A Phase II, Randomised, Double Blind, Placebo Controlled, Three Way Crossover Study to Assess the Bronchodilator Effect of RPL554 Administered in Addition to Open Label Tiotropium in Patients with COPD

Verona Pharma Plc Study No: RPL554-CO-202
Syne qua non Ltd Study No: VMA16001

Statistical Analysis Plan

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Date: 19 September 2017

For Syne qua non Ltd – Lead Statistician

For Verona Pharma Plc

If Signing manually, please include: Signature + Date + Full Name + Position

Contents

LIST OF ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
AUC	Area under the curve
AUC_{0-t}	Area Under the Plasma Drug Concentration Versus Time Curve (AUC) up to the Last Measurable Time Point
AUC_{0-12h}	Area Under the Plasma Drug Concentration Versus Time Curve (AUC) up to 12 Hours
AUC_{0-24h}	Area Under the Plasma Drug Concentration Versus Time Curve (AUC) up to 24 Hours
AUC_{0-4h} FEV	Area Under the FEV Curve (AUC) up to 4 Hours
AUC_{0-12h} FEV	Area Under the FEV Curve (AUC) up to 12 Hours
AUC_{0-24h} FEV	Area Under the FEV Curve (AUC) up to 24 Hours
AUC_{0-4h} Heart Rate	Area Under the Heart Rate Curve (AUC) up to 4 Hours
AUC_{0-4h} Pulse Rate	Area Under the Pulse Rate Curve (AUC) up to 4 Hours
BMI	Body Mass Index
BP	Blood Pressure
CAS	Completer Analysis Set
C_{max}	Maximum Observed Plasma Drug Concentration
CI	Confidence Interval
CL/F	The apparent clearance
COPD	Chronic Obstructive Pulmonary Disease
CV	Coefficient of Variation
DPI	Dry Powder Inhaler
DRM	Data Review Meeting
ECG	Electrocardiogram
FAS	Full Analysis Set
FEV_1	Forced Expiratory Volume in One Second
FRC	Functional Residual Capacity
HIV	Human Immunodeficiency Virus
K_{el}	The First Order Apparent Elimination Rate Constant
KG	Kilogram
LLOQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities

PK	Pharmacokinetic
PT	Preferred Term
QTcF	QT interval corrected using Fridericia's formula
R_{aw}	Airway Resistance
RV	Residual Volume
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard Deviation
SEM	Standard Error of Mean
sG_{aw}	Specific Airway Conductance
SOC	System Organ Class
$t_{1/2}$	Terminal Elimination Half-Life
TEAE	Treatment Emergent Adverse Event
TLC	Total Lung Capacity
t_{max}	Time of the Maximum Observed Plasma Drug Concentration
V_z/F	The (Apparent) Volume of Distribution
WHO Drug	World Health Organization Drug Dictionary

1 INTRODUCTION

This document details the statistical analysis of the data that will be performed for the Verona Pharma Plc study: A Phase II, Randomised, Double Blind, Placebo Controlled, Three way Crossover Study to Assess the Bronchodilator Effect of RLP554 Administered in Addition to Open Label Tiotropium in Patients with Chronic Obstructive Pulmonary Disease (COPD).

The proposed analysis is based on the contents of the Final Version of the protocol, Version 2.0 (dated 26 April 2017). In the event of future amendments to the protocol, this statistical analysis plan (SAP) may be modified to account for changes relevant to the statistical analysis.

The table, listing and figure shells are supplied in a separate document.

2 STUDY OBJECTIVES AND DESIGN

2.1 Study Objectives

The primary objective of the study is to investigate the bronchodilator effect on peak forced expiratory volume in one second (FEV_1) (measured in first 4 hours after dosing) and average (measured as the area under the curve (AUC)) FEV_1 over 12 hours of nebulised RPL554 dosed twice daily for 3 days (five total doses), as compared to placebo, when administered in addition to once daily tiotropium.

The secondary objectives of the study are:

- To investigate the effect of twice daily nebulised doses of RPL554, as compared to placebo, when administered in addition to tiotropium on lung volumes
- To assess the bronchodilator effect on peak FEV_1 (measured in first 4 hours after dosing) and average (measured as the AUC) FEV_1 over 12 hours of nebulised RPL554 after the first dose as compared to placebo, when administered in addition to tiotropium
- To analyse plasma concentrations and assess the steady state pharmacokinetics of RPL554 when administered in addition to tiotropium
- To assess the tolerability and safety of twice daily nebulised doses of RPL554 in addition to tiotropium
- To assess the dose response of two different doses of RPL554 on peak, average (0 to 12 hours), and morning trough FEV_1 on Day 3 when dosed in addition to tiotropium
- To determine the onset of action of RPL554 when administered with tiotropium (after the first dose)
- To investigate the bronchodilator effect on nebulised RPL554, or placebo, administered in addition to tiotropium on average FEV_1 over 4 hours after each morning dose

- To investigate the effects of RPL554 on specific airway conductance (sG_{aw}) and lung volumes (residual volume [RV], functional residual capacity [FRC]) when administered in addition to tiotropium.

The exploratory objectives are:

- To examine the effect of RPL554 on top of tiotropium on average FEV_1 over 24 hours after 3 days of dosing
- To assess morning trough FEV_1 prior to the final dose of study treatment.

2.2 Study Endpoints

The primary endpoints of the study are:

- Peak FEV_1 (measured in first 4 hours after dosing) and $AUC_{0\text{-}12h}$ FEV_1. These will be measured after dosing on Day 3.

The secondary endpoints are:

- Determination of $AUC_{0\text{-}4h}$ FEV_1 after morning dosing
- Peak FEV_1 (measured in first 4 hours after dosing) and $AUC_{0\text{-}12h}$ FEV_1. These will be measured after dosing on Day 1
- Determination of onset of action (>10% increase in FEV_1, from pre-first dose, censored at 120 minutes) on Day 1
- RV, FRC, and sG_{aw} at 1.25 hours after dosing on Day 2
- RPL554 steady state pharmacokinetics (AUC, maximum observed concentration (C_{max}), time to maximum concentration (t_{max}), half-life)
- Safety and tolerability:
  - Continuous monitoring of adverse events
  - Laboratory safety tests [haematology, biochemistry and urinalysis]
  - 12-lead electrocardiogram (ECG) (including QT interval corrected using Fridericia's formula (QTcF) and heart rate), supine vital signs [blood pressure and pulse rate]
  - Peak (measured in first 4 hours after dosing) and $AUC_{0\text{-}4h}$ pulse rate for each treatment period
  - Holter monitor results

The exploratory endpoints are:

- Pre-dose FEV_1 on Day 3

2.3 Study Design

This is a phase IIb, randomised, double blind, placebo controlled, complete block three-way crossover study to investigate treatment with nebulised RPL554 and tiotropium together in patients with moderate to severe COPD. It is planned to randomise up to 30 patients to have 24 evaluable patients at one study centre. The study comprises the following: screening, three treatment periods each lasting 3 days and an end of study visit.

2.4 Visit Structure

The visit structure and scheduled assessments are detailed in the study protocol section 6.

3 SAMPLE SIZE

This is a complete block three-way crossover study. Assuming a residual coefficient of variation (CV) of 6% for peak FEV_1, 24 patients will give an 80% power to detect a pairwise difference in peak FEV_1 of 5.1%. Assuming a mean baseline FEV_1 of 1.3 litres this will correspond to a difference of about 66mL. The detectable difference for FEV_1 $AUC_{0\text{-}12h}$ is expected to be similar as for the peak.

4 RANDOMISATION

All patients consented will be assigned a screening number using the study centre's standard convention.

Patients will receive three different treatment combinations (dry powder inhaler (DPI) plus nebulised treatment) shown in Table 1 in a randomised sequence during Treatment Periods 1 to 3. All study treatments will be administered using the inhaled route. In each case, the DPI treatment will be administered first, followed immediately (within 2 minutes) by the nebulised RPL554 or placebo using a standard Jet nebuliser (PARI LC SPRINT® attached to a PARI TurboBOY® SX compressor unit). The DPI treatment will be open label and the nebulised treatment will be double blind.

Table 1: Treatment Combinations in RPL554-CO-202

TREATMENT COMBINATION	DPI TREATMENT	NEBULISED TREATMENT
1	Tiotropium 18 mcg qd	RPL554 6 mg bid
2	Tiotropium 18 mcg qd	Placebo bid
3	Tiotropium 18 mcg qd	RPL554 1.5 mg bid
Abbreviation: bid=twice daily; DPI=dry powder inhaler; qd=once daily		

Patients will be equally randomised to one of six treatment sequences (using a Latin Square design with the three different potential medication combinations) before the first study treatment administration in Treatment Period 1.

Tiotropium will be provided in trade dress and will not be blinded.

RPL554 and placebo will be administered double blind. It has not been possible to completely match the placebo to RPL554, as the visual appearance is slightly different. The study personal preparing the RPL554 and placebo, placing them into the nebuliser, and supervising dosing will therefore not to blinded to treatment identity. The dosing cup on each nebuliser will be obscured with tape to visually blind the study treatment. The sponsor, Investigator (defined as Principal Investigator and all study physicians), all patients and all other research personnel (except

bioanalytical personnel performing the pharmacokinetic assays) will therefore be blinded to the treatment allocation.
The blind should be broken only if specific emergency treatment would be dictated by knowing the treatment status of the patient. If the blind needs to be broken, the Investigator should discuss it with the Sponsor's Medical monitor in advance.

Otherwise, all blinding will be maintained until all queries are resolved and the database is locked.

5 INTERIM ANALYSIS

No formal interim analysis is planned for this study.

6 ANALYSIS PLAN

6.1 General

Summary statistics for continuous variables will consist of number of non-missing observations (n), mean, standard deviation (SD), minimum, median and maximum, unless specified otherwise. The precision of these variables is defined in the table, figure and listing shells document.

For categorical variables, the number and percentage of patients in each category will be presented, based on the number of non-missing observations apart from disposition of patients, protocol deviations, background and demographic characteristics, prior and concomitant medications/procedures and adverse events where the percentage will be based on the number of patients in the analysis set.

All statistical tests will be performed using a two-tailed 5% overall significance level, unless otherwise stated. The null hypothesis at all times will be that the treatments are equivalent. All comparisons between the treatments will be reported with 95% confidence intervals for the difference.

6.2 Derived data

- Definition of baseline

Baseline is defined as the last non-missing value (including scheduled and unscheduled assessments) prior to the patient receiving study treatment for each of the respective study treatments.

For ECG and vital signs, time-matched baselines from Day 1 of each Treatment Period will be used for the analysis of data.

- Incomplete dates

For calculation purposes, incomplete dates will be completed using worst case. Further details are detailed in the relevant sections as required.

- Non-numeric values for numeric variables

In the case where the result of a variable is recorded as “>x”, “≥x”, “<x” or “≤x”, then for analysis purposes a value of x will be taken. Where a range of values is quoted the midpoint of the range will be taken.

- Methods for handling withdrawals and missing data

Patients withdrawn after only one treatment period will not be included in the efficacy analyses. If a patient has baseline data missing for a parameter, within a treatment period, data for that patient and parameter will not be derived or analysed for that treatment period.

No imputation of missing data will be performed.

All available data from all dosed 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 treatment day is rescheduled due to variability in FEV_1 or other reason, the rescheduled treatment day will be listed and summarised as the valid treatment day.

6.3 Analysis Sets

The **Enrolled Set** includes all patients who passed screening irrespective of whether they received the study treatment.

The **Completer Analysis Set** (CAS) includes all randomised patients who complete all treatment periods.

The **Full Analysis Set** (FAS) includes all randomised patients with sufficient data collected after intake of study treatment to compute the pharmacodynamics parameters on at least two treatment periods. Patients will be analysed according to the treatment they are assigned to at randomisation, irrespective of what treatment they actually received.

The **Safety Analysis Set** (SAF) consists of all patients who take at least one administration of study treatment. Patients will be analysed according to the treatment actually taken.

The **Pharmacokinetic (PK) Set** will consist of those patients in the SAF who have at least one pre-dose and one post-dose RPL554 PK concentration and with sufficient data to calculate PK parameters. Patients will be analysed according to the treatment actually taken.

The list of patients included in the FAS, CAS, SAF and PK will be agreed prior to database lock once all study data are available.

6.4 Data presentations

The data will be summarised in tabular form by treatment group apart from disposition of patients, background and demographic characteristics which will be summarised by treatment sequence and overall patients.

Only scheduled post-baseline laboratory, vital signs and ECG values will be tabulated. Post-baseline repeat/unscheduled assessments will be disregarded, although they will be listed and in particular all clinically significant values will be noted.

All listings apart from eligibility and analysis sets will be based on SAF. Eligibility and analysis sets will be listed using the enrolled set. Listings will be sorted by treatment sequence, patient number and date/time of assessment. Treatment sequences will be presented in the following order:

- Placebo - RPL554 1.5 mg – RPL554 6 mg
- RPL554 1.5 mg – RPL554 6 mg – Placebo
- RPL554 6 mg – Placebo – RPL554 1.5 mg
- RPL554 6 mg - RPL554 1.5 mg – Placebo
- RPL554 1.5 mg – Placebo – RPL554 6 mg
- Placebo – RPL554 6 mg – RPL554 1.5 mg

Graphical presentations of the data will also be provided where appropriate.

Disposition of patients will be summarised using the enrolled set. Background and demographic characteristics will be summarised using the SAF. The primary efficacy endpoints will be summarised using the FAS and CAS. Secondary endpoints will be summarised using the FAS. Protocol deviations, prior/concomitant medications and safety will be summarised using the SAF.

6.5 Disposition of patients

The number and percentage of all patients enrolled, randomised, included in the FAS, CAS, SAF, PK analysis set who completed the study and prematurely discontinued study treatment and study duration will be summarised. The number and percentage of patients will be summarised by their reasons for withdrawal where applicable. Eligibility for each of the analysis sets along with reasons for exclusion will be listed. Study completion/withdrawal data will be listed.

6.6 Protocol Deviations

Prior to database lock, Verona Pharma Plc may review the individual deviations and classify them as major (which includes those described in section 6.3 above) or minor during a data review meeting (DRM).

Details of all protocol deviations (date, deviation category, specific details and classification of major or minor) and patient eligibility will be listed.

The number and percentage of patients with protocol deviations will be summarized by category.

6.7 Background and Demographic Characteristics

6.7.1 Demography

Demographic characteristics (age, sex, ethnic origin and race), body measurements (height, weight and body mass index (BMI)) and smoking status collected at Screening will be summarised.

Age is calculated in years from the date of first administration of study treatment.

BMI is calculated as (weight (kilogram(kg))/height (m)²).

All patient demographic data including informed consent will be listed.

6.7.2 Medical History

Medical history events will be coded using the latest Medical Dictionary for Regulatory Activities (MedDRA) dictionary version. The number and percentage of patients will be presented by system organ class (SOC), and preferred term (PT), where SOC and PT will be presented in decreasing frequency of the total number of patients with medical history events. All events will be listed, which will include a flag for ongoing conditions.

6.7.3 Diagnosis of COPD

Details of the diagnosis of COPD including date of diagnosis, date of onset of symptoms, any hospitalisations, history of a chronic cough with phlegm for at least 3 months a year for at least 2 years and number of exacerbations in the prior year (defined as needing oral steroids and/or antibiotics) taken at screening will be summarised and listed. Where possible, duration of symptoms (years) will be derived as (date of enrolment – date of symptom +1). Duration of diagnosis (years) will be derived as (date of enrolment – date of diagnosis + 1). Dates will not be imputed, instead, where days and months are missing but year known, duration can be derived from years, where months and year are known, duration can be derived using the month and year.

6.7.4 Pregnancy test

Details of the pregnancy test conducted at Screening, Treatment Period 1 - Day 1, Treatment Period 2 - Day 1, Treatment Period 3 - Day 1 and End of Study will be listed.

6.7.5 Alcohol breath test

Details of the alcohol breath test conducted at Screening will be listed.

6.7.6 Serology

Details of the screen for human immunodeficiency virus (HIV), hepatitis B and hepatitis C conducted at Screening will be listed.

6.7.7 Chest X-Ray

Details of the chest x-ray conducted at Screening will be listed.

6.7.8 Training

Details of the whole-body plethysmography and inhalation training conducted at Screening will be listed.

6.7.9 Spirometry: Reversibility Test

Reversibility in response to salbutamol will be assessed at Screening as an eligibility measure. Spirometry (FEV_1 and FVC) assessment before and after four puffs (400 mcg) of salbutamol administered using a pMDI will be performed.

Three technically acceptable measurements should be made and recorded in the eCRF. Spirometry assessments may be performed up to eight times to obtain three acceptable readings according to ATS guidelines. The highest reading from each assessment will be used for calculation of predicted values and increase from baseline.

The following must be confirmed for inclusion:
• Post-bronchodilator FEV_1/FVC ratio of ≤0.70 (derived as the highest of the 3 post-bronchodilator FEV_1 measurements divided by the highest of the 3 post-bronchodilator FVC measurements)
• Post-bronchodilator FEV_1 ≥40 % and ≤80% of predicted normal*
• Demonstrates ≥150 mL increase from pre-bronchodilator FEV_1 (derived as the highest of the 3 post-bronchodilator FEV_1 measurements – the highest of the 3 pre-bronchodilator FEV_1 measurements)

*NHANES III will be used as a reference for normal predicted values.

These data will be summarised and listed.

6.8 Prior, Rescue and Concomitant Medications and COPD Therapies

Medications will be coded using the latest World Health Organization Drug dictionary (WHO Drug) version.

Prior therapies and prior medications are defined as those that started and ended prior to the first administration of study treatment. Medications that are ongoing at the first administration of study treatment or started after time of first administration will be deemed to be concomitant medications. If medication dates are incomplete and it is not clear whether the medication was concomitant, it will be assumed to be concomitant.

The following rules will be used for defining whether a medication is concomitant and which treatment they should be assigned to:

1. If the start date is after the period first administration of study treatment and before the start of the next period, then the study treatment is concomitant and assigned to the treatment for that period.
2. The start date is before the period first administration of study treatment and the end date is after the first administration of study treatment of the period, then the medication is concomitant and assigned to the treatment for that period.
3. Any medication taken after the end of the final period (i.e. in the follow-up period) will be concomitant and will be assigned to the treatment for the final period.
4. Any medication that starts and ends during the washout period counts as part of the previous treatment period.
5. Any medication that starts during the washout period and ends after the start of the next treatment period will be assigned to the previous and current treatment period,

The number and percentage of patients taking prior and separately concomitant medications will be summarised by medication class and standardised medication name, where medication class and standardised medication name will be presented

in decreasing frequency of the total number of patients with medications. In summary tables, patients taking multiple medications in the same medication class or having the same standardised medication recorded multiple times in the study will be counted only once for that specific medication class and standardised medication name.

Medication data will be listed, where concomitant medications will be flagged.

6.9 Lifestyle Restrictions

The date and time of the lifestyle restrictions assessments, information regarding refrain from xanthine for at least 24 hours prior to visit (yes/no), information regarding refrain from alcohol for 24 hours prior to visit (yes/no) and whether the patient refrained from strenuous exercise for 72 hours prior to visit will be listed.

6.10 Administration of Study treatment and Exposure

Study treatment administration will take place at the study centre and will be administered by the investigator or designated and trained study centre personnel.

Patients will be dosed with open label tiotropium DPI 18 mcg (two inhalations from one capsule) followed immediately (within 2 minutes) with either blinded RPL554 or placebo using a nebuliser according to the randomisation scheme. At 12 hours (±30 minutes) after the morning dose, the second dose of RPL554 or placebo will be administered by nebulisation.

A summary of whether treatment was received (yes/no), whether the patient fasted from 2 hours pre-dose, and continue until 2 hours post-dose (yes/no) and has the patient refrained from smoking within 1 hour of dosing (yes/no) will be presented by timepoint and treatment day.

The number of doses of study treatment administered (0 if no treatment administered, 1 if only first or second dose taken and 2 if first and second doses taken) during each period and the number of days of exposure to the study treatment (last date of dosing minus first day of dosing + 1) during each period will be summarised.

The duration of the first and second nebulisation of RPL554 in minutes will be summarised by timepoint and treatment day and listed. Time of tiotropium dosing will be included in the listing.

Details of the first compressor unit number, volume of first residual product (mL), second compressor unit number and volume of second residual product (mL) will be summarised and listed.

6.11 Primary Endpoints

Peak FEV_1 (measured in first 4 hours after dosing) and $AUC_{0\text{-}12h}$ FEV_1. These will be measured after dosing on Day 3

The peak effect on FEV_1 during these time intervals will be computed as the maximum value within the 4 hours after dosing minus the pre-dose Day 1 baseline value.

The average effect will be calculated as the AUC divided by the length of the time interval of interest. The AUC parameters will be calculated using the linear trapezoidal rule.

Pre-dose treatment day 1 value for each treatment period will be used as the baseline value.

Changes from baseline in AUC average effect parameters will be derived by deducting the pre-dose treatment day 1 value from the average effect.

Peak effect and average effect will be $\log_e$ transformed in order to perform a multiplicative (Analysis of Covariance) ANCOVA as follows:

Log_e (Max FEV_1)

Log_e ($AUC_{0\text{-}12h}$ FEV_1 /12)

Spirometry performed within 8 hours after the use of a rescue bronchodilator (salbutamol) will not be utilized in calculations of peak or AUC FEV_1.

Observed FEV_1 data will be summarised as actual and change from baseline using descriptive statistics over time.

Hypothesis to be tested:

H_0 = tiotropium + RPL554 in patients with COPD has the same effect on FEV_1(first 4 hours)/ $AUC_{0\text{-}12h}$ FEV_1 as tiotropium + Placebo

The primary comparisons are tiotropium + RPL554 6mg vs tiotropium + RPL554 placebo, followed by tiotropium + RPL554 1.5mg vs tiotropium + RPL554 placebo.

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

6.11.1 Primary Analysis

The primary analysis will be based on the FAS and CAS. Peak FEV_1 and $AUC_{0\text{-}12h}$ FEV_1 measured after dosing on Day 3 will be analysed.

Log_e (Max FEV_1) and $\log_e$ ($AUC_{0\text{-}12h}$ FEV_1/12) will be compared between the three study treatments using ANCOVA models with fixed factors for treatment, period and patient, and using the $\log_e$ baseline FEV_1 of the treatment period (pre-first dose in a treatment period) as a covariate. FEV_1 will be analysed using multiplicative models, and the result then transformed back to the linear scale giving treatment differences as ratios of geometric means. Primarily the combination tiotropium + RPL554 will be compared to tiotropium + Placebo RPL554 for each of the two doses of RPL554. Results of the comparisons will be expressed as the mean geometric ratio with 95% confidence intervals and associated, 2-sided, p-value.

Derived FEV_1 data will be summarised and listed.

Example SAS code for Peak FEV_1 analysis is as follows:

```
PROC GLM DATA=dataset;
   CLASS TREATMENT PATIENT PERIOD;
   MODEL LOGE(MAX 4HRS POSTDOSE VALUE) = TREATMENT PATIENT PERIOD
    LOGE(BASELINE);
   LSMEANS TREATMENT / DIFF PDIFF CL;
RUN;
```

Example SAS code for $AUC_{0\text{-}12h}$ FEV_1 analysis is as follows:

```
PROC GLM DATA=dataset;
   CLASS TREATMENT PATIENT PERIOD;
   MODEL LOG_E(AUC/12) = TREATMENT PATIENT PERIOD LOG_E(BASELINE);
   LSMEANS TREATMENT / DIFF PDIFF CL;
RUN;
```

6.12 Secondary Endpoints

The following endpoints will be analysed based on the FAS:

AUC_{0-4h} FEV_1 after morning dosing on Day 1, Day 2 and Day 3

Log_e (AUC_{0-4h} FEV_1/4)

Peak FEV_1 (measured in first 4 hours after dosing) and AUC_{0-12h} FEV_1 Day 1

Peak effect and average effect will be log_e transformed in order to perform a multiplicative ANCOVA as follows:

Log_e (Max FEV_1)

Log_e (AUC_{0-12h} FEV_1/12)

Onset of action

Time to onset of action will be determined for each patient on Day 1 of each treatment period. The scheduled timepoint at which ≥10% increase from baseline was achieved and the scheduled timepoint before that will be interpolated to determine the time relative to dosing at which 10% increase occurred for that patient on that treatment day. Subjects who do not achieve a 10% increase from baseline by 120 minutes (2 hours postdose) on Day 1 will be censored.

The following formulae will be used to derive the time to onset:

Time to Onset = Time A + (FEV_1 target - FEV_1 A)/ (FEV_1 B - FEV_1 A) * (Time B - Time A)

Where:

- Time A is the scheduled timepoint before 10% increase is met
- Time B is the scheduled timepoint at which 10% increase is met
- FEV_1 target is the FEV_1 value corresponding to a 10% increase or, if FEV_1 is expressed as percentage increases from baseline, it is the value 10 itself
- FEV_1 A is the maximum FEV_1 measurement at the timepoint before 10% increase is met
- FEV_1 B is the maximum FEV_1 measurement at the timepoint at which 10% increase is met

RV, FRC, sG_{aw} and R_{aw} at 1.25 hours after dosing on Day 2

Ability to adequately perform whole body plethysmography will be conducted at screening. Whole body plethysmography assessments will be conducted at pre-dose Day 1, pre-dose and 1.25 hours post-dose on Day 2 of each treatment period. Patients will be placed on a body box for plethysmographic determination of lung volumes, to include RV, FRC and total lung capacity (TLC). Additionally,

measurements will be made of airway conductance (sG_{aw}) and airway resistance (R_{aw}).

Plethysmographic endpoints will be calculated on Day 2, at both pre-dose and 1.25 hours post dose of each treatment period. The change from pre-dose Day 1 will be calculated for RV, FRC, sG_{aw} and R_{aw} at 1.25 hours post-dose on Day 2 of each treatment period.

AUC, C_{max}, t_{max} and half-life

RPL554 steady state pharmacokinetics (AUC, maximum observed concentration (C_{max}), time to maximum concentration (t_{max}) and half-life) will be determined. Analysis of PK data is detailed in section 6.16.

Rescue Medication

The use of rescue medication during the study will documented. Short acting bronchodilators (e.g. salbutamol, ipratropium or Combivent) may be used as rescue medication. Salbutamol should be considered the primary rescue medication and ipratropium secondary. The time to the first rescue medication use is defined as the duration from the date of first dose of study treatment to the date of the first administration of a rescue medication for each study period calculated in hours. Subjects who do not use rescue medication will be right-censored at the date of the last timepoint of the last treatment day of each study period.

Tolerability and Safety

Assessment of safety and tolerability of twice daily nebulised doses of RPL554 in addition to tiotropium is detailed in section 6.17.

6.12.1 Secondary Analysis

Continuous variables will be compared between the three study treatments using analysis of covariance (ANCOVA) models with fixed factors for treatment, period and patient, and using the log_e baseline measurement of the treatment day (pre-first dose in a treatment period) as a covariate. The analysis uses multiplicative models.

The combination of tiotropium + RPL554 will be compared to tiotropium alone for each of the two doses of RPL554. Results of the comparisons will be expressed as the mean geometric ratio with 95% confidence intervals and associated, 2-sided, p-value.

$AUC_{0\text{-}4h}$ FEV_1 after morning dosing will be determined separately for Day 1, Day 2 and Day 3 and analysed as above, as average effect ($AUC_{0\text{-}4h}$ /4 FEV_1).
Median time to onset of action for each study treatment will be calculated. The median time to onset across all patients will only be reported if at least 50% of patients achieve a 10% increase from baseline in FEV1 from pre-first dose within 120 minutes on Day 1. 95% CI of the median time to onset will also be presented. Kaplan-Meier plot illustrating time to onset will also be constructed. Where possible, the median time to onset of action data will be compared using a Wilcoxon signed rank test. P-values will be generated for comparisons of the median time to onset of the two tiotropium + RPL554 doses against tiotropium + Placebo.

Log_e transformed Plethysmographic endpoints will be compared in the same way as FEV_1. The log_e baseline of the treatment day (pre-first dose in a treatment period) will be used as covariate in these models. Results of the comparisons will be back

transformed and expressed as the mean geometric ratio with 95% confidence intervals and associated, 2-sided, p-value.

All plethysmography data will be listed and summarised where applicable. A histogram of change from baseline (pre-dose day 2 to pre-dose day 1) and (post-dose day 2 to pre-dose day 1) mean plethysmography data will be presented for actual values and percent predicted values of RV, FRC and actual values for sGaw and R_{aw} for each study treatment. All treatments will be presented on the same figure.

The use of rescue medication during the study timepoints will be summarised by treatment, as the number and percentage of patients who used rescue medication, and, if appropriate, a Kaplan-Meier plot illustrating time to first use of rescue constructed. Rescue medication data will also be listed.

6.13 Exploratory Endpoints

The following endpoint will be analysed based on the FAS:

- Pre-dose FEV_1 on Day 3

6.13.1 Exploratory Analysis

Log_e (pre-dose FEV_1) on Day 3 will be compared between the three study treatments using analysis of covariance (ANCOVA) models with fixed factors for treatment, period and patient, and using the baseline measurement of the treatment day (pre-first dose in a treatment period) as a covariate. The analysis uses multiplicative models and the result will be transformed back to the linear scale giving treatment differences as ratios of geometric means.

The combination of tiotropium + RPL554 will be compared to Placebo tiotropium + RPL554 for each of the two doses of RPL554. Results of the comparisons will be expressed as the mean geometric ratio with 95% confidence intervals and associated, 2-sided, p-value.

6.14 Summary of AUC Data

Changes from baseline in AUC parameters will be derived using the following definitions for baseline:

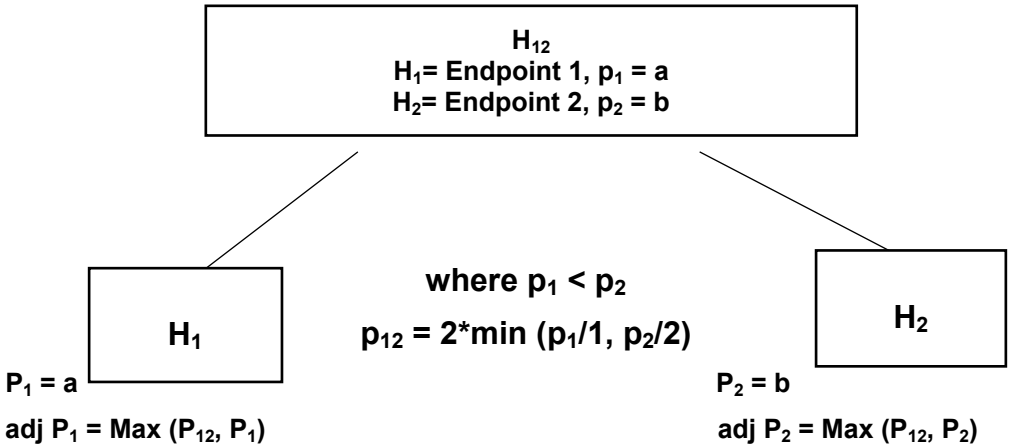
- $AUC_{0\text{-}4h}$: 4 * pre-dose treatment day 1 value
- $AUC_{0\text{-}12h}$: 12 * pre-dose treatment day 1 value
- $AUC_{0\text{-}24h}$: 24 * pre-dose treatment day 1 value

6.15 Multiplicity

For the primary endpoints analyses, a closed test procedure will be used to maintain the overall type 1 error rate starting with the highest dose of RPL554. The method is illustrated in Figure 1 below.

FIGURE 1: CLOSED TESTING PROCEDURE

For the RPL554 6mg dose compared to RPL554 Placebo for each of peak and AUC $FEV_{1,}$ a significant result will be required for both variables to proceed to testing the lower dose.



If H_1 and H_2 are rejected, then analysis of the primary endpoints will stop here. However, if both H_1 and H_2 are accepted then the above closed test procedures will be repeated for the lower dose of RPL554 compared to placebo RPL554. If either H_1 or H_2 are accepted, testing will continue for the hypothesis that was accepted for the lower dose of RPL554 compared to placebo RPL554.

The adjusted p-value of the relevant endpoint from above, will be used to assess significance.

All secondary endpoints and the supportive analyses will be considered as descriptive evidence of efficacy and will be analysed without any procedures to account for multiple comparisons.

6.16 Spirometry: Pulmonary Function Tests

Spirometry (FEV_1 and FVC) will be performed at screening, the end of study visit and the following time points in each treatment period:
• Day 1: pre-dose; 5, 15 and 30 minutes and 1, 1.5, 2, 4, 6, 8 and 12 hours
• Day 2: pre-dose; 15 and 30 minutes and 1, 2, 3 and 4 hours
• Day 3: pre-dose; 5, 15 and 30 minutes and 1, 1.5, 2, 4, 6, 8, 12, 15 and 24 hours
Post-dose measurements will be taken in relation to the morning dose of tiotropium DPI and RPL554 or placebo. The 12-hour measurement will therefore be taken pre-dose before the evening dose of RPL554 or placebo. The 24-hour measurement on Day 3 will be taken in the morning on Day 4 prior to discharge from the study centre.

Spirometry assessments will be made in accordance with ATS/ERS guidelines (Miller et al, 2005). At all timepoints, three technically acceptable measurements should be made and recorded. Spirometry assessments may be performed up to eight times to obtain three acceptable readings according to ATS guidelines (Miller, 2005). The highest FEV_1 and FVC readings from each assessment will be used for analysis even if the FEV_1 and FVC values come from two different forced exhalations.

Pulmonary function tests data (FEV_1 and FVC) including change from baseline will be listed and summarised.

Mean (±Standard error of mean (SEM)) figures will be presented for FEV_1 and FVC data separately using change from baseline data where baseline is the pre-dose Day 1 value. Separate figures will be generated for FEV_1 and FVC data collected from Day 1 to Day 3. The three treatments will be represented on the figures using different colours.

6.17 Pharmacokinetics

Plasma concentrations of RPL554 will be listed and summarised over time. Values below the lower limit of quantification (LLOQ) of 5 pg/mL will be set to 0.5 *LLOQ for calculation of summary plasma concentration statistics. Individual plasma concentration profiles will be plotted both on the original scale and on the log scale separately. Mean (±SD) and median plasma concentration profiles will be plotted on the original scale and on the log scale.

The following steady state pharmacokinetic parameters will be calculated from plasma concentrations of RPL554 using standard non-compartmental methods by LGC Ltd and transferred to SQN for analysis.

- Area under the plasma drug concentration versus time curve (AUC) up to 12 hours ($AUC_{0\text{-}12h}$ (pg.h/mL))
- Area under the plasma drug concentration versus time curve (AUC) up to 24 hours ($AUC_{0\text{-}24h}$ (pg.h/mL))
- Area under the plasma drug concentration versus time curve (AUC) up to the last measurable time point ($AUC_{0\text{-}t}$ (pg.h/mL))
- Maximum plasma drug concentration (C_{max} (pg/mL))
- Time of observed maximum plasma drug concentrations (t_{max} (h))
- The first-order apparent elimination rate constant (K_{el} (hr^{-1}))
- Half-life ($t_{1/2}$(h)) The apparent clearance (CL/F (mL/min))
- The (apparent) volume of distribution (V_z/F (L))

These PK parameters will be summarised by treatment using descriptive statistics (n, geometric mean, coefficient of variation (CV), minimum, maximum and median for AUC parameters, C_{max}, half-life, and n, arithmetic mean, standard deviation, minimum, maximum and median for.: $t_{1/2}$, t_{max}, C_{max}, $AUC_{0\text{-}12h}$, $AUC_{0\text{-}24h}$ and $AUC_{0\text{-}t}$, CL/F, V_z/F. $K_{el.}$

The PK parameters will be listed.

6.18 SAFETY EVALUATION

Safety and tolerability will be assessed by assessments including physical examinations, vital signs, 12-lead ECGs, 24-Hour Holter monitoring, adverse event recording and laboratory safety tests.

Safety data will be summarised by treatment group and time point of collection when appropriate. For continuous variables, the change from baseline (pre-dose at each treatment day) 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 selected time points. Separate listings will be generated of abnormal values occurring after the first dose of study treatment.

6.18.1 Adverse Events

Adverse events (AEs) will be coded using the latest MedDRA dictionary version.

A treatment-emergent adverse event (TEAE) is defined as an AE that started on or after the start of the administration study treatment. If adverse event dates or times are incomplete and it is not clear whether the adverse event was treatment-emergent, it will be assumed to be treatment-emergent.

Where there are partial dates, if the start date and other data mean that the event could have occurred during more than one treatment, then the event will be recorded under the lowest possible active study treatment excluding placebo that the TEAE could be assigned to.

Adverse events are treatment emergent and accountable to period x treatment if they started on or after the start of the administration study treatment in period x up to the first administration of study treatment in period x+1. However, for treatment period 3, adverse events are treatment emergent and accountable to the last treatment period if they started on or after the start of the administration study treatment in the last period up to the end of the follow-up period.

If an adverse event starts in period x and continues on till after period x+1, the adverse event will be counted in period x, i.e. the period where it first occurred. If the start time of adverse event is missing, the adverse event will be taken to have started post dose.

A treatment-related TEAE is defined as a TEAE that is definitely, possibly and unlikely related to the study treatment. If the TEAE has a missing relationship it is assumed to be related to the study treatment for analysis purposes.
System organ class and preferred term will be ordered by decreasing frequency of the total number of patients with TEAEs.

A summary table will present the following:

- TEAEs (events and patients).
- Serious TEAEs (events and patients).
- Serious study treatment related TEAEs (events and patients).
- TEAEs by severity (mild/moderate/severe) (events and patients).
- TEAEs by relationship (definitely/probable/possible/unrelated) to study treatment, the pooled study treatment related category and the pooled study unrelated category (events and patients).
- TEAEs leading to withdrawal from study (patients only).
- TEAEs leading to discontinuation of study treatment (patients only).
- Study treatment related TEAEs leading to discontinuation of study treatment (patients only).
- TEAEs leading to death (patients only).

In the above summaries, if a patient experienced more than one TEAE, the patient will be counted once using the most related event for the “by relationship to study treatment” and “related to study treatment” summaries and at the worst severity for the “by severity” summary.

The following tables will be presented:

- TEAEs by system Organ Class (SOC) and Preferred Term (PT) and by treatment group.
- TEAEs by PT.
- TEAEs by SOC, PT and severity.
- TEAEs by SOC, PT and relation to study treatment.

For all of the above, SOC and PT will be presented in decreasing frequency of the total number of patients with TEAEs.

Further details of the above four tables are given below:

1. If a patient experienced more than one TEAE, the patient will be counted once for each SOC and once for each PT.
2. If a patient experienced more than one TEAE, the patient will be counted once for each PT.
3. If a patient experienced more than one TEAE, the patient will be counted once for each SOC and once for each PT at the worst severity.
4. If a patient experienced more than one TEAE, the patient will be counted once for each SOC and once for each PT using the most related event.

Adverse event data will be listed in full and this will also include a treatment emergent flag, the time of onset and cessation of event relative to first dosing of study treatment and duration of AE. All SAEs, AEs leading to withdrawal and AEs leading to study treatment discontinuation will be listed separately.

6.18.2 Clinical Laboratory Evaluation

Observed values of haematology and biochemistry assessments will be summarised over time. If the test results are reported in categorical format, the results will be summarised by patient counts and percentage for each category.

Haematology, biochemistry and urinalysis data will be listed separately and clinical significance will be flagged.

6.18.3 Vital Signs

The AUC parameters will be calculated using the linear trapezoidal rule. The peak pulse rate will be computed as the maximum value in the 4 hours after dosing minus the pre-dose Day 1 baseline value.

Peak (measured in first 4 hours after dosing) and $AUC_{0\text{-}4h}$ pulse rate will be derived.

Vital sign observed values and change from baseline by parameter (unit) will be summarised over time.

In addition, ‘substantial’ changes from baseline will be categorised as follows: change from baseline in systolic/diastolic blood pressure (BP) (systolic BP [>±40 mmHg], diastolic BP [>±20 mmHg]) and pulse rate [>±30 bpm]). The number

and percentage of patients with changes from baseline as categorised above will be summarised separately for positive and negative changes over time and at any post-baseline time point.

All vital sign data will be listed including change from baseline and flags for substantial changes from baseline.,

A listing of clinically significant vital signs recorded throughout the study will be provided.

Mean (±SEM) figures will be presented for changes from baseline blood pressure and pulse rate data for each treatment day. The three study treatments will be represented by different colours on the figures at each postdose timepoint.

6.18.4 Electrocardiography

The AUC parameters will be calculated using the linear trapezoidal rule. The peak heart rate will be computed as the maximum value in the 4 hours after dosing.

Peak (measured in first 4 hours after dosing) and AUC_{0-4h} heart rate will be derived.

ECG data including QTcF and heart rate data will be summarised and listed.

Shift table in relation to the normal range from baseline over time for the absolute values will be presented for ECG data. Number and percentage of subjects will be presented for the change from baseline values. The absolute values will be categorised as: ≤450 msec, >450 msec, >480 msec and >500 msec. The change from baseline values will be categorised as: increase >30 msec and increase >60 msec.

Mean (±SEM) figures will be presented for changes from baseline QTcF and heart rate data for Day 1 and Day 3 separately. The three study treatments will be represented by different colours on the figures at each postdose timepoint.

All ECG overall interpretation data will be listed. Abnormal ECG data will be listed separately.

6.18.5 24-Hour Holter Monitoring

All 24-hour Holter monitoring and parameter data will be listed. Data for time in AFIB percentage, supraventricular runs, total QRS complexes, ventricular runs, ventricular singles and RR > 2 seconds will be summarised.

6.18.6 Physical Examination

The number and percentage of patients with physical examination findings will be presented for each body system over time in the form of a shift table. The shift table will show change from normal to abnormal across body systems. Data will be classed as normal, abnormal and missing.

Individual patient physical examination data will be listed.

6.18.7 Safety Analyses

Peak (measured in first 4 hours after dosing) and AUC_{0-4h} pulse rate and heart rate will be analysed.

Peak and AUC_{0-4h} for the pulse rate and heart rate will be analysed by comparing between the three study treatments using ANCOVA models with fixed factors for

treatment, period and patient, and using the baseline measurement of the treatment day (pre-first dose in a treatment period) as a covariate. The combination of tiotropium + RPL554 will be compared to tiotropium alone for each of the two doses of RPL554. Results of the comparisons will be expressed as the least squared means with 95% confidence interval (CI) and associated, 2-sided p-value.

6.19 Changes from the Protocol Planned Analysis

The definition of the PK analysis set has been edited to clarify that patients are required to have at least one pre-dose and one post-dose PK concentration to be part of the PK analysis set.

It was clarified that the Peak parameters will be derived for inclusion in analysis models as the $\log_e$ (maximum value measured within the first 4 hours) as they are assumed not to be normally distributed.

$AUC_{0\text{-}24h}$ Day 3 will not be analysed.

An additional plethysmography endpoint, R_{aw} will now be analysed.
Details of derivation of time to first use of rescue medication was added.