacerbation of a pre-existing condition or illness is defined as a more frequent occurrence or as an increase in the severity of the pre-existing condition or illness during the study. Planned or elective surgical or invasive procedures for pre-existing conditions that have not worsened are not adverse events. However, any complication that occurs during a planned or elective surgery is an adverse event (if the event fits the serious criteria, such as an extended hospitalisation, it will be considered to be serious). Conditions leading to unplanned surgical procedures may be adverse events.

Adverse reaction is defined as all untoward and unintended responses to study treatment related to any dose administered.

Serious adverse event (SAE) is any adverse experience that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity, OR
- Is a congenital anomaly/birth defect
- Other medical events*

*Important medical events that may not be immediately life-threatening or result in death or hospitalisation may be considered a SAE when, based on appropriate

medical judgement, they may jeopardise the patient or may require medical or surgical intervention to prevent one of the outcomes listed above.

An unexpected adverse reaction is an adverse reaction in which the nature or severity of which is not consistent with the Investigator Brochure.

Suspected unexpected serious adverse reactions (SUSAR) is any suspected adverse reaction related to the study drug that is both unexpected and serious.

All adverse events, whether reported spontaneously by the patient, in response to open questioning on treatment days or observed by the investigator or his/her staff, will be recorded from informed consent until the end of study visit (Visit 5). The start and stop time will be recorded and adverse events will be assessed by the investigator for the following:

- severity (mild, moderate, severe)
- chronicity (single occasion, intermittent, persistent)
- causality (not related, unlikely, possibly, definitely)
- action taken with study treatment (none, study treatment stopped, study treatment temporarily interrupted)
- other actions (none, concomitant medication, study discontinuation, hospitalisation, other)
- outcome and date of outcome (recovered or resolved, recovering or resolving, not recovered or not resolved, recovered or resolved with sequelae, fatal, unknown)
- seriousness (yes, no)

4.5.2 Laboratory Safety Measurements and Variables

All female patients will have a urine pregnancy test at screening (Visit 1), pre-dose at Visit 2 to Visit 4 and at the end of study visit (Visit 5) according to the study centre's standard operating procedures (SOPs).

All other laboratory safety assessments will be performed at the study centre's local laboratory. Two samples will be collected at each time point, one sample for haematology using a ethylenediaminetetraacetic acid (EDTA) whole blood collection tube and one sample for biochemistry using a serum tube. A midstream urine sample will also collected in a sterile container. Samples will be taken at screening (Visit 1) and end of study visit (Visit 5) and analysed for:

Haematology: haemoglobin, haematocrit, total white cell count, leukocyte differential count and platelet count

Biochemistry: creatinine, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine transaminase, gamma-GT, lactate dehydrogenase, creatine kinase, thyroid stimulating hormone, triiodothyronine and thyroxine, glucose, potassium, sodium and calcium

Urinalysis: leukocytes, blood, ketones, bilirubin, urobilinogen, protein and glucose using a dipstick. In the event of an abnormal dipstick urinalysis result, microscopic urinalysis may be conducted. The referral criteria will be specified in the laboratory manual

In addition, unscheduled and/or repeat testing may be performed at the discretion of the Investigator. Laboratory results will be provided to the Investigator for each

patient and each visit. The Investigator should assign whether each abnormal result is not clinically significant or clinically significant.

4.5.3 Vital Signs

Blood pressure and pulse rate will be measured at screening (Visit 1) and pre-dose, 30 minutes and 1, 2, 4, 6 and 8 hours post-dose at Visit 2 to Visit 4

At each time point, supine vital signs will be assessed while the patient has been at rest for at least 5 minutes.

4.5.4 Physical Examination

A full physical examination, covering major body systems (assessments of the nose, throat, skin, thyroid gland, neurological system, respiratory system, cardiovascular system, abdomen [liver and spleen], lymph nodes and extremities) will be performed at screening (Visit 1). Results will be recorded in the CRF as normal, abnormal not clinically significant or abnormal clinically significant. The physical examination will be repeated at the end of study visit (Visit 5), and any changes only recorded.

4.5.5 12-Lead ECG

12-lead ECGs will be taken at screening (Visit 1) and pre-dose and 1, 2, 4 and 8 hours post-dose at Visit 2 to Visit 4.

At each time point, 12-lead ECGs should be taken after at least 5 minutes in the supine position. An ECG printout must be taken at each time point and signed and dated by the Investigator. An overall assessment (normal, abnormal not clinically significant or abnormal clinically significant) and continuous variables, including QT interval, QTcF and heart rate, will be recorded in the CRF.

5 DATA MANAGEMENT

Data management will be performed by Syne-qua-non. Data handling will be described in a Data Management Plan and a Data Validation Plan.

6 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

6.1 Statistical Evaluation

The statistical analysis will be conducted by StatMind AB using SAS version 9.3 or higher. Validation will be performed using Gauss 6.25.

The statistical analyses and reporting of results (tables, figures and listings [TFL]) for this study follows the International Conference on Harmonisation (ICH) guidelines.

6.2 Description of Analysis Sets

Allocation of patients to the analysis populations (and whether any patients or specific data from a patient will be excluded) will be determined at the pre-database lock meeting.

The full analysis set [FAS] will consist of all randomised patients with sufficient data collected after intake of study treatment to compute the pharmacodynamic parameters on at least two study visits.

The completer analysis set [CAS] will consist of all randomised patients with sufficient data collected after intake of study treatment to compute the pharmacodynamic parameters on all three study visits.

The safety set will consist of all randomised patients with safety data collected after intake of study treatment during at least one visit.

The pharmacokinetic data set [PKS] will consist of all randomised patients with blood sampling performed after at least one dose of RPL554 and with data sufficient to calculate pharmacokinetic parameters.

6.3 Summary Statistics

Continuous variables will be summarised using descriptive statistics (number of patients [N], arithmetic mean, standard deviation [SD], median, minimum and maximum values) and for categorical (nominal) variables, the number and percentage of patients will be used. For continuous variables with an expected skew distribution (plasma RPL554 concentrations, some pharmacokinetic parameters [AUC₀₋₂₄, C_{max}], inflammatory cell mediators [IL-8, TNF- α , MPO]), geometric mean and coefficient of variation (CV) will be given instead of arithmetic mean and SD.

6.4 Methods of Statistical Analysis

6.4.1 Disposition

A summary of the patient flow (number and percentage) will be made of all patients enrolled in the study, randomized in the study, belonging to each of the analysis sets, completed the study or prematurely discontinued the investigational product.

The primary reason for withdrawal from the study will be summarized into the following categories: consent withdrawn, adverse event, protocol violation, investigator's decision, sponsor decision or other reason. Patient disposition data including individual reasons for withdrawal will be listed.

6.4.2 Demographic and Other Baseline Characteristics

Demographics and baseline characteristics including age, sex, race, ethnic origin, height, weight, BMI, smoking status, pack years, time since CF diagnosis, CF mutations, FEV₁ [both in litres and in percentage of predicted normal], FVC and FEV₁ reversibility will be listed and summarised by descriptive statistics.

Prior and concomitant CF therapies will be listed and summarised by ATC class for all enrolled patients (long-acting and short-acting LABAs and LAMAs will be separated).

Other medical history and other prior and concomitant medications will be listed. Medical history events will be coded using the MedDRA Dictionary in addition to the verbatim.

6.4.3 Extent of Exposure and Treatment Compliance

All administration of study treatment will be done at the clinic under supervision of the study staff; therefore no formal analysis of compliance will be performed. The

RPL554 exposure will be calculated based on residual volume in the nebuliser cup and summarised by treatment group. The exposure would equal (nominal volume – residual volume) times the drug concentration.

6.4.4 Protocol Deviations

Protocol deviations will be categorized as eligibility deviation, sample handling error, missed assessment, late or early assessment, outside visit window or other category. All protocol deviations collected will be divided into major or minor categories. Prior to database lock protocol deviations will be reviewed and consequences for inclusion of patients in various analysis population sets determined and documented. All details of protocol deviations in the study will be listed. Further protocol deviation will be summarised by category and minor/major assessment.

6.4.5 Pharmacokinetic Analysis

Blood sampling for pharmacokinetic assessments will be done pre-dose and up to 24 hours post-dose. From the plasma concentrations of RPL554 collected, the following pharmacokinetic parameters will be calculated using standard non-parametric methods.

- AUC_{0-24} is the area under the plasma concentration curve from time 0 to 24 hours post-dose, computed using the linear trapezoidal method
- C_{max} denotes the highest plasma concentration measured
- t_{max} denotes the time point corresponding to C_{max}

Pharmacokinetic parameters will be summarised by dose level using descriptive statistics. Graphics will include individual and mean plasma concentration – time curves (both on linear and semi-log scale) and scatter plots of computed pharmacokinetic parameters with mean levels indicated.

6.4.6 Pharmacodynamic Analysis

6.4.6.1 Spirometry

For FEV_1 the average effect, (E_{av}), will be calculated as the Area Under the Curve (AUC) divided by the length of the time interval of interest. The AUC will be computed using the linear trapezoidal rule. Linear interpolation/extrapolation will be used to fill in a missing value (at most one missing value allowed) at end of the computational interval prior to calculation. In addition, the peak effect on FEV_1 will be computed as the maximum value (E_{max}) within 4 hours post-dosing. In analysis, E_{av} and E_{max} will be normalized using the baseline value.

Computed pharmacodynamic parameters for FEV_1 will be compared between placebo and the two doses of RPL554 using analysis of covariance (ANCOVA) models with fixed factors for treatment, period and patient, and using the baseline of the day as a covariate. FEV_1 will be analysed using multiplicative models, which means that data is logged prior to analysis and the result then transformed back to the linear scale giving treatment differences as ratios of geometric means. All three pairwise comparisons will be stated and expressed as the geometric mean ratio with 95% confidence interval and associated, 2-sided, p-value.

All spirometry data will be listed and summarized by treatment and assessment point as well as the change from baseline. Pharmacodynamic parameters will be

summarized by treatment. Graphics will include individual and mean FEV₁ and FVC curves over time (expressed as change from baseline on absolute scale) and scatter plots of computed pharmacodynamic parameters with the mean levels indicated.

Onset of effect will be defined as time to reach a 10% increase in FEV₁ counted from start/end of nebulization. Patients not reaching a response after 2 hours will be censored. Outcome will be illustrated using a Kaplan-Meier plot and, if appropriate, the median time to onset by (active) treatment group determined.

Duration of effect will be assessed by consecutively testing effect of active treatment versus placebo for increasing time points using similar models as the analysis of the pharmacodynamics parameters. If missing values exist, these will be filled in using linear interpolation/extrapolation prior to analysis.

6.4.6.2 Sputum inflammatory mediators, rheology and exhaled breath pH

Il-8, TNF- α , MPO, exhaled breath pH and sputum viscoelasticity (log G) collected 6 and 24 h after dose administration will be compared between treatments using similar ANCOVA models as for the FEV₁ parameters. Multiplicative models will be used for the sputum inflammatory mediators, additive (linear) models for exhaled breath pH and sputum viscoelasticity (log G) that already are expressed on log scales.

All data will be listed and summarized by treatment and assessment point as well as the change from baseline. Graphics will include individual and mean value curves over time for each variable.

6.4.7 Safety Analysis

6.4.7.1 Adverse Events

Adverse events (AEs) will be collected throughout the study and will be coded according to the current version of the MedDRA dictionary. A treatment-emergent adverse event (TEAE) is defined as an AE that started or worsened after intake of study treatment in each treatment period. If the start date is unknown then the AE will be assumed to be treatment emergent unless the partial start date, or other data (i.e. stop date) indicates differently. The TEAEs will be assigned to the treatment given prior to onset of the event. In summaries, causally related AEs will consist of AEs classified as Unlikely, Probably or Definitely related to study treatment.

Coded adverse event terms will be presented by system organ class (SOC) and preferred term and summarised by treatment group. A summary table by treatment group with total number and number of patients with adverse events, SAEs, adverse events leading to discontinuation of study treatment, causally related adverse events and severe adverse events will be produced. Further SAEs, causally related adverse events and adverse events of each intensity will be summarised by SOC and preferred term.

AEs will be presented in decreasing frequency of the total number of patients with TEAEs. All AEs, including those which are not treatment emergent, will be listed. If appropriate, SAEs and AEs leading to discontinuation of study treatment will also be separately listed.

6.4.7.2 Laboratory tests

Safety laboratory test data will be summarised by treatment group and time point. For continuous variables, the change from baseline (pre-dose at each treatment

visit) to each post-dose time point will also be calculated and summarised. Data will further be illustrated by shift tables (showing changes from low/normal/high) and shift plots for change over the study.

The baseline value will be defined as the last scheduled, unscheduled or repeat value collected prior to first dosing. The tabulation will only include scheduled visits; all unscheduled assessments will be displayed in the listing as appropriate.

All laboratory values will be listed showing reference ranges and flagging all abnormal findings and their clinical significance (CS or NCS). Out of reference range values will be flagged as high (H) or low (L). A similar listing including all post-dose laboratory values found abnormal, including the baseline result and clinical significance, will also be produced.

6.4.7.3 Vital Signs

Vital signs data will be summarised by treatment group and time point. The change from baseline (pre-dose at each treatment visit) to each post-dose time point will also be calculated and summarised. Data will further be illustrated by shift tables (showing changes from low/normal/high) and shift plots for the change from baseline to worst case during the treatment period.

The baseline value will be defined as the last scheduled, unscheduled or repeat value collected prior to dosing in a period. The tabulation will only include scheduled visits; all unscheduled assessments will be displayed in the listing as appropriate.

Vital signs will be listed showing reference ranges and flagging all abnormal findings and their clinical significance (CS or NCS). Out of reference range values will be flagged as high (H) or low (L). A similar listing including all post-dose vital signs values found abnormal, including the baseline result and clinical significance, will also be produced.

Further, for data collected during treatment days, graphics will include individual and mean value curves over time for each variable expressed as change from baseline.

6.4.7.4 12-Lead ECG

12-lead ECG data will be summarised and illustrated in the same way as vital signs data. In addition, for QTcF, a plot showing the baseline value of the treatment day on the x-axis versus the post-dose value indicating the largest absolute change on the y-axis will be constructed. QTcF data will also be summarised according to frequencies of values >450 ms, >480 ms and >500 ms and according to frequencies of shifts from baseline of the treatment day to post-dose of >30 ms and >60 ms.

6.4.7.5 Physical Examination

Physical examination data (changes in status between Visit 1 and Visit 5) will be listed.

6.4.7.6 Rescue Medication

Rescue medication used during the treatment visits will be summarised by treatment group and all individual use listed.

6.4.7.7 Concomitant Medications

Prior medications are defined as those with a stop date prior to the date of the first dose of study treatment. Concomitant medications are defined as those medications with either a start date or a stop date that is after the date of first dose of study

treatment. If the start date is unknown then it will be assumed to be concomitant unless the partial start date, or other data (i.e. stop date) indicates differently.

All prior and concomitant medications will be listed by reported name, medication class, standardised medication name, indication, dose, dose unit, route of administration, dosing frequency, start and end dates.

6.4.7.8 Pregnancy tests

Pregnancy test results, or reasons for not performing the test(s), from females at screening, pre-dose at each treatment visit and at end-of-study will be listed.

6.5 Determination of Sample Size

This is a sample size of convenience. No calculation of power was performed.

6.6 Statistical Issues

All hypothesis testing will be done using two-sided alternative hypotheses. P-values less than 5% will be considered statistically significant.

No adjustment for multiple testing will be done. A closed testing procedure will be applied: first the higher dose of RPL544 will be tested versus placebo and then, if statistically significant, the lower dose of RPL554 versus placebo. Finally the two doses of RPL554 will be compared.

Patients withdrawn after only one treatment period will not be included in the efficacy analyses. No imputation of missing data other than described under Section 6.4 will be performed.

Sensitivity analyses of pharmacodynamic data will be done to explore the impact of outliers or, for FEV₁ parameters, the use of rescue medication during the clinic visits.

All available data from all patients who have received study treatment will be listed and summarised. Any unscheduled or unplanned readings will be presented within the patient listings, but only the scheduled readings will be used in any summaries. If a visit is rescheduled due to variability in FEV₁ or other reason, the rescheduled visit will be listed and summarised as the valid visit.

6.7 Interim Analyses

No formal interim analysis is planned for the study.

6.8 Changes in the Conduct of the Study or Planned Analyses

Clinical Study Protocol version 2.2 stated that the pharmacokinetic parameters should include AUC_{0-∞}, C_{max}, T_{max} and the terminal half-life, t_{1/2}. Due to the sampling scheme was considered insufficient to capture an appropriate t_{1/2} estimate, this parameter was omitted and AUC_{0-∞} was replaced with an AUC₀₋₂₄ (area under the scheduled sampling period).

7 REFERENCES

Miller MR, Hankinson J, Brusasco V et al. Standardisation of spirometry. Eur Respir J 2005;26(2):319-338

Quanjer PH, Tammeling GJ, Cotes JE et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl. 1993 Mar; 16:5-40

8 DATA PRESENTATION PLAN

8.1 Tables for Section 14.1-3

Safety set used for tables if not otherwise indicated

- 14.1.1.1 Patient disposition
- 14.1.1.2 Patient flow
- 14.1.2.1 Summary of protocol deviations
- 14.1.3.1 Summary of demographic data and baseline characteristics
- 14.1.3.2 Summary of prior cystic fibrosis medications
- 14.1.3.3 Summary of life-style restrictions and nebuliser training
- 14.1.3.4 Summary of drug exposure data from nebulisations
- 14.2.1.1 Summary of plasma RPL554 concentrations [PKS]
- 14.2.1.2 Summary of pharmacokinetic parameters based on plasma RPL554 concentrations [PKS]
- 14.2.2.1 Summary of spirometry (FEV₁ and FVC) at clinic visits [FAS and CAS]
- 14.2.2.2 Summary of pharmacodynamic parameters based on FEV₁ [FAS and CAS]
- 14.2.2.3 Statistical analysis of pharmacodynamic parameters based on FEV₁ (multiplicative models) [FAS and CAS]
- 14.2.2.4 Statistical analysis of FEV₁ by assessment point (duration of action, multiplicative models) [FAS]
- 14.2.3.1 Summary of inflammatory mediators, sputum rheology and exhaled breath pH at clinic visits [FAS]
- 14.2.3.2 Statistical analysis of inflammatory mediators (multiplicative models) [FAS]
- 14.2.3.3 Statistical analysis of sputum rheology and exhaled breath pH (additive models) [FAS]
- 14.3.1.1 Summary of treatment emergent adverse events
- 14.3.1.2 Summary of adverse events by system organ class and preferred term
- 14.3.1.3 Summary of adverse events causally related to treatment by system organ class and preferred term
- 14.3.1.4 Summary of adverse events of moderate or severe intensity by system organ class and preferred term

- 14.3.1.5 Summary of adverse events with chronicity intermittent or persistent by system organ class and preferred term
- 14.3.2.1 Summary of serious adverse events by system organ class and preferred term
- 14.3.4.1 Summary of laboratory tests (continuous variables) at clinic visits
- 14.3.4.2 Summary of laboratory tests (categorical variables) at clinic visits
- 14.3.4.3 Summary of abnormal findings on laboratory data
- 14.3.5.1 Summary of vital signs at clinic visits
- 14.3.5.2 Summary of abnormal findings on vital signs
- 14.3.5.3 Summary of 12-lead ECG data at clinic visits
- 14.3.5.4 Summary of abnormal findings on 12-lead ECG
- 14.3.5.5 Summary of abnormal findings on physical examination
- 14.3.5.6 Summary rescue medication use during clinic visits
- 14.3.5.7 Summary of pregnancy tests

8.2 Graphs for Section 14.2-3

Safety set used for graphics if not otherwise indicated

- 14.2.1.1 Individual plasma concentration-time curves for RPL554 [PKS]
- 14.2.1.2 Mean plasma concentration-time curves for RPL554 [PKS]
- 14.2.1.3 Scatter plots of computed pharmacokinetic parameters [PKS]
- 14.2.2.1 Individual curves for FEV₁ on absolute scale [FAS]
- 14.2.2.2 Individual curves for FEV₁ as change from baseline [FAS]
- 14.2.2.3 Mean value curves for FEV₁ on absolute scale [FAS and CAS]
- 14.2.2.4 Mean value curves for FEV₁ as change from baseline [FAS and CAS]
- 14.2.2.5 Scatter plots of pharmacodynamic parameters based on FEV₁ [FAS]
- 14.2.2.6 Individual curves for FVC, absolute and as change from baseline [FAS]
- 14.2.2.7 Mean value curves for FVC, absolute and as change from baseline [FAS]
- 14.2.3.1 Individual curves for inflammatory mediators, exhaled breath pH and sputum rheology by treatment [FAS]
- 14.2.3.2 Mean value curves for inflammatory mediators, exhaled breath pH and sputum rheology, absolute and change from baseline [FAS]
- 14.3.4.1 Shift plots for laboratory data
- 14.3.5.1 Individual curves for vital signs, absolute and change from baseline
- 14.3.5.2 Mean value curves for vital signs, absolute and change from baseline
- 14.3.5.3 Individual curves for 12-lead ECG, absolute and change from baseline

- 14.3.5.4 Mean value curves for 12-lead ECG, absolute and change from baseline
- 14.3.5.5 Scatter plot of baseline QTcF versus post-dose assessment indicating largest absolute change from baseline

8.3 Listings for Section 16.2

Safety set used for listings if not otherwise indicated

- 16.2.1.1 Randomised patients that completed the study
- 16.2.1.2 Randomised patients that were withdrawn the study
- 16.2.1.3 Visit dates by patient
- 16.2.1.4 Eligibility by patient
- 16.2.2.1 Listing of protocol deviations
- 16.2.3.1 Analysis dataset allocation by patient
- 16.2.4.1 Demographic data and baseline characteristics
- 16.2.4.2 Lung function at baseline and diagnosis of cystic fibrosis
- 16.2.4.3 Listing of medical history
- 16.2.5.1 Listing of drug exposure
- 16.2.5.2 Life-style restrictions to adhere to prior to visits and nebuliser training
- 16.2.5.3 Plasma concentrations of RPL554 [PKS]
- 16.2.5.4 Pharmacokinetic parameters [PKS]
- 16.2.6.1 Listing of spirometry data [FAS]
- 16.2.6.2 Derived pharmacodynamic parameters based on FEV₁ [FAS]
- 16.2.6.3 Listing of inflammatory mediators, sputum rheology and exhaled breath pH [FAS]
- 16.2.7.1 Listing of adverse events
- 16.2.7.2 Listing of serious adverse events
- 16.2.7.3 Listing of adverse events leading to discontinuation of study treatment
- 16.2.8.1 Listing of laboratory data
- 16.2.9.1 Listing of vital signs
- 16.2.9.2 Listing of 12-lead ECG
- 16.2.9.3 Listing of physical examination
- 16.2.9.4 Concomitant medications at entry and during the study
- 16.2.9.5 Rescue medication used during clinic visits
- 16.2.9.6 Listing of pregnancy tests (female patients)

APPENDICES

Appendix 1 Inclusion and Exclusion Criteria

Inclusion Criteria:

1. Sign an informed consent document indicating they understand the purpose of and procedures required for the study and are willing to participate in the study.
2. Male or female aged ≥ 18 years at the time of informed consent. Females of childbearing potential must have been using a consistent and reliable form of contraception from the last menses before the first study treatment administration, and must commit to continue to do so during the study and for 3 months after the last dose of study treatment.
3. Have a 12-lead electrocardiogram (ECG) recording at screening (Visit 1) and Visit 2 pre-dose showing the following:
 - Heart rate between 45 and 90 beats per minute
 - QT interval corrected for heart rate using Fridericia's formula (QTcF) interval ≤ 450 msec
 - QRS interval ≤ 120 msec
 - PR interval ≤ 220 msec
 - No clinically significant abnormality including morphology (e.g. left bundle branch block, atrioventricular nodal dysfunction, ST segment abnormalities)
4. Capable of complying with all study restrictions and procedures including ability to use the study nebuliser correctly.
5. Body mass index (BMI) between 18 and 30 kg/m² (inclusive) with a minimum weight of 40 kg.
6. Patients with a genetic diagnosis of CF
7. Spirometry at screening demonstrating an FEV₁ $\geq 40\%$ and $\leq 80\%$ of predicted normal.
8. Capable of withdrawing from long acting bronchodilators for 48 hours prior to study visits, and short acting bronchodilators for 8 hours prior to study visits.
9. Clinically stable CF in the 2 weeks prior to randomisation (Visit 2).

Exclusion Criteria:

1. History of cirrhotic liver disease or portal hypertension.
2. CF exacerbation requiring hospitalization in the month prior to screening (Visit 1) or prior to randomisation (Visit 2).
3. Use of oral or intravenous antibiotics (in addition to usual maintenance therapy) in the 2 weeks prior to screening (Visit 1) or randomisation (Visit 2).

4. Other non-CF related respiratory disorders: Patients with a current diagnosis of asthma, active tuberculosis, lung cancer, sarcoidosis, sleep apnoea, known alpha-1 antitrypsin deficiency or other active pulmonary diseases.
5. Previous lung resection or lung transplant.
6. History of, or reason to believe a patient has, drug or alcohol abuse within the past 3 years.
7. Received an experimental drug within 3 months or five half-lives, whichever is longer.
8. Patients with a history of chronic uncontrolled disease including, but not limited to, cardiovascular (including arrhythmias), endocrine, active hyperthyroidism, neurological, hepatic, gastrointestinal, renal, haematological, urological, immunological, or ophthalmic diseases that the Investigator believes are clinically significant.
9. Documented cardiovascular disease: angina, recent or suspected myocardial infarction, congestive heart failure, a history of unstable, or uncontrolled hypertension, or has been diagnosed with hypertension in last 3 months.
10. Has had major surgery, (requiring general anaesthesia) in the 6 weeks prior to screening (Visit 1), or will not have fully recovered from surgery, or planned surgery through the end of the study.
11. Infection with nontuberculous mycobacteria, methicillin-resistant Staphylococcus aureus (MRSA), B. Cepecia, or B. cenocepecia.
12. Use of immune-suppression; long term use of prednisolone >10 mg/day
13. History of malignancy of any organ system within 5 years with the exception of localised skin cancers (basal or squamous cell)
14. Clinically significant abnormal values for safety laboratory tests (haematology, biochemistry or urinalysis) at screening, as determined by the Investigator.
15. A disclosed history or one known to the Investigator, of significant non-compliance in previous investigational studies or with prescribed medications.
16. Requires oxygen therapy on a regular basis.
17. Pregnancy or lactation (female patients only)
18. Any other reason that the Investigator considers makes the patient unsuitable to participate.

Appendix 2 Allowed and Disallowed Concomitant Medication

Patients taking inhaled steroids may continue their medication only if the dose is stable from at least four weeks prior to screening and is expected to remain stable.

All long acting (once or twice daily) bronchodilators will be stopped at the screening visit and are not allowed during the study. Patients should not use a long acting bronchodilator (long acting muscarinic antagonists [LAMA], long acting beta₂-agonists [LABA], or combination LABA/inhaled steroid) on the day of screening.

- Patients taking LAMAs and LABAs should be placed on short acting bronchodilators (e.g. salbutamol, ipratropium or Combivent) as per the discretion of the Investigator. These can be dosed on a regular scheduled basis and/or as needed use
- Patients taking combination products should be prescribed the inhaled steroid at the same or equivalent dose contained in the combination product to allow continuation of steroid use regularly throughout the study while stopping the LABA component

Patients currently taking terbutaline will be switched to other short acting bronchodilators (e.g. salbutamol).

Short acting bronchodilators must be withheld for at least 8 hours prior to spirometry at each study visit (Visit 1 to Visit 4). If this withhold is not met, the patient should be rescheduled for a repeat visit within 7 days.

Patients may continue other prescribed non-respiratory therapies during the study that the Investigator considers to neither compromise patient safety nor affect study data. Pulmonary rehabilitation programs should not be started or completed during this period. Oxygen therapy is an exclusion criterion for this study.