Verona Pharma

CLINICAL STUDY PROTOCOL

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

STUDY NO. RPL554-010-2015

Version:	**7.0**
Date:	**19 October 2017**
Phase	**IIa**
Investigational Medicinal Product:	**RPL554**
EudraCT Number	**2015-004263-36**

THIS STUDY WILL BE CONDUCTED IN ACCORDANCE WITH THE INERNATIONAL CONFERENCE ON HARMONISATION GUIDELINES FOR GOOD CLINICAL PRACTICE (DIRECTIVE CPMP/ICH/135/95), THE DECLARATION OF HELSINKI (1964) AS AMENDED AND APPLICABLE REGULATORY REQUIREMENTS

SPONSOR SIGNATURE PAGE

THIS DOCUMENT HAS BEEN APPROVED BY VERONA PHARMA PLC:

Name and Title	Signature	Date
[redacted]		

INVESTIGATOR SIGNATURE PAGE

I, the undersigned, am responsible for the conduct of the study at my study centre and agree to the following:

I understand that this protocol is a confidential document for the use of the Investigator's team and other persons involved in the study only, and for the information of the ethics committee. The information contained herein must not be communicated to a third party without prior written approval from the Sponsor.

I understand and will conduct the study according to the protocol, any approved protocol amendments, ICH GCP and all applicable regulatory authority requirements and national laws. To ensure compliance with the guidelines, the study will be monitored by a representative of the Sponsor and may be audited by an independent body. I agree, by written consent to the protocol, to fully co-operate with compliance checks by allowing access to all documentation by authorised individuals.

I have read and understand fully the Investigator Brochure and I am familiar with the study treatment and its use according to this protocol.

Name and Title	Signature	Date
Dr. med. Irene Heimbeck Coordinating Investigator	[redacted]	

CONFIDENTIALITY STATEMENT

The contents of this document are the property of Verona Pharma plc and should be regarded as confidential.

It is intended to provide information on the investigational medicinal product for the use of clinical Investigators, their research associates, members of ethics committees as well as others directly concerned in the conduct of clinical studies.

It may not be copied, reproduced or cited as a reference without the permission of Verona Pharma plc from whom additional copies can be obtained if needed.

No unpublished information contained herein may be disclosed without the prior written approval of Verona Pharma plc.

CONTACT LIST

Investigator: Study Location:	Prof Andres Floto Cambridge Centre for Medical Research Papworth Hospital [redacted]
Investigator: Study Location:	Dr Irene Heimbeck Inamed GmbH [redacted]
Sponsor:	Verona Pharma plc 3 More London Riverside London, SE1 2RE UK
Sponsor's Medical Monitor:	[redacted]
E-mail address for reporting SAEs:	
Contract Research Organisation:	
Local Laboratories: (Safety Analysis)	

Local Laboratory: (Biomarker Analysis for both centres)	[redacted]
Central Laboratory: (Pharmacokinetic Analysis for both centres)	

DETAIL OF AMENDMENTS SINCE THE PREVIOUS VERSION

Protocol Synopsis, Inclusion Criteria for ECG: The lower limit of heart rate is changed from 45 to 50 beats per minute. The upper limit of PR interval is reduced from 220 msec to 200 msec.

Protocol Synopsis, new Exclusion Criteria: Patient treated with ivacaftor or ivacaftor/ lumacaftor will be excluded from study participation.

Section 4.1, Inclusion Criteria #3 (ECG): The lower limit of heart rate is changed from 45 to 50 beats per minute. The upper limit of PR interval to be reduced from 220 msec to 200 msec.

Section 4.2, new Exclusion Criteria: Patients treated with ivacaftor or ivacaftor/lumacaftor will be excluded from study participation.

SYNOPSIS

Title of Study:	A Phase IIa, randomised, double blind, placebo controlled, three way crossover study to assess the pharmacokinetics of RPL554 administered to adult patients with Cystic Fibrosis.
Protocol Number:	RPL554-010-2015
EudraCT Number:	2015-004263-36
Phase:	IIa
Sponsor:	Verona Pharma plc
Principal Investigators:	Prof Andres Floto Dr Irene Heimbeck
Study Centres:	Cambridge Centre for Lung Infection [redacted] Inamed GmbH [redacted]
Planned Study Period:	January 2017 to December 2017
Objectives:	**Primary Objective** To investigate pharmacokinetics of single nebulised doses of RPL554 in patients with Cystic Fibrosis (CF) **Secondary Objectives** • To investigate the bronchodilator effect on peak forced expired volume in 1 second (FEV_1) after single nebulised doses of RPL554 as compared to placebo • To investigate the bronchodilator effect on area under the curve (AUC) FEV_1 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 **Exploratory Objectives** • To examine the anti-inflammatory effects of single nebulised doses of RPL554 in patients with CF
Study Design and Methodology:	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 ensure that 10 complete all three treatment periods at two centres. 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 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 FEV_1 at Visit 3 and Visit 4 must be within ±20% of the pre-dose FEV_1 at Visit 2 in order to ensure consistent baseline FEV_1 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 (FEV_1 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 (at UK centre only) • 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).
Number of Patients Planned:	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.
Main Criteria for Eligibility:	***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 old, 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 ECG recording at screening (Visit 1) and Visit 2 pre-dose showing the following: • Heart rate between 50 and 100 beats per minute • QT interval corrected for heart rate using Fridericia's formula (QTcF) interval ≤450 msec • QRS interval ≤120 msec • PR interval ≤200 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 between 18 and 30 kg/m^2 (inclusive) with a minimum weight of 40 kg. 6. Patients with a genetic diagnosis of CF. 7. Spirometry demonstrating an FEV_1 of ≥40% and ≤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 hospitalisation in the month prior to screening (Visit 1) or prior to randomisation (Visit 2). 3. Use of oral or intravenous antibiotics (in additional 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 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, unless CF related, 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) or Burkholderia species. 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 (Visit 1), 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. Patients treated with ivacaftor or ivacaftor/lumacaftor will be excluded. 17. Requires oxygen therapy on a regular basis. 18. Pregnancy or lactation (female subjects only). 19. Any other reason that the Investigator considers makes the patient unsuitable to participate.
Study Treatments:	Patients will receive the following three single doses of study treatment, one at each treatment visit (Visit 2 to Visit 4), in a randomised sequence: 1.5 mg RPL554, 6mg RPL554 or placebo. All study treatments will be administered using the inhaled route using a standard Jet nebuliser (PARI LC Sprint plus a PARI TurboBOY SX compressor unit). [redacted]
Duration of Treatment:	The approximate planned duration for each patient will be up to 52 days: 3 to 14 days screening, up to 28 days treatment (three treatment visits each usually separated by 3 to 14 days) and an end of study visit 3 to 10 days after the last treatment visit.
Statistical Methods:	This is a sample size of convenience. No powering calculation was performed. ***Pharmacodynamics:*** Treatments will be compared using analysis of covariance adjusting for treatment, period, patient and baseline. Multiplicative models will be used for FEV_1 and additive models for blood pressure, pulse rate and ECG heart rate. Active treatments will first be compared to placebo using a closed test procedure (within substance) starting with the highest dose. ***Other Analyses:*** Biomarker measurements and exhaled breath pH will be analysed by Student's T test. Other data will be analysed descriptively. Adverse events will be presented by system organ class and preferred term and summarised by study treatment and further by intensity and relationship to study treatment.

TABLE OF CONTENTS AND LIST OF TABLES AND FIGURES

Table of Contents

SPONSOR SIGNATURE PAGE 2
INVESTIGATOR SIGNATURE PAGE 3
CONFIDENTIALITY STATEMENT 3
CONTACT LIST 4
DETAIL OF AMENDMENTS SINCE THE PREVIOUS VERSION 6
SYNOPSIS 7
TABLE OF CONTENTS AND LIST OF TABLES AND FIGURES 10
Table of Contents 10
List of Tables and Figures 13
LIST OF ABBREVIATIONS AND DEFINITION OF TERMS 14
1 INTRODUCTION 16
1.1 Disease and Study Treatment Review 16
1.2 Summary of Risks and Benefits 18
2 OBJECTIVES 19
2.1 Primary Objective 19
2.2 Secondary Objectives 19
2.3 Exploratory Objectives 19
3 INVESTIGATIONAL PLAN 20
3.1 Overall Study Design and Plan Description 20
3.2 Discussion of Study Design, including the Choice of Control Groups 21
3.3 Planned Duration of the Study 22
3.4 Definition of the End of the Study 22
4 SELECTION OF STUDY POPULATION 22
4.1 Inclusion Criteria 22
4.2 Exclusion Criteria 23
4.3 Removal of Patients from Therapy or Assessment 23
4.3.1 Study Treatment Discontinuation 24
4.3.2 Patient Withdrawal 24
4.3.3 Study Discontinuation 24
4.3.4 Replacement Policy 25
5 STUDY TREATMENTS 25
5.1 Study Treatments Administered 25
5.2 Identity of Study Treatments 25
5.3 Preparation and Labelling 26
5.4 Selection of Doses, Dosing Schedule and Route of Administration 26
5.4.1 Selection of Doses in the Study 26
5.4.2 Selection and Timing of Dose for each Patient 26
5.5 Storage 27
5.6 Accountability 27

5.7 Method of Assigning Patients to Treatment Groups....28
5.8 Blinding....28
5.9 Prior and Concomitant Therapies and Medications....29
5.9.1 Prior and Concomitant Therapies....29
5.9.2 Prior and Concomitant Medications....29
5.10 Rescue Medications....29
5.11 Treatment Compliance....29
6 STUDY PROCEDURES AT EACH VISIT....30
6.1 Screening (Visit 1)....32
6.2 Visit 2....32
6.2.1 Pre-Dose Assessments....32
6.2.2 Study Treatment Administration....33
6.2.3 Post-Dose Assessments....33
6.3 Visit 3 to 4....33
6.3.1 Pre-Dose Assessments....33
6.3.2 Study Treatment Administration....34
6.3.3 Post-Dose Assessments....34
6.4 End of Study (Visit 5)....34
7 STUDY METHODOLOGY....35
7.1 Demographics, Baseline Characteristics and Eligibility Assessments....35
7.1.1 Demographic Variables....35
7.1.2 Medical/Surgical and Disease History....35
7.1.3 Reversibility Test....35
7.1.4 Screening Laboratory Eligibility Assessments....35
7.1.5 Prior and Concomitant Medications and Therapies....35
7.1.6 Eligibility Check....35
7.2 Pharmacodynamic Assessments....36
7.2.1 Pulmonary Function Tests....36
7.2.2 Sputum Measurements....36
7.2.3 Exhaled breath pH....36
7.3 Pharmacokinetic Assessments....36
7.4 Safety Assessments....37
7.4.1 Adverse Events....37
7.4.2 Laboratory Safety Assessments....37
7.4.3 Vital Signs....37
7.4.4 Physical Examination....37
7.4.5 12-Lead ECG....38
7.5 Blood Sampling....38
7.6 Appropriateness of Measurements....38
8 HANDLING OF ADVERSE EVENTS AND PREGNANCIES....38
8.1 Adverse Event Definitions....38

8.2 Recording and Assessing Adverse Events 39
8.2.1 Severity 39
8.2.2 Chronicity 40
8.2.3 Causality 40
8.2.4 Action and Outcome 41
8.3 Reporting Procedure for SAEs 41
8.4 Management of Pregnancies 41
9 QUALITY ASSURANCE AND QUALITY CONTROL 42
9.1 Audit and Inspection 42
9.2 Monitoring and Source Document Verification 42
9.3 Data Management and Coding 43
10 STATISTICAL METHODS 44
10.1 Statistical and Analytical Plans 44
10.2 Populations to be Analysed 44
10.3 Study Endpoints 44
10.3.1 Primary Endpoints 44
10.3.2 Secondary Endpoints 44
10.3.3 Exploratory Endpoints 45
10.4 Statistical Methods 45
10.4.1 Patient Disposition 45
10.4.2 Protocol Deviations 45
10.4.3 Demographics and Other Baseline Characteristics 45
10.4.4 Extent of Exposure and Treatment Compliance 45
10.4.5 Pharmacodynamics 45
10.4.6 Pharmacokinetics 46
10.4.7 Safety 46
10.4.8 Handling of Withdrawals or Missing Data 46
10.4.9 Interim Analyses 47
10.5 Determination of Sample Size 47
11 ETHICAL CONSIDERATIONS 47
11.1 Guidelines 47
11.2 Ethics and Regulatory Approval 47
11.3 Informed Consent Process 47
11.4 Patient Confidentiality 47
11.5 Record Maintenance/Retention 48
12 FINANCE AND INSURANCE 48
13 PUBLICATION POLICY 48
14 REFERENCES 49
15 APPENDICES 51
Appendix 1 Acceptable Methods of Contraception 51

List of Tables and Figures

Figure 1 Study Flow Chart 20
Table 1 Composition of Nebulised RPL554 and Placebo Formulations 25
Table 2 Schedule of Assessments in RPL554-010-2015 31

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ANCOVA	Analysis of covariance
ATS	American Thoracic Society
AUC	Area under the curve
BMI	Body mass index
BD	Becton Dickinson
C_{max}	Maximum concentration
CF	Cystic Fibrosis
CFR	Code of Federal Regulations
CFTR	Cystic Fibrosis transmembrane conductance regulator
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CRF	Case report form
E_{av}	Average effect
EBC	Exhaled breath condesate
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
E_{max}	Maximum effect
ERS	European Respiratory Society
ETFE	Ethylene tetrafluoroethylene
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
FEV_1	Forced expired volume in 1 second
FVC	Forced vital capacity
ICH	International Conference on Harmonisation
IL-8	Interleukin 8
IUPAC	International Union of Pure and Applied Chemistry
LABA	Long acting $beta_2$-agonist
LAMA	Long acting muscarinic antagonist
LPS	Lipopolysaccharide
MAD	Multiple ascending dose(s)

MedDRA	Medical Dictionary for Regulatory Activities
MPO	Myeloperoxidase
MRSA	Methicillin-resistant Staphylococcus aureus
PC_{20}MCh	Provocative concentration of methacholine chloride causing a fall in FEV_1 of 20% from baseline
PDE	Phosphodiesterase
QTcF	QT interval corrected for heart rate using Fridericia's formula
SAE	Serious adverse event
SAD	Single ascending dose(s)
SAS	Statistical analysis software
SD	Standard deviation
SOC	System organ class
SOP	Standard operating procedure
SUSAR	Suspected, unexpected serious adverse reaction
$t_{1/2}$	Half-life
t_{max}	Time to maximum concentration
TNF-α	Tumour necrosis factor alpha
UK	United Kingdom

1 INTRODUCTION

1.1 Disease and Study Treatment Review

RPL554, a small molecule isoquinolone derivative, is a dual inhibitor of two isoforms (type 3 and 4) of the phosphodiesterase (PDE) family of enzymes. PDE3 and PDE4 are known to have a role in modulating the inflammatory airway response in respiratory diseases, including chronic obstructive pulmonary disease (COPD), allergic asthma and allergic rhinitis. In general, PDE3 inhibitors act as bronchodilators (through interaction with smooth muscle cells), whilst PDE4 inhibitors have anti-inflammatory properties and there is also evidence to suggest that combined inhibition of PDE3 and PDE4 can have additive or synergistic anti-inflammatory and bronchodilator effects (reviewed by Abbott-Banner & Page, 2014). Pharmacological evidence from non-clinical models in which dual PDE3/4 inhibitors have been examined, suggests that RPL554 may have potential therapeutic activity in allergic asthma, COPD, allergic rhinitis and Cystic Fibrosis (CF). Dual PDE3/PDE4 inhibitors (administered by inhalation) and PDE4 inhibitors (when administered orally) have exhibited bronchodilator and anti-inflammatory actions respectively in clinical studies (Ukena et al, 1995; Grootendorst et al, 2007); however, oral administration of PDE4 inhibitors has been associated with unfavourable gastrointestinal side effects such as nausea, emesis, diarrhoea, abdominal pain, loss of appetite and weight loss. Indeed, these were some of the most common side effects of the PDE4 inhibitors, roflumilast and cilomilast observed in clinical studies involving patients with asthma or COPD (Harbinson et al, 1997; van Schalkwyk et al, 2005; Compton et al, 2001; Rabe et al, 2005; Rennard et al, 2006; Calverley et al, 2007; Gamble et al, 2003; Grootendorst et al, 2007). It is plausible that increased efficacy with reduced side effects may be achievable with administration of a dual PDE3/4 inhibitor by the inhaled route compared to orally administered PDE3 or PDE4 inhibitors.

Furthermore, in isolated human bronchi pre-contracted with acetylcholine, concomitant administration of low concentrations of RPL554 and salbutamol, or low concentrations of RPL554 and anti-muscarinic antagonists (atropine and glycopyrrolate), induced additive and synergistic relaxation, respectively. In addition, the concomitant administration of low concentrations of RPL554 plus glycopyrrolate also produced a significant synergistic relaxant effect of bronchi pre-contracted with histamine (Calzetta et al, 2013). RPL554 and glycopyrronium also caused a synergistic interaction in relaxing both human medium and small isolated bronchi, not just in terms of peak relaxation but also with regards to an extended duration of action (Calzetta et al, 2015). RPL554 has also been shown to interact with $beta_2$-agonists and anti-muscarinic antagonists in vivo. Specifically the combination of RPL554 and atropine or RPL554 and salbutamol caused a greater relaxation of bombesin-induced contraction in guinea pig airways than RPL554 alone (Keir et al, 2014)

Preliminary data has demonstrated that RPL554 can activate the Cystic Fibrosis transmembrane conductance regulator (CFTR), the basic defect in CF, and also that it is additive with Kalydeco (ivacaftor; VX770) CFTR potentiation in F508del/R117H-CFTR human bronchial epithelial cells (Turner et al, 2016). It is hypothesised that RPL554 should be of significant benefit to CF patients by virtue of its proven bronchodilator and anti-inflammatory activities in man, thus reducing symptoms and preventing lung tissue destruction.

The safety, bronchodilator, bronchoprotective and anti-inflammatory activities of the novel dual PDE3/4 inhibitor, RPL554 has been evaluated and reported in four completed studies in healthy subjects, patients with mild-moderate persistent asthma and those with COPD

(Franciosi et al, 2013). These studies are described in detail in the Investigator's Brochure.

[redacted]

inhalation using this solution formulation was low and somewhat variable, with maximum concentration (C_{max}) values ranging from about 0.9 ng/mL following administration at 0.018 mg/kg to about 4 ng/mL at 0.072mg/kg. Area under the curve $(AUC)_{0-t}$ values ranged from about 1.5 ng.h/mL to 11 ng.h/mL over the same dose range. Mean half-life ($t_{1/2}$) values ranged from approximately 3 to 7 hours.

RPL554 delivered by inhalation as a nebulised solution was well tolerated. Adverse events were mild and generally of equal frequency between placebo and active treatment groups. RPL554 produced a rapid bronchodilation in asthmatic patients with a maximal forced expired volume in one second (FEV_1) increase of 520 mL (95% confidence interval [CI]: 320-720 mL; p<0.0001), which was a 14% increase from baseline and increased the provocative concentration of methacholine chloride causing a fall in FEV_1 of 20% from baseline (PC_{20}MCh) by 1.5 doubling doses (95% CI: 0.63-2.28; p=0.004). When inhaled once daily for 6 days, RPL554 produced a similar magnitude of bronchodilation each time. RPL554 was absorbed into the blood stream, but had limited systemic bioavailability reaching a geometric mean C_{max} of RPL554 in blood of 1706 pg/mL (Day 1) and 1904 pg/mL (Day 6), with rapid plasma clearance and no drug accumulation. In COPD patients, RPL554 produced clinically meaningful bronchodilation with a mean maximal FEV_1 increase of 17%. In healthy subjects, RPL554 also produced a significant inhibition of the lipopolysaccharide (LPS)-induced recruitment of the total number of inflammatory cells to the airways (p=0.002), as well as an inhibition of the absolute numbers of neutrophils (p=0.002), eosinophils (p=0.001), lymphocytes (p=0.001) and macrophages (p=0.04) in sputum.

[redacted]

The suspension formulation has been tested in three completed studies (Bjermer et al, 2016; Singh et al, 2016; Singh et al, 2016). Study RPL554-007-2014 was a Phase I randomised, double blind, placebo controlled study in which single ascending doses (SAD) in the range 1.5 mg to 24 mg were administered to 35 healthy subjects, multiple ascending doses (MAD) in the range 6 mg to 24 mg twice daily for up to 5.5 days were administered to 21 healthy subjects and MAD in the range 1.5 mg to 12 mg twice daily for 5.5 days were administered to 23 COPD patients.

Study RPL554-008-2014 was a Phase II double blind, placebo controlled seven way complete block crossover study. This study enrolled 29 patients with mild-moderate chronic asthma and patients received four single doses of RPL554 (0.4 mg, 1.5 mg, 6 mg and 24 mg), two doses of nebulised salbutamol (2.5 mg and 7.5 mg) and placebo in a randomised sequence. RPL554 produced a dose-dependent bronchodilation with a magnitude that was comparable to a maximal dose of salbutamol, but with fewer of the well described salbutamol side effects (e.g. hypokalaemia, tachycardia, tremor and palpitations).

Study RPL554-009-2015 was a Phase II randomised, double blind, double dummy, placebo controlled, six way complete block crossover study in moderate to severe COPD patients. This study enrolled 36 subjects who received salbutamol (200 μg), ipratropium (40 μg) or placebo using a pressurised metered dose inhaler followed immediately by nebulised RPL554 (6 mg) or placebo. RPL554 alone was as effective as salbutamol or ipratropium as a bronchodilator, and importantly produced significant additive bronchodilation (peak and average over 8 hours) when dosed with either salbutamol or ipratropium (p<0.001). RPL554 also resulted in an additive and significant reduction in lung volumes and airway resistance.

RPL554 was well tolerated in all three studies. There were no serious adverse events or adverse events of concern.

The pharmacokinetics of RPL554, following single nebulised inhaled doses of this suspension formulation, were characterised by approximately dose proportional systemic exposure in all three studies. Values of C_{max} were generally attained around 1 to 1.5 hours after dosing, suggesting a steady and somewhat prolonged absorption of the RPL554 dose from the lungs into the systemic circulation; plasma concentrations declined slowly with a mean terminal half-life in the range 8 to 13 hours. Peak plasma concentrations obtained with the suspension formulation were one third to one quarter of those seen with the solution formulation. In study RPL554-007-2014, the twice daily dosing regimen adopted for the MAD phase led to some accumulation and steady state exposure appeared to be achieved by Day 6 of twice daily dosing in both healthy subjects and COPD patients. The observation that systemic exposure to RPL554 was generally lower in COPD patients was in line with expectations. It is considered that this, and the degree of inter-subject variability in pharmacokinetic parameters seen in all cohorts are mainly a reflection of differences in the extent of lung deposition between study subjects. The pharmacokinetic data obtained in the two Phase II studies were consistent with the profiles established in the Phase I study; again it was observed that mean systemic exposure parameters were lower in mild to moderate asthmatic patients and in patients with stable COPD, than in healthy subjects. Overall, the studies performed with inhaled nebulised suspension doses of RPL554 have shown reproducible pharmacokinetic behaviour between studies and across patient cohorts.

Based on these data, the new suspension formulation seems to be i) well tolerated, possibly due to lower peak plasma levels ii) an effective bronchodilator in COPD and asthma patients as well as healthy subjects and iii) have a prolonged half-life compared to the solution formulation, allowing for twice daily dosing.

The purpose of this study is to investigate if RPL554 has similar pharmacokinetics in CF patients to that seen in healthy subjects and patients with asthma or COPD. A secondary outcome is to look for pharmacodynamic endpoints, predominantly $FEV_{1,}$ to determine the bronchodilator effect of RPL554 in stable patients with CF.

1.2 Summary of Risks and Benefits

Data from non-clinical studies suggest a potential for hypotension and tachycardia. However, RPL554 has been administered to about 249 subjects in clinical studies to date and has been well tolerated in COPD patients, healthy subjects, asthmatics and allergic rhinitics. No serious adverse events (SAEs) during scheduled treatment and follow-up have been reported with RPL554.

Adverse events that have been reported at least twice in subjects who have received single or multiple doses of the solution formulation of RPL554 were general mild and in descending order of frequency are headache, nasal congestion, dizziness, somnolence, rhinorrhoea, fatigue, larynx irritation, vasodilation, asthma, dry throat, respiratory tract infection, and sneezing. Many of the events also occurred with a similar frequency with placebo. The only other observation noted was a modest increase in standing heart rate in one study.

The suspension formulation of RPL554 is pH balanced, and has favourable non-clinical toxicology and pharmacokinetic data. The pharmacokinetics with the suspension formulation is dramatically different from that with the solution formulation. The absorption is much slower, with a T_{max} of 1 to 1.5 hours, and the terminal half-life much longer (8 to 13 hours). The C_{max} is lower than with the solution formulation, although the total exposure is greater.

There has been no evidence of significant adverse events related to the cardiovascular or gastrointestinal systems, except associated with an increase in heart rate at high doses (12 mg to 24 mg). These small increases in heart rate may relate to the PDE3 inhibitory activity of the compound. In single dose studies there appears to be an increase in the rate of headache, which is most pronounced at doses over 6 mg. There was no other apparent dose related adverse events, with the exception of palpitations in patients dosed at 24 mg. Results from multiple dose data in patients with COPD suggests a transient increase in dizziness; the majority of which occurred during spirometry or dosing, but otherwise the rate of adverse events were similar in RPL554 or placebo treated patients. There was an apparent increase in mild adverse events in healthy subjects treated with high doses of RPL554, which may be associated with the higher serum levels in healthy individuals than in those with obstructive lung disease.

Improvements in lung function can be expected in patients who are dosed with RPL554. However, subjects will not individually benefit from participation in this study.

2 OBJECTIVES

2.1 Primary Objective

To investigate pharmacokinetics of single nebulised doses of RPL554 in patients with CF.

2.2 Secondary Objectives

- To investigate the bronchodilator effect on peak FEV_1 after single nebulised doses of RPL554 as compared to placebo
- To investigate the bronchodilator effect on AUC FEV_1 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

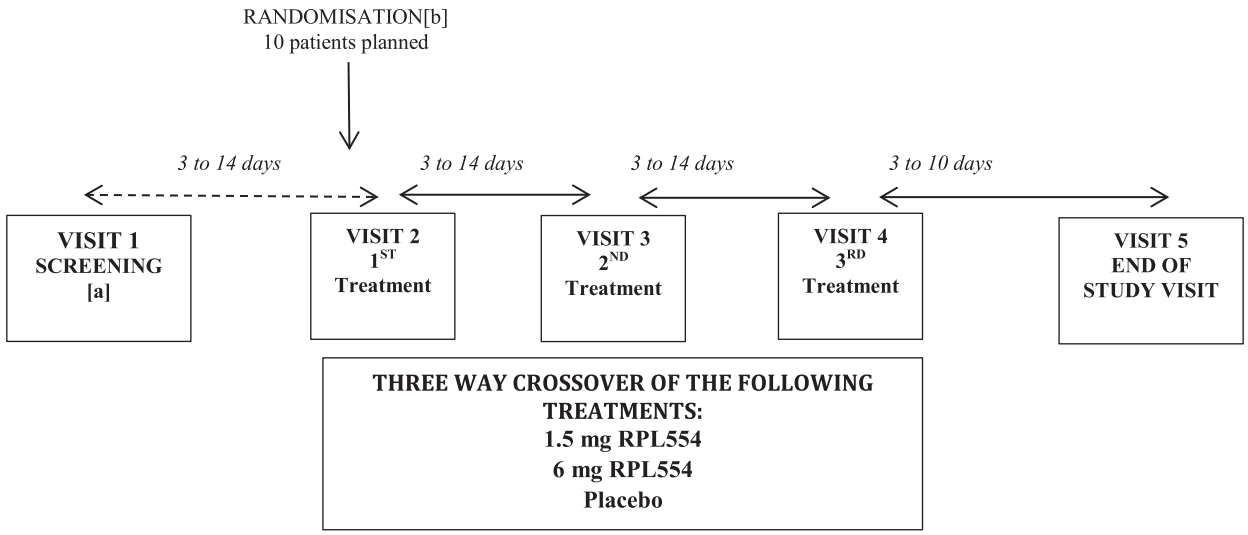
- To examine the anti-inflammatory effects of single nebulised doses of RPL554 in patients with CF

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan Description

This is a Phase IIa, randomised, double blind, placebo controlled, double dummy, complete block three way crossover study to investigate the pharmacokinetics of nebulised RPL554 in adult patients with CF. It is planned to enrol sufficient patients to ensure that 10 complete all three treatment periods at two centres. The study comprises the five visits shown in Figure 1: screening (Visit 1), three treatment visits (Visit 2 to Visit 4) and an end of study visit (Visit 5). The procedures performed at each visit are summarised in Section 6 and the study assessments are described in Section 7.

Figure 1 Study Flow Chart



[a] May be performed as a single visit or more than one visit (for example if the patient is taking medications that require washout for the reversibility test)
[b] Patients will be randomised pre-dose at Visit 2

Patients will be screened for eligibility (Visit 1), including a reversibility test with salbutamol between 3 and 14 days before 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 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. 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 (FEV_1 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] and myeloperoxidase [MPO])

- Exhaled breath pH measurement pre-dose and at 8 and 24 hours post-dose (at UK centre only)
- Urine pregnancy test pre-dose (female patients only)
- Vital signs and 12-lead electrocardiograms (ECG) 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).

3.2 Discussion of Study Design, including the Choice of Control Groups

A total of approximately 10 CF patients aged ≥18 years will be randomised. The purpose of the study is to investigate the pharmacokinetics of RPL554 in patients with CF. In addition, the acute bronchodilator effect of RPL554 will be studied in these patients.

Single nebulised doses of RPL554 or placebo will be administered. All patients will receive all three treatments in this study in a randomised, crossover design therefore each patient will act as his or her own control in the study. This design makes it possible to obtain unbiased inferences about differences between treatments, based on intra-patient differences. Treatments will be administered double blind with the Investigator and patient unaware of the treatment identity to further minimise any potential bias in the overall assessment of treatment effect and safety.

The washout period between the three treatments was selected based upon the available single dose pharmacokinetics of the suspension formulation RPL554 in healthy male subjects (SAD part of Study RPL554-007-2014). The mean (range) half-lives were 8.3 (6.0 to 10.5) hours at the 1.5 mg dose, 8.5 (7.6 to 9.21) hours at the 3.0 mg dose, 10.5 (9.1 to 11.7) hours at the 6 mg dose, 9.4 (7.8 to 11.8) hours at the 12 mg dose and 10.2 (8.1 to 12.5) hours at the 24 mg dose. Following the MAD part of the same study to healthy subjects, the mean (range) half-lives following the final dose on Day 6 were 13.2 (8.17 to 26.3) hours at the 6 mg dose and 12.8 (9.8 to 15.8) hours at the 12 mg dose. Five half-lives is considered as the time for elimination of study drug from the body. In this study, a washout period of 3 to 14 days was deemed adequate to ensure there was no overlap between the pharmacokinetic profiles of consecutive treatments.

RPL554 nebulisation, pharmacodynamics, safety and tolerability assessments will be performed whilst patients are resident at the study centre. This is to ensure standardised conditions for dosing and other study procedures. The pre-dose FEV_1 at Visit 3 and Visit 4 must be within ±20% of the pre-dose FEV_1 at Visit 2 in order to ensure consistent baseline FEV_1 for each study treatment. If the FEV_1 is greater than 20% different, then the visit may be rescheduled (within 2 weeks). Patients must be off antibiotics for an acute infection for at least 2 weeks prior to each visit. If patients become unstable between visits, their attending physician should give them appropriate therapy and reschedule the visit for 2 weeks after recovery (last dose of antibiotic).

3.3 Planned Duration of the Study

The approximate planned duration for each patient will be up to 52 days: 3 to 14 days screening, up to 28 days treatment (three treatment visits each separated by 3 to 14 days) and an end of study visit 3 to 10 days after the last treatment visit.

3.4 Definition of the End of the Study

The end of the study is defined as the date of the last end of study visit of the last patient in the study.

4 SELECTION OF STUDY POPULATION

The population to be recruited into this study is stable patients with CF and without significant heart or liver disease. Subjects with all CFTR mutations are eligible for the study, and there are no excluded CF medications other than bronchodilators and antibiotics as noted.

4.1 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 (see Appendix 1) 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 ECG recording at screening (Visit 1) and Visit 2 pre-dose showing the following:
   - Heart rate between 50 and 100 beats per minute
   - QT interval corrected for heart rate using Fridericia's formula (QTcF) interval ≤450 msec
   - QRS interval ≤120 msec
   - PR interval ≤200 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/m^2 (inclusive) with a minimum weight of 40 kg.
6. Patients with a genetic diagnosis of CF.
7. Spirometry at screening demonstrating an FEV_1 ≥40% and ≤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).

4.2 Exclusion Criteria

1. History of cirrhotic liver disease or portal hypertension.
2. CF exacerbation requiring hospitalisation in the month prior to screening (Visit 1) or prior to randomisation (Visit 2).
3. Use of oral or intravenous antibiotics (in additional 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 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, unless CF related, 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), or Burkholderia species.
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 (Visit 1), 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. Patients treated with ivacaftor or ivacaftor/lumacaftor will be excluded.
17. Requires oxygen therapy on a regular basis.
18. Pregnancy or lactation (female subjects only).
19. Any other reason that the Investigator considers makes the patient unsuitable to participate.

4.3 Removal of Patients from Therapy or Assessment

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.

4.3.1 Study Treatment Discontinuation

Study treatment must be discontinued for the following reasons:

- Unacceptable toxicity related to study treatment
- Intolerable or persistent adverse events of any severity
- Pregnancy in female patients
- General or specific changes in the patient's condition rendering the patient unacceptable for further treatment in the judgment of the Investigator
- Clinically significant progression of disease

4.3.2 Patient Withdrawal

The patient has the right to withdraw at any time and for any reason, without explanation and without jeopardising any subsequent treatment by the clinician, if applicable. However, anyone withdrawing should be encouraged to offer an explanation for their withdrawal particularly if it relates or is perceived to relate in any way to the study treatment, or to the conduct of the study. Patients can also be withdrawn in case of protocol violations and non-compliance.

It should be made clear that the patient is free to withdraw from the study at any time.

4.3.3 Study Discontinuation

Conditions that may warrant termination of the study include, but are not limited to:

- The discovery of an unexpected, serious, or unacceptable risk to patients enrolled in the study
- The decision on the part of the Sponsor to suspend or discontinue testing, evaluation, or development of RPL554
- Serious failure of the Investigator to comply with the International Conference on Harmonisation (ICH) Guidelines on Good Clinical Practice (GCP) or local regulations
- Submission of knowingly false information from the research facility to the Sponsor, the ethics committee or any national regulatory officials
- Major, repeated non-adherence to the protocol

The Sponsor must be informed immediately in the event of any major protocol violation or serious breach of GCP.

Study termination and follow-up will be performed in compliance with the conditions set forth in ICH GCP. The decision to discontinue the study is at the discretion of the Sponsor, the Investigator, the regulatory authority or ethics committee and should if possible be taken by mutual agreement. A record of such a discussion will be prepared and stored in the Study File. The Sponsor will ensure the regulatory authorities and ethics committees are notified.

4.3.4 Replacement Policy

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 treatment sequence.

5 STUDY TREATMENTS

5.1 Study Treatments Administered

Patients will receive the following three single doses in a randomised sequence at Visit 2 to Visit 4:

1. 1.5 mg RPL554
2. 6 mg RPL554
3. Placebo

All three study treatments will be administered using the inhaled route with nebulised RPL554 or placebo administered using a standard Jet nebuliser (PARI LC Sprint plus a PARI TurboBOY SX compressor unit) as described in Section 5.4.2.

5.2 Identity of Study Treatments

RPL554 and placebo will be manufactured using aseptic manufacturing techniques to Good Manufacturing Practice (GMP). The International Union of Pure and Applied Chemistry (IUPAC) name for RPL554 drug substance is 9,10-dimethoxy-2-(2,4,6-trimethylphenylimino)-3-(*N*-carbamoyl-2-aminoethyl)-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-a]isoquinolin-4-one.

The composition of the nebulised RPL554 and placebo stock formulations is shown in Table 1.

Table 1 Composition of Nebulised RPL554 and Placebo Formulations

[redacted]

5.3 Preparation and Labelling

Stock glass vials of RPL554 and placebo will be labelled in compliance with GMP, released by a qualified person and then shipped to the study centres.

[redacted]

5.4 Selection of Doses, Dosing Schedule and Route of Administration

5.4.1 Selection of Doses in the Study

The doses of 1.5 mg RPL554 and 6 mg RPL554 were selected based on the results from three completed clinical studies. Both of these doses were included in Study RPL554-007-2014 investigating SAD and MAD of RPL554 in healthy subjects and MAD in COPD patients, (ii) Study RPL554-008-2014 and Study RPL554-008-2014 in patients with asthma. The 6 mg dose was also investigated in Study RPL554-009-2015 in patients with COPD. These doses were well tolerated and also caused bronchodilation in healthy subjects, asthmatic patients and COPD patients.

5.4.2 Selection and Timing of Dose for each Patient

All three treatments should be given at approximately (±1 hour) the same time of day at each visit (Visit 2 to Visit 4).

The following restrictions in relation to study visits should be adhered to:

- Patients should refrain where possible, from xanthine (chocolate, caffeine containing drinks and food), for at least 24 hours before and during all visits. Decaffeinated beverages are permitted
- Patients should refrain from alcohol for 24 hours before and during all visits (including visits for safety laboratory tests) and until all procedures for that study visit are completed
- Patients must fast (water permitted) from 2 hours pre-dose until 2 hours post-dose at all treatments visits (Visit 2 to Visit 4). Standardised meals will be served during the visit day

- Patients should refrain from smoking on the day of the visit, at the very least abstain from smoking in the 2 hours prior to dosing and within 1 hour of all spirometry assessments
- Patients should refrain from strenuous exercise for 72 hours prior to all study visits and should undertake no unaccustomed strenuous exercise from screening (Visit 1) until the end of study visit (Visit 5)

RPL554 or placebo will be administered by inhalation of an aerosol generated by a PARI LC Sprint® jet nebuliser attached to a PARI TurboBOY® SX compressor. Wherever possible, the same compressor unit should be used for each dose within a patient.

A filter valve set will be attached to the PARI LC Sprint® jet nebuliser and a separate filter pad will be used for each dose (see separate PARI instructions provided with the filter valve set).

The dosing cup on each nebuliser will be obscured with tape to visually blind the study treatment.

Patients must wear a protective gown during the dosing procedure to prevent contamination of the cannula for pharmacokinetic samples.

The end time of nebulisation (sputtering) of the study treatment will be considered Time 0 for the purposes of scheduling all post-dose study procedures. Nebulisation time should not exceed 10 minutes.

The following must be recorded in the CRF:

- Compressor unit number
- Start and end times of nebulisation (times will be rounded down to the nearest minute)
- The volume of residual product at the end of nebulisation

5.5 Storage

RPL554 and placebo should be stored below 25°C and should not be frozen. The expiry date will be indicated on the box label.

Temperature logs should be maintained in areas where study treatment is stored. If temperature conditions have been seriously compromised or any study treatment has not been stored appropriately, this should be documented, and the study treatment quarantined until the Sponsor has been notified and confirmed whether it may be used.

Study treatments will be stored under the control of the Investigator or designee in a secure facility appropriate for the advised storage conditions. Study treatments that are to be returned or destroyed by the Investigator/staff or have expired must be stored separately from the unused study treatments.

5.6 Accountability

The Investigator will be responsible for the dispensing, inventory and accountability of study treatment, exercising accepted medical and pharmaceutical practices and ensuring that an accurate, timely record of the disposition is maintained. The study treatment supplies and inventory must be available for inspection by the designated representatives of the Sponsor upon request.

Upon receipt of the study treatment, the Investigator or designee will inspect the contents and return the completed acknowledgement of receipt. Copies of all study treatment inventory records must be retained for accountability of study products and supplies. Accountability must be documented from the time of initial receipt at the study centre to their final removal from the centre or destruction.

Written records must also be maintained to confirm the purpose and reason for any study treatment disposal, e.g. the amount contaminated, broken, or lost, and the name/signature of the personnel responsible for destruction. No study material (including used vials) may be destroyed until final study treatment accountability has been performed and written approval has been received from the Sponsor.

At the end of the study, the unused study treatment will either be destroyed locally or returned to the Sponsor for destruction after accountability has been verified.

5.7 Method of Assigning Patients to Treatment Groups

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

Patients will receive three different treatments in the study (see Section 5.1) at Visit 2 to Visit 4 in a randomised sequence. Randomisation numbers will be allocated using a three number format.

A dummy randomisation schedule will be approved by the Sponsor before the final randomisation schedule is produced.

5.8 Blinding

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 different. The study personnel preparing the RPL554 and placebo, placing them into the nebuliser and supervising study treatment administration will therefore not be 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.

Individual tamper evident code break envelopes will be generated for each patient identifying the treatment allocation. A set of envelopes will be held by the Sponsor's Medical Monitor and each Investigator for use in the event of the need for emergency unblinding. In both cases, the code break envelopes must be held in a secure restricted-access location that is accessible 24 hours a day.

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 open, sign and date the correct envelope, if possible having discussed 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.

5.9 Prior and Concomitant Therapies and Medications

5.9.1 Prior and Concomitant Therapies

Prior therapies for CF taken in the last 3 months and all concomitant therapies for CF will be recorded in the CRF, with the medication, route of administration, dose and start and stop date(s) and time(s) clearly recorded to document all required washout periods and compliance with the inclusion and exclusion criteria. Any oral medications for CF used at screening should be continued during the study, preferably at a stable dose, as deemed appropriate by the Investigator.

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.

Long acting bronchodilators must be withheld for at least 48 hours prior to each study visit. Short acting bronchodilators must be withheld for at least 8 hours prior to each study visit. If this withhold is not met, the patent should be rescheduled for a repeat visit within 7 days.

Patients must be off antibiotics for an infectious exacerbation for at least 2 weeks prior to each treatment visit (Visits 2 to Visit 4). If patients become unstable between visits, their attending physician should give them appropriate therapy and reschedule the next treatment visit for 2 weeks after the last dose of antibiotics. In this instance the washout period between consecutive treatments is permitted to be longer than 14 days.

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

5.9.2 Prior and Concomitant Medications

Other prior prescription or non-prescription medications (medication, dose, route of administration, treatment duration and indication) taken 3 months before the first study treatment administration must be recorded in the CRF in order to confirm compliance with the inclusion and exclusion criteria.

All concomitant medications must also be documented in the CRF. The impact of any concomitant medications will be evaluated during the pre-database lock review and the decision taken whether to exclude the patient from any analysis populations.

5.10 Rescue Medications

Standard procedures for emergency care should be followed for any individual adverse event, whenever clinically needed (decision to be taken by the Investigator). Short acting bronchodilators may be used as rescue medication. If this is inadequate to control their symptoms they should contact the Investigator. Rescue medication use during each treatment visit must be separately documented in the CRF (medication, dose, route of administration, date and time of each administration). Protocol procedures must still continue even if rescue medication has been taken.

5.11 Treatment Compliance

Study treatment administration will take place at the study centre and will be administered by the Investigator or designated and trained study centre personnel. The precise date and time

of administrations shall be documented in the CRF. The study will be monitored by a monitor approved by the Sponsor. During these visits, all procedures will be monitored for compliance with the protocol. Source documents will be reviewed and compared with the data entries in the CRFs to ensure consistency.

6 STUDY PROCEDURES AT EACH VISIT

The study will consist of five visits:

- Screening (Visit 1) will take place in the period between 3 and 14 days prior to the first study treatment administration. This may be performed as a single visit or more than one visit. Eligible subjects may be re-screened at the discretion of the Investigator, and following discussion with the Sponsor, if the reason for screen failure was considered temporary in nature. All screening data must be obtained within 14 days prior to administration of study treatment
- Three treatment visits (Visit 2 to Visit 4) each separated by 3 to 14 days
- An end of study visit (Visit 5) between 3 and 10 days after the last study treatment administration

Repeat, rescheduled and unscheduled visits and procedures are permitted at the discretion of the Investigator.

The schedule of assessments at each visit is shown in Table 2 and listed in Section 6.1 to Section 6.4. The study assessments are described in Section 7.

Table 2 Schedule of Assessments in RPL554-010-2015

	Screen		Treatment										EoS
Visit	**1[a]**		**2 to 4 (each visit separated by 3-14 days)**										**5[b]**
Timepoint		**Pre-Dose**	**0**	**15 min**	**30 min**	**1 h**	**2 h**	**4 h**	**6 h**	**8 h**	**12 h [c]**	**24 h**	
Informed consent	X												
Demographics	X												
Medical / surgical and disease history	X												
Urine pregnancy test (female patients only)	X	X											X
Inclusion/exclusion criteria	X	X[d]											
Nebulisation training		X[d]											
Randomisation		X[d]											
Study treatment inhalation (RPL554 or placebo)			X										
Reversibility test [e]	X												
Spirometry (FEV_1 and FVC)		X[f]		X	X	X	X	X	X	X	X	X	
Prior/concomitant medications/therapies		Throughout											
Rescue medication				Throughout									
Laboratory safety tests	X												X
Vital signs (blood pressure and pulse rate)	X	X			X	X	X	X	X	X	X		
Physical examination	X												X
12-lead ECG	X	X				X	X	X		X			
Exhaled breath pH [g]		X								X		X	
Sputum inflammatory mediators (IL-8, TNF-α MPO)		X								X		X	
Sputum rheology		X								X		X	
Pharmacokinetic blood sample		X		X	X	X	X	X	X	X		X	
Adverse event questioning		Throughout											

Abbreviations: ECG=electrocardiogram; EoS=end of study; FEV_1=forced expired volume in 1 second; FVC=forced vital capacity; h=hour; IL-8=interleukin-8; min=minute; MPO=myeloperoxidase; pMDI=pressurised metered dose inhaler; TNF-α=tumour necrosis factor alpha

[a] 3 to 14 days prior to Visit 2. Screening may be performed over more than 1 day
[b] EoS will occur 3-10 days after the last dose of study treatment
[c] German centre only
[d] Visit 2 only
[e] Spirometry (FEV_1 and FVC) before and 30 minutes and 1 hour after pMDI salbutamol administration. The pre-bronchodilator FEV_1 must be ≥40% and ≤80% of predicted normal for inclusion.
[f] One measurement at -15 minutes
[g] UK centre only

6.1 Screening (Visit 1)

Written informed consent will be obtained by the Investigator as specified in Section 11.3 prior to any study related procedures being performed.

Patients will be screened to determine eligibility against the inclusion and exclusion criteria between 3 and 14 days before the first dose of study treatment. There are no fasting requirements for the screening visit. Patients must withhold CF therapies prior to screening as defined in Section 5.9.1.

The following assessments will be performed:

- Reversibility test: spirometry before and 30 minutes and 1 hour after salbutamol administration
- Recording of demographic information
- Recording of medical/surgical and disease history
- Recording of prior medications and therapies
- Vital signs (blood pressure and pulse rate)
- Physical examination
- 12-lead ECG
- Blood and urine samples for laboratory safety tests
- Urine pregnancy tests (female patients only)
- Questioning for adverse events

If the patient meets the inclusion criteria and none of the exclusion criteria, they will be instructed to return in 3 to 14 days for Visit 2.

6.2 Visit 2

Patients must withhold short and long acting bronchodilators prior to Visit 2 as defined in Section 5.9.1. Patients should fast from 2 hours pre-dose until 2 hours post-dose (except for water) and other restrictions defined in Section 5.4.2 should be adhered to.

Patients will be evaluated to ascertain if the inclusion and exclusion criteria are still met.

6.2.1 Pre-Dose Assessments

- Confirm that respiratory medications were withheld as required. If not reschedule the visit for within the required window
- Questioning for adverse events
- Concomitant medication check
- Urine pregnancy test (female patients only)
- Nebuliser inhalation training
- 12-lead ECG
- Vital signs (blood pressure and pulse rate)

- Blood sample for pharmacokinetics
- Exhaled breath pH (UK centre only)
- Sputum for rheology and inflammatory mediator measurements
- Spirometry (one measurement at -15 minutes)

Eligible patients will then be randomised and receive a randomisation number.

6.2.2 Study Treatment Administration

Patients will be dosed either RPL554 or placebo using a nebuliser according to randomisation.

6.2.3 Post-Dose Assessments

- 12-lead ECGs at 1, 2, 4 and 8 hours
- Vital signs at 30 minutes and 1, 2, 4, 6 and 8 hours
- Blood samples for pharmacokinetics at 15, 30 minutes and 1, 2, 4, 6, 8 and 24 hours
- Spirometry at 15 and 30 minutes and 1, 2, 4, 6, 8 and 24 hours
- Questioning for adverse events
- Exhaled breath pH at 8 and 24 hours (UK centre only)
- Sputum obtained for rheology and inflammatory mediator measurement at 8 and 24 hours

Patients completing the visit will be instructed to return in 3 to 14 days for Visit 3.

6.3 Visit 3 to 4

Patients must withhold short and long acting bronchodilators prior each visit as defined in Section 5.9.1. Patients should fast from 2 hours pre-dose until 2 hours post-dose (except for water) and other restrictions defined in Section 5.4.2 should be adhered to. The following assessments will be performed:

6.3.1 Pre-Dose Assessments

- Confirm that respiratory medications were withheld as required. If not reschedule the visit for within the required window
- Questioning for adverse events
- Concomitant medication check
- Urine pregnancy test (female patients only)
- 12-lead ECG
- Vital signs (blood pressure and pulse rate)
- Blood sample for pharmacokinetics
- Exhaled breath pH (UK centre only)
- Sputum for rheology and inflammatory mediator measurements

- Spirometry (at -15 minutes), including confirmation that FEV_1 is within 20% of that at Visit 2

6.3.2 Study Treatment Administration

Patients will be dosed either RPL554 or placebo using a nebuliser according to randomisation.

6.3.3 Post-Dose Assessments

- 12-lead ECGs at 1, 2, 4 and 8 hours
- Vital signs at 30 minutes and 1, 2, 4, 6 and 8 hours
- Blood samples for pharmacokinetics at 15, 30 minutes and 1, 2, 4, 6, 8, 12 and 24 hours
- Spirometry at 15 and 30 minutes and 1, 2, 4, 6, 8 and 24 hours
- Questioning for adverse events
- Exhaled breath pH at 8 and 24 hours (UK centre only)
- Sputum obtained for rheology and inflammatory mediator measurement at 8 and 24 hours

Patients completing Visit 3 will be instructed to return in 3 to 14 days for Visit 4. Patients completing Visit 4 will be instructed to return in 3 to 10 days for Visit 5.

6.4 End of Study (Visit 5)

The following will be performed:

- Questioning for adverse events
- Concomitant medication check
- Physical examination
- Blood and urine samples for laboratory safety tests
- Urine pregnancy test (female patients only)

7 STUDY METHODOLOGY

7.1 Demographics, Baseline Characteristics and Eligibility Assessments

Safety assessments (laboratory safety assessments, vital signs, 12-lead ECG and physical examination) will be performed at screening (Visit 1) as described in Section 7.4.2 to Section 7.4.5 as part of the eligibility assessment.

7.1.1 Demographic Variables

Demographic variables, including date of birth, sex, height, weight, BMI (weight [kg]/height [m]^2), race and smoking status will be collected at screening (Visit 1).

7.1.2 Medical/Surgical and Disease History

Active medical conditions and all surgeries will be recorded at screening (Visit 1). Disease history, including date of diagnosis of CF and the specific CF mutations will also be recorded.

7.1.3 Reversibility Test

A reversibility test in response to salbutamol will be performed at screening (Visit 1). Spirometry (FEV_1 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 will be performed and recorded. The pre-brochodilator FEV_1 must be ≥40% and ≤80% of predicted normal for inclusion. The reversibility response is not an eligibility measure.

At all three timepoints, 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. GLI Quanjer, 2012 will be used as a reference for predicted normal values.

7.1.4 Screening Laboratory Eligibility Assessments

Laboratory safety assessments will be performed as described in Section 7.4.2.

Unscheduled and/or repeat testing may be performed at the discretion of the Investigator.

7.1.5 Prior and Concomitant Medications and Therapies

Prior respiratory therapies and medications taken in the last 3 months will be recorded at screening (Visit 1) and concomitant use during the study recorded as described in Section 5.9.1.

Other prior medications taken in the last 3 months will be separately recorded at screening (Visit 1) and concomitant use during the study recorded as described in Section 5.9.2.

7.1.6 Eligibility Check

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.

7.2 Pharmacodynamic Assessments

7.2.1 Pulmonary Function Tests

Spirometry assessments (FEV_1 and FVC) will be made at the following timepoints at Visit 2 to Visit 4: pre-dose (at -15 minutes), 15 and 30 minutes and 1, 2, 4, 6, 8 and 24 hours post-dose in accordance with ATS/European Respiratory Society (ERS) guidelines (Miller, 2005). At all timepoints, 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 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.

7.2.2 Sputum Measurements

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 -20ºC 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).

Results will be provided to the Investigator for entry into the CRF.

7.2.3 Exhaled breath pH

Exhaled breath pH (UK centre only) 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 RTubeTM (Respiratory Research Inc, Charlottesville, Virginia). A 200 µL aliquot of EBC will be used for the pH assay. Measurement of pH will be performed after de-aeration by bubbling argon through the sample at 2 L/min while monitoring pH until the reading stabilised, usually after 8 minutes of de-aeration with argon.

7.3 Pharmacokinetic Assessments

Pharmacokinetic analysis will be performed on samples taken from all subjects. 4 mL blood samples will be collected at the following timepoints 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 30 minutes of collection. The plasma will be separated in a refrigerated centrifuge (about 4°C) at 1100g 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.

7.4 Safety Assessments

7.4.1 Adverse Events

Recording and reporting adverse events is described in detail in Section 8.

7.4.2 Laboratory Safety Assessments

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 each study centre's local laboratory. Samples will be handled and analysed according to the study centre and local laboratory standard procedures.

Monovette-S® (Sarstedt) tubes will be used to collect safety laboratory blood samples. Two samples will be collected at each timepoint, one sample for haematology using a 2.6 mL ethylenediaminetetraacetic acid (EDTA) whole blood collection tube and one 4.7 mL sample for biochemistry using a serum tube. A midstream urine sample will also be 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, alanine transaminase, gamma-GT, potassium, sodium, urea

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.

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 a clinically significant.

7.4.3 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 timepoint, supine vital signs will be assessed while the patient has been at rest for at least 5 minutes.

7.4.4 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.

7.4.5 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 timepoint, 12-lead ECGs should be taken after at least 5 minutes in the supine position. An ECG print out must be taken at each timepoint and signed and dated by the Investigator. An overall assessment (normal, abnormal not clinically significant or abnormal clinically significant) and continuous variables, including QT, heart rate and QTcF (or RR to derive QTcF) will be recorded in the CRF.

7.5 Blood Sampling

The maximum volume of blood taken from any subject during the study which includes laboratory safety tests and pharmacokinetic samples will be up to approximately 200 mL, allowing for any repeat sampling required.

7.6 Appropriateness of Measurements

The assessments planned in this study are recognised as reliable, accurate and relevant.

Plasma concentrations of RPL554 will be evaluated using a validated assay and quality control samples will be analysed throughout the study. The concentrations will be used to determine within run, between run and overall precision and accuracy of the method. The procedures, including the limits of quantitation and results will be described in detail in a separate bioanalytical protocol and report. The timing of pharmacokinetic samples is deemed appropriate to establish the exposure of patients to single doses of study treatment.

Spirometry is a standard lung function test used to screen for, and monitor, respiratory disease. Spirometry and daily calibrations will be performed in accordance with ATS/ERS task force standardisation guidelines (Miller et al, 2005) using the study centre's standard spirometry equipment and SOPs.

The measurement of sputum inflammatory mediators and exhaled breath pH will be performed in accordance with SOPs in place at Addenbrookes and Papworth Hospitals respectively.

Physical examinations, vital signs, 12-lead ECGs, adverse event recording and laboratory safety tests are standard assessments of safety and tolerability. The range of assessments to be performed is deemed appropriate to detect any safety signals.

8 HANDLING OF ADVERSE EVENTS AND PREGNANCIES

8.1 Adverse Event Definitions

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.

8.2 Recording and Assessing Adverse Events

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:

8.2.1 Severity

Mild:	Resolved without treatment
Moderate:	Resolved or was tolerated with specific treatment without affecting study activities
Severe:	Did not resolve or was not tolerated with treatment

8.2.2 Chronicity

Single occasion:	Single event with limited duration
Intermittent:	Several episodes of an event, each of limited duration
Persistent:	Event which remained indefinitely

8.2.3 Causality

Not Related — This category applies to those adverse events which, after careful consideration, are clearly due to extraneous causes (disease, environment, etc.)

Unlikely — In general, this category can be considered applicable to those adverse events which, after careful medical consideration at the time they are unrelated to the test drug. An adverse event may be considered unlikely to be related if or when (must have two of the following):

1. It does not follow a reasonable temporal sequence from administration of the study treatment.
2. It could readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patients.
3. It does not follow a known pattern of response to the study treatment.
4. It does not reappear or worsen when the study treatment is re-administered.

Possibly — This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, a connection with the study treatment administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possibly related if or when (must have two of the following):

1. It follows a reasonable temporal sequence from administration of the study treatment.
2. It could not readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patients.
3. It follows a known pattern of response to the study treatment.

Definitely — This category applies to those adverse events which the Investigator feels are incontrovertibly related to study treatment. An adverse event may be assigned an attribution of definitely related if or when (must have all of the following):

1. It follows a reasonable temporal sequence from administration of the study treatment.
2. It could not be reasonably explained by the known characteristics of the patient's clinical state, environmental, or toxic factors, or other modes of therapy administered to the patients.
3. It disappears or decreases on cessation or reduction in dose and recurs with re-exposure to drug. (Note: this is not to be construed

as requiring re-exposure of the patient; however, a category of definitely related can only be used when a recurrence is observed.)

4. It follows a known pattern of response to the study treatment.

8.2.4 Action and Outcome

- Action taken with study treatment (none, study treatment stopped, study treatment temporarily interrupted)
- Other actions (none, concomitant medication, study discontinuation, hospitalisation, other)
- The outcome and date of outcome according to the following definitions:
  - Recovered or resolved (adverse event disappeared)
  - Recovering or resolving (patient is recovering)
  - Not recovered or not resolved (adverse event remains without signs of improvement)
  - Recovered or resolved with sequelae (adverse event has resulted in permanent disability or incapacity)
  - Fatal
  - Unknown (only applicable if patient has been lost to follow-up)
- Seriousness (yes or no)

8.3 Reporting Procedure for SAEs

The Investigator must report all SAEs to the Sponsor by telephone and in writing using the Sponsor's reporting form and emailed (email address included on Page 2) to as soon as practical, but at least within 24 hours of awareness. Any fatal SAEs notified in the 30 day period after the last dose of study treatment must also be reported

SUSARs will be determined by the Sponsor's Medical Monitor.

SAEs will be reported to the ethics committee and regulatory authority according to local requirements.

All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the patient's general physician or a medical specialist.

It is the responsibility of the Sponsor and of the Investigator to ensure that any necessary additional therapeutic measures and follow-up procedures are performed.

8.4 Management of Pregnancies

Should a female patient become pregnant during the study or in the 30 days after the end of the study they must inform the Investigator immediately. The Investigator will report this information to the Sponsor within 7 days of awareness. The Investigator will make all reasonable efforts to ascertain the progress and outcome of the pregnancy. If the outcome meets the criteria for immediate classification of a SAE (e.g. spontaneous or therapeutic

abortion, stillbirth, neonatal death, congenital anomaly, birth defect), the Investigator must follow the procedure for reporting SAEs.

9 QUALITY ASSURANCE AND QUALITY CONTROL

The study will be conducted in accordance with the current approved protocol, SOPs and all applicable guidelines and requirements.

9.1 Audit and Inspection

The Sponsor, or its designee may conduct a quality assurance audit. An inspection of this study may also be carried out by regulatory authorities at their discretion. Such audits or inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the Investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his time and the time of his staff to the auditor or inspector to discuss findings and any relevant issues.

9.2 Monitoring and Source Document Verification

The Sponsor will arrange for the study to be monitored in accordance with the principles of ICH GCP. The frequency of monitoring visits will be determined by the rate of patient recruitment.

The following are examples of items that will be reviewed at these visits:

- Compliance with the protocol
- Consent procedure
- Source documents
- Adverse event procedures
- Storage and accountability of materials

The monitoring visits also provide the Sponsor with the opportunity to ensure that timely patient accrual and the other Investigator's obligations and all applicable requirements are being fulfilled.

The Investigator must permit the study monitor, the ethics committee, the Sponsor's auditors and representatives from regulatory authorities direct access to all source documents for confirmation of the accuracy and reliability of data contained within the CRFs (source document verification). Patient confidentiality will be protected at all times.

Source documents are defined as the results of original observations and activities of a clinical investigation, including medical notes. All source documents produced in this study will be maintained by the Investigator and made available for inspection. The original signed informed consent form for each patient will be retained by the Investigator and the second signed original given to the patient.

Source data include, but is not limited to, the following and will be identified in a source data location log:

- Screening/enrolment log
- Medical notes - which should be updated after each visit to include visit dates, medical history, diagnosis of cystic fibrosis, concomitant medication, any clinically relevant

findings of clinical examinations or clinically relevant adverse events/medication changes, SAEs and information on patient withdrawal

- Informed consent form
- 12-lead ECGs
- Laboratory reports
- Visit dates
- Study treatment accountability/inventory forms

The study monitor will carry out source document verification at regular intervals. This is an essential element of quality control, as it allows the rectification of transcription errors and omissions.

9.3 Data Management and Coding

An electronic CRF will be designed and produced by the Sponsor (or designee) and should be completed in accordance with instructions. The Investigator is responsible for maintaining adequate and accurate medical records from which accurate information will be transcribed directly into the CRFs using a secure internet connection. The CRFs should be filled out completely by the Investigator (or designee). The CRF system will be Food and Drug Administration (FDA) Code of Federal Regulations (CFR) 21 Part 11 compliant.

Data entered into the CRF will be validated, which includes, but is not limited to, validity checks (e.g. range checks), consistency checks and customised checks (logical checks between variables to ensure that study data are accurately reported) for CRF data and external data (e.g. laboratory data). A majority of edit checks will be triggered during data entry and will therefore facilitate efficient 'point of entry' data cleaning.

The Sponsor (or designee) will perform both manual CRF review and review of electronic edit checks to ensure that the data are complete, consistent and reasonable. The electronic edit checks will run continually throughout the course of the study and the issues will be reviewed manually online to determine what action needs to be taken.

Manual queries may be added to the system the Sponsor (or designee). Representatives of the Sponsor are able to remotely and proactively monitor the CRFs to improve data quality.

Central laboratory pharmacokinetic data will be transferred electronically into the study database. Discrepancies will be queried to the study centre and/or the laboratory until the electronic data and the database are reconciled.

All updates to queried data will be made by authorised study centre personnel only and all modifications to the database will be recorded in an audit trail. Once all the queries have been resolved, CRFs will be locked by password protection. Any changes to locked CRFs will be approved by the Investigator.

Once the full set of CRFs have been completed and locked, the Sponsor will authorise database lock and all electronic data will be sent to the designated statistician for analysis. Subsequent changes to the database will then only be made only by written agreement of the Sponsor.

Adverse events and medical/cancer history terms will be coded from the verbatim description (Investigator term) using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and therapies will be coded according to the WHO drug

dictionary. Coding review will be performed by the Sponsor (or designee) prior to database lock.

The clinical data (in statistical analysis software [SAS] format) will be transferred to the Sponsor at the end of the study.

10 STATISTICAL METHODS

10.1 Statistical and Analytical Plans

This section presents a summary of the planned statistical analyses. A detailed plan describing the analyses to be conducted will be defined before the first patient is enrolled. Any deviation from the analysis specified in the protocol or the statistical analysis plan will be detailed and justified in the clinical study report.

10.2 Populations to be Analysed

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 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 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 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.

10.3 Study Endpoints

10.3.1 Primary Endpoints

RPL554 pharmacokinetic parameters (AUC, C_{max}, t_{max}, $t_{1/2)}$.

10.3.2 Secondary Endpoints

- Peak and AUC FEV_1 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 rate] over 8 hours

10.3.3 Exploratory Endpoints

- Levels of inflammatory mediators in sputum and sputum rheology

10.4 Statistical Methods

In general, unless stated otherwise, continuous variables will be summarised using descriptive statistics (number of patients, arithmetic mean, standard deviation, median, minimum and maximum values) and for categorical (nominal) variables, the number and percentage of patients will be used.

10.4.1 Patient Disposition

The number of patients enrolled, randomised, completed or withdrawn (with reason for withdrawal) will be summarised.

10.4.2 Protocol Deviations

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.

10.4.3 Demographics and Other Baseline Characteristics

Demographics and baseline characteristics including FEV_1 [both in litres and in percentage of predicted normal], FEV_1 reversibility, disease history and prior and concomitant therapies will be listed and summarised appropriately.

Other medical history, other prior and concomitant medications, viral serology results and pregnancy test results from female patients will be listed.

10.4.4 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.

10.4.5 Pharmacodynamics

For FEV_1 the average effect, (E_{av}), will be calculated as the AUC divided by the length of the time interval of interest. In addition the peak effect on FEV_1 during these time intervals will be computed as the maximum value (E_{max}).

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.

Results of the comparisons will be expressed as the mean geometric ratio with 95% confidence intervals and associated, 2-sided, p-value.

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

10.4.6 Pharmacokinetics

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.

- $AUC_{0-\infty}$ is the area under the plasma concentration curve from time 0 to infinity, computed as $AUC_{0-t}+C_t/\lambda_z$, where C_t denotes the last plasma concentration above the lower limit of quantitation and λ_z denotes the terminal elimination rate. AUC_{0-t} will be computed using the linear trapezoidal method
- C_{max} denotes the highest plasma concentration measured
- t_{max} denotes the time point corresponding to C_{max}
- $t_{1/2}$ denotes the estimated half-life and is computed as $\ln(2)/\lambda_z$

Pharmacokinetic parameters will be summarised by dose level using descriptive statistics (number of patients, geometric mean, coefficient of variation, minimum, maximum and median for AUC parameters, C_{max}, $t_{1/2;}$ and number of patients, arithmetic mean, standard deviation, minimum, maximum and median for t_{max}).

10.4.7 Safety

Safety data including safety laboratory tests, 12-lead ECG parameters, vital signs and physical examinations, will be summarised by treatment group and time point, when appropriate. 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 selected time points.

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.

10.4.8 Handling of Withdrawals or Missing Data

Patients withdrawn after only one treatment period will not be included in the pharmacodynamic analyses. Imputation of data for calculation of average (AUC) effects for FEV_1 will be described in the statistical analysis plan. No other imputation of missing data will be performed.

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 FEV_1 or other reason, the rescheduled visit will be listed and summarised as the valid visit.

10.4.9 Interim Analyses

No formal interim analysis is planned for the study.

10.5 Determination of Sample Size

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

11 ETHICAL CONSIDERATIONS

11.1 Guidelines

The study will be performed in accordance with the ICH GCP guidelines, the principles outlined in the Declaration of Helsinki (1996), the protocol and applicable regulatory requirements.

11.2 Ethics and Regulatory Approval

The Sponsor will supply all background data necessary to enable submission to the appropriate ethics committee and regulatory authority. The study will not commence before formal ethical and regulatory approvals have been granted.

All changes or revisions of this protocol will be documented. The reason for the amendment will be stated. The Sponsor will ensure ethical and regulatory approval is obtained for all substantial amendments to the original approved documents.

11.3 Informed Consent Process

It is the responsibility of the Investigator to obtain written informed consent from patients. All consent documentation must be in accordance with applicable regulations and GCP. Each patient is requested to sign and date the informed consent form after (s)he has received and read the patient information sheet and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences and the patient's rights and responsibilities. Patients will be given adequate time to evaluate the information given to them before signing the informed consent form.

One original of the signed informed consent form must remain on file and must be available for verification by the study monitor at any time. A second original of the informed consent form plus the patient information sheet must be given to the patient or the patient's legally authorised representative.

11.4 Patient Confidentiality

Data collected during this study may be used to support the development, registration or marketing of the study treatment. The Sponsor will control all data collected during the study, and will abide by the European Union Directive on Data Privacy concerning the processing and use of patient's personal data. For the purpose of data privacy legislation, the Sponsor will be the data controller.

After patients have consented to take part in the study their medical records and the data collected during the study will be reviewed by the Sponsor and/or its representatives. These records and data may, in addition, be reviewed by the following: independent auditors who

validate the data on behalf of the Sponsor; regulatory authorities and the ethics committee which gave its approval for this study to proceed.

Although patients will be known by a unique number, their initials will also be collected and used to assist the Investigator to reconcile data clarification forms, for example, that the results of study assessments are assigned to the correct patient. The results of this study containing the unique number, but not the patient's initials and relevant medical information may be recorded and transferred to and used in other countries throughout the world, which may not afford the same level of protection that applies within the European Union. The purpose of any such transfer would be to support regulatory submissions made by the Sponsor in such countries.

11.5 Record Maintenance/Retention

The Investigator will retain the originals of all source documents generated at his hospital unit office for a period of 2 years after the report of the study has been finalised, after which all study-related documents will be archived according to GCP regulations.

12 FINANCE AND INSURANCE

Financial arrangements are detailed in the Investigator Agreement between the Sponsor and Investigator.

The Sponsor will arrange clinical study insurance to compensate patients for any potential injury or death caused by the study.

13 PUBLICATION POLICY

The publication policy is detailed in the Investigator Agreement between the Sponsor and Investigator.

14 REFERENCES

Abbott-Banner KH, Page CP. Dual PDE3/4 and PDE4 inhibitors: novel treatments for COPD and other inflammatory airway diseases. Basic Clin Pharmacol Toxicol. 2014 May;114(5):365-76. Doi: 10.1111/bcpt.12209. Epub 2014 Mar 6.

Bjermer L, Stewart J, Abbott-Banner K, Newman K. RPL554, a first-in-class dual PDE3/4 inhibitor is equieffective as a bronchodilator to maximal doses of salbutamol in asthmatics but with fewer adverse events. Am J Respir Crit Care Med 2016, A7770

Calverley PM, Sanchez-Toril F, McIvor A et al. Effect of 1-year treatment with roflumilast in severe chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2007; 176(2):154-161.

Calzetta L, Page CP, Spina D, et al**.** The effect of the mixed phosphodiesterase 3/4 inhibitor RPL554 on human isolated bronchial smooth muscle tone. J Pharmacol Exp Ther 2013; Sep;346(3):414-423

Calzetta L, Cazzola M, Page CP et al. Pharmacological characterization of the interaction between the dual phosphodiesterase (PDE) 3/4 inhibitor RPL554 and glycopyrronium on human isolated bronchi and small airways. Pulm Pharmacol Ther 2015 Apr 18. pii: S1094-5539(15)00042-5.

Celli BR, MacNee W, ATS/ERS Task Force. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J, 2004; Vol 2:932–946.

Compton CH, Gubb J, Nieman R et al. Cilomilast, a selective phosphodiesterase-4 inhibitor for treatment of patients with chronic obstructive pulmonary disease: a randomised, dose-ranging study. Lancet 2001; 358(9278):265-270.

Downey DG, Martin SL, Dempster M, et al. The relationship of clinical and inflammatory markers to outcome in stable patients with cystic fibrosis. Pediatr Pulmonol. 2007 Mar;42(3):216-220.

EU Directive 95/46/EC: The Data Protection Directive.

Franciosi LG, Diamant Z, Banner KH, et al. Efficacy and safety of RPL554, a dual PDE3 and PDE4 inhibitor, in healthy volunteers and in patients with asthma or chronic obstructive pulmonary disease: findings from four clinical trials. Lancet Respiratory Medicine 2013; 1(9): 714-727.

Gamble E, Grootendorst DC, Brightling CE et al. Anti-inflammatory effects of the phosphodiesterase-4 inhibitor cilomilast (Ariflo) in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2003; 168(8):976-982.

Grootendorst D, Gauw SA, Verhoosel RM, et al. Reduction in sputum neutrophil and eosinophil numbers by the PDE4 inhibitor roflumilast in patients with COPD. Thorax 2007; 62(12); 1081-1087.

Harbinson PL, MacLeod D, Hawksworth R et al. The effect of a novel orally active selective PDE4 isoenzyme inhibitor (CDP840) on allergen-induced responses in asthmatic subjects. Eur Respir J 1997; 10(5):1008-1014.

Horsley A, Rousseau K, Ridley C, et al. Reassessment of the importance of mucins in determining sputum properties in cystic fibrosis. J Cyst Fibros. 2014 May;13(3):260-6. doi: 10.1016/j.jcf.2013.11.002. Epub 2013 Dec 12.

International Conference on Harmonisation Topic E6. ICH Harmonised Tripartite Guideline. Good Clinical Practice.

Keir S and Page C. RPL554, a dual phosphodiesterase (PDE) 3/4 inhibitor acts synergistically with muscarinic receptor antagonists and beta-adrenoceptor agonists to produce bronchodilator in vivo. Am J Respir Crit Care Med 189: 2014 A4218

MacNee W, Rennard SI, Hunt JF, et al. Evaluation of exhaled breath condensate pH as a biomarker for COPD. Respiratory Medicine 2011; 105:1037e1045

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

Quanjer PH, Stanojevic S, Cole TJ et al. Multi-ethnic reference values for spirometry for the 3-95 year age range: the global lung function 2012 equations. Report of the Global Lung Function Initiative (GLI), ERS Task Force to establish improved Lung Function Reference Values. Eur Respir J 2012 Dec; 40 (6):1324-1343.

Rabe KF, Bateman ED, O'Donnell D, et al. Roflumilast--an oral anti-inflammatory treatment for chronic obstructive pulmonary disease: a randomised controlled trial. Lancet 2005; 366(9485):563-571.

Rennard SI, Schachter N, Strek M, et al. Cilomilast for COPD: results of a 6-month placebo controlled study of a potent, selective inhibitor of phosphodiesterase 4. Chest 2006; 129(1):56 66.

Singh, D, Abbott-Banner K, Newman K. A Phase I, randomised, double blind, placebo controlled, study to assess the safety, tolerability and pharmacokinetics of single inhaled doses of the dual phosphodiesterase 3/4 (PDE3/4) inhibitor RPL554 administered by nebuliser to healthy male subjects. Am J Respir Crit Care Med 2016, A6839

Singh, D, Abbott-Banner K, Newman K. A Phase I, randomised, double blind, placebo controlled, study to assess the safety, tolerability and pharmacokinetics of multiple inhaled doses of RPL554 administered by nebuliser to healthy male subjects and stable COPD patients. Am J Respir Crit Care Med 2016, A6838

Turner MJ, Matthes E, Billet A, et al. The dual phosphodiesterase 3 and 4 inhibitor RPL554 stimulates CFTR and ciliary beating in primary cultures of bronchial epithelia. Am J Physiol Lung Cell Mol Physiol. 2016 Jan 1;310(1):L59-70. doi: 10.1152/ajplung.00324.2015. Epub 2015 Nov 6.

Ukena D, Rentz K, Reiber C, et al. Effects of the mixed phophodiesterase III/IV inhibitor, zardaverine, on airway function in patients with chronic airflow obstruction. Respir Med 1995; 89 (6): 441-444.

van Schalkwyk E, Strydom K, Williams Z et al. Roflumilast, an oral, once-daily phosphodiesterase 4 inhibitor, attenuates allergen-induced asthmatic reactions. J Allergy Clin Immunol 2005; 116 (2):292 298.

Verona Pharma plc . RPL554 Investigator Brochure (current version).

Verona Pharma plc . RPL554 Investigational Medicinal Product Dossier (current version).

World Medical Association, Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. Last amended by the 48th World Medical Association General Assembly, Somerset West, 1996.

15 APPENDICES

Appendix 1 Acceptable Methods of Contraception

Females of childbearing potential must have been using a consistent and reliable form of contraception according to the following list 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:

- Male partner who is sterile and is the sole sexual partner for that female subject
- Abstinence from sexual intercourse.
- Implants of levonorgestrel or etonogestrel
- Injectable progestogen
- Oral contraceptive
- Oestrogenic vaginal ring
- Percutaneous contraceptive patches
- Any intrauterine device with a documented failure rate of less than 1% per year
- Male condom combined with a vaginal spermicide
- Male condom combined with a female diaphragm.