ON-LINE SUPPLEMENT

Methods

RPL554 manufacture and administration

RPL554 and placebo were 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 RPL554 formulation is a sterile suspension for nebulisation, supplied as sterile stock suspensions of micronised RPL554 in pH 7 phosphate buffered saline with surfactants to aid suspension. The placebo is the same as the RPL554 suspension except that the active RPL554 ingredient is omitted, i.e. it consists of pH 7 phosphate buffered saline and surfactants only. It is thus a clear solution rather than a pale yellow suspension; there is no known inert yellow solid that would be acceptable for inhalation, and therefore a visually matching placebo cannot be developed. The placebo was used as a diluent, enabling the preparation of the range of active suspension concentrations required for the study. The study drugs were manufactured by Intertek Melbourn (Melbourn, UK).

Treatment allocation was concealed and kept in a secure location at study sites and was not available to site staff involved in the clinical trial until all participants had completed the study and the database had been finalised. RPL554 and placebo were prepared by unmasked pharmacy staff that were not involved in the study assignments. It was not possible to completely match the placebo to RPL554 as the visual appearance is slightly different, but it was only seen by the staff preparing and administering the study drug. The dosing cup on each nebuliser was obscured with tape to visually blind the study treatment. This procedure successfully allowed 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) to be blinded to the treatment allocation.

The RPL554 study drug was administered by inhalation through a mouthpiece of an aerosol generated by a reusable PARI LC Sprint® jet nebuliser attached to a PARI TurboBOY® SX compressor which produced particles of 1-5µm. The dosing cup on each nebuliser was obscured with tape to visually blind the study treatment. RPL554 or placebo was administered until sputtering, with nebulisation not exceeding 10 minutes.

Salbutamol and ipratropium and pMDIs were blinded using a double dummy technique with matched placebo pMDIs that were identical in appearance.

Inclusion / Exclusion Criteria for Study 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 between 40 and 70 years inclusive, at the time of informed consent.
3. If male: must be willing, able and agree to meet the following from the first dose up to 1 month after the last dose of study treatment:

   - Not donate sperm
   - *Either:* be sexually abstinent in accordance with a patient's usual and preferred lifestyle (but agree to abide by the contraception requirements below should their circumstances change)

     Or: use a condom with all sexual partners. If the partner is of childbearing potential the condom must be used with spermicide and a second reliable form of contraception must also be used (e.g. diaphragm/cap with spermicide, established hormonal contraception, intra-uterine device)

   If female: be of non-childbearing potential defined as being:

   Either: post-menopausal (being spontaneously amenorrhoeic for at least 1 year with an appropriate clinical profile [e.g. age appropriate, history of vasomotor symptoms]. However, if indicated, this should be confirmed by follicle stimulating hormone levels consistent with post-menopausal status [according to local laboratory ranges])

   Or: permanently sterilised e.g. tubal occlusion, hysterectomy, bilateral oophorectomy, bilateral salpingectomy.
4. Have a 12-lead ECG recording at screening (Visit 1) and Visit 2 pre-dose showing the following:

   - Heart rate between 45 and 90 beats per minute
   - QT interval corrected for heart rate using Fridericia's formula (QTcF) interval ≤450 msec
   - QRS interval ≤120 msec
   - PR interval ≤220 msec
   - No clinically significant abnormality including morphology (e.g. left bundle branch block, atrioventricular nodal dysfunction, ST segment abnormalities).
5. Capable of complying with all study restrictions and procedures including ability to use the study nebuliser correctly.
6. Body mass index (BMI) between 18 and 33 kg/m2 (inclusive) with a minimum weight of 45 kg.
7. COPD diagnosis: Patients with a diagnosis of COPD as defined by the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines (Celli and MacNee, 2004) with symptoms compatible with COPD for at least 1 year prior to screening (Visit 1).
8. Post-bronchodilator (two puffs of salbutamol followed by two puffs of ipratropium) spirometry at screening (Visit 1):

   The following must be confirmed at 1 hour post-dose for inclusion:

   - Post-bronchodilator FEV1/FVC ratio of ≤0.70
   - Post-bronchodilator FEV1 ≥40 % and ≤80% of predicted normal

   The following must be confirmed at either 30 minutes or 1 hour post-dose for inclusion:

   - Demonstrates ≥150 mL increase from pre-bronchodilator FEV1
9. Clinically stable COPD in the 4 weeks prior to screening (Visit 1) and randomisation (Visit 2).
10. A chest X-ray (post-anterior [PA]) at screening, or in the 12 months prior to screening showing no abnormalities, which are both clinically significant and unrelated to COPD.

11. Meet the concomitant medication restrictions and be expected to do so for the rest of the study.
12. Smoking history of ≥10 pack years.
13. Capable of withdrawing from long acting bronchodilators, as defined in Section 5.9, until the end of the treatment period, and short acting bronchodilators for 8 hours prior to administration of study treatment.

Exclusion Criteria

1. A history of life-threatening COPD including Intensive Care Unit admission and/or requiring intubation.
2. COPD exacerbation requiring oral steroids in the 3 months prior to screening (Visit 1) or prior to randomisation (Visit 2).
3. A history of one or more hospitalisations for COPD in the 12 months prior to screening (Visit 1).
4. Respiratory tract infection (both upper and lower) treated with antibiotics within 12 weeks of screening (Visit 1) or prior to randomisation (Visit 2).
5. Evidence of cor pulmonale or clinically significant pulmonary hypertension.
6. Other respiratory disorders: Patients with a current diagnosis of asthma, active tuberculosis, lung cancer, bronchiectasis, sarcoidosis, lung fibrosis, interstitial lung diseases, sleep apnoea, known alpha-1 antitrypsin deficiency or other active pulmonary diseases.
7. Previous lung resection or lung reduction surgery.
8. Oral therapies for COPD (e.g. oral steroids, theophylline, and roflumilast) in the 3 months prior to screening (Visit 1) and throughout the study.
9. History of, or reason to believe a subject has, drug or alcohol abuse within the past 3 years.
10. Received an experimental drug within 3 months or five half-lives, whichever is longer.
11. Prior exposure to RPL554.
12. Patients with a history of chronic uncontrolled disease including, but not limited to, cardiovascular (including arrhythmias), endocrine, active hyperthyroidism, neurological, hepatic, gastrointestinal, renal, haematological, urological, immunological, or ophthalmic diseases that the Investigator believes are clinically significant.
13. 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.
14. 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.
15. History of malignancy of any organ system within 5 years with the exception of localised skin cancers (basal or squamous cell).
16. Clinically significant abnormal values for safety laboratory tests (haematology, biochemistry or urinalysis) at screening (Visit 1), as determined by the Investigator.
17. A disclosed history, or one known to the Investigator, of significant non-compliance in previous investigational studies or with prescribed medications.
18. Requires oxygen therapy, even on an occasional basis.
19. Inability to adequately perform whole body plethysmography.
20. Any other reason that the Investigator considers makes the subject unsuitable to participate.

21. Patients with known hypersensitivity to atropine or its derivatives, or to ipratropium bromide or its excipients.
22. Patients with known hypersensitivity to salbutamol or its excipients.
23. Patients with known hypersensitivity to RPL554 or its excipients/components.

Inclusion / Exclusion Criteria for Study 2

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 between 40 and 75 years inclusive, at the time of informed consent.
3. If male: must agree to meet the following from the first dose up to 2 months after the last dose of study treatment:

- Not donate sperm
- *Either:* be sexually abstinent in accordance with a patient's usual and preferred lifestyle (but agree to abide by the contraception requirements below should their circumstances change)

  Or: use a condom with all sexual partners. If the partner is of childbearing potential the condom must be used with spermicide and a second highly effective form of contraception must also be used (as defined in Section 8.4)

4. If female: either be:
   a) Of non-childbearing potential defined as being:
      - *Either:* post-menopausal (being spontaneously amenorrhoeic for at least 1 year with an appropriate clinical profile [e.g. age appropriate, history of vasomotor symptoms]
      - *Or:* permanently sterilised e.g. tubal occlusion, hysterectomy, bilateral oophorectomy, bilateral salpingectomy
   b) Of childbearing potential and agreeing to use a highly effective method of contraception (as defined in Section 8.4) until completion of the end of study visit.
5. Have a 12-lead ECG recording at screening and randomisation (pre-dose in Treatment Period 1) showing the following:
   - Heart rate between 45 and 90 beats per minute (bpm)
   - QT interval corrected for heart rate using Fridericia's formula (QTcF) ≤450 msec for males and ≤470 ms for females
   - QRS interval ≤120 msec
   - No clinically significant abnormalities (as judged by the Investigator) including morphology (e.g. left bundle branch block, atrio-ventricular nodal dysfunction, ST segment abnormalities)
6. Have a screening Holter report with a minimum of 18 hours recording that is able to be evaluated for rhythm analysis which shows no abnormality which indicates a significant impairment of patient safety or which may significantly impairs interpretation in the opinion of the Investigator including:
   - Significant arrhythmias including atrial flutter, atrial fibrillation, ventricular tachycardia
   - Any symptomatic arrhythmia (except isolated extra systoles)
   - Any sustained second or third degree heart block
7. Capable of complying with all study restrictions and procedures including ability to use the study nebuliser and HandiHaler® DPI correctly.
8. Body mass index (BMI) between 18 and 33 kg/m^2 (inclusive) with a minimum weight of 45 kg.

9. COPD diagnosis: Patients with a diagnosis of COPD as defined by the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines (Celli and MacNee, 2004) with symptoms compatible with COPD for at least 1 year prior to screening.
10. Post-bronchodilator (four puffs of salbutamol) spirometry at screening:
    - Post-bronchodilator FEV_1/FVC ratio of ≤ 0.70
    - Post-bronchodilator FEV_1 ≥ 40 % and ≤ 80% of predicted normal
    - Demonstrates ≥ 150 mL increase from pre-bronchodilator FEV_1
11. Clinically stable COPD in the 4 weeks prior to screening and randomisation (pre-dose in Treatment Period 1).
12. A chest X-ray (post-anterior) at screening, or in the 12 months prior to screening showing no abnormalities, which are both clinically significant and unrelated to COPD.
13. Meet the concomitant medication restrictions and be expected to do so for the rest of the study.
14. Smoking history of ≥ 10 pack years.
15. Capable of withdrawing from long acting bronchodilators for the duration of the study, and short acting bronchodilators for 8 hours prior to spirometry.

Exclusion Criteria

1. A history of life-threatening COPD exacerbation including Intensive Care Unit admission and/or requiring intubation.
2. COPD exacerbation requiring oral steroids, or lower respiratory tract infection requiring antibiotics, in the 3 months prior to screening or randomisation (pre-dose in Treatment Period 1).
3. A history of one or more hospitalisations for COPD in the 12 months prior to screening or randomisation (pre-dose in Treatment Period 1).
4. Lactation (female patients only).
5. Positive urine or serum pregnancy test at screening, or a positive urine pregnancy test prior to randomisation (female patients of childbearing potential only).
6. Known hypersensitivity to RPL554 or its components.
7. Intolerance or hypersensitivity to tiotropium.
8. Evidence of cor pulmonale.
9. Other respiratory disorders: Patients with a current diagnosis of asthma, active tuberculosis, lung cancer, bronchiectasis, sarcoidosis, lung fibrosis, interstitial lung diseases, sleep apnoea, known alpha-1 antitrypsin deficiency or other active pulmonary diseases.
10. Previous lung resection or lung reduction surgery.
11. Use of oral COPD medications (e.g. oral steroids, theophylline and romifulast) in the 3 months prior to screening or randomisation (pre-dose in Treatment Period 1).
12. History of, or reason to believe, a patient has drug or alcohol abuse within the past 3 years.
13. Inability to perform technically acceptable spirometry or whole body plethysmography (at screening or randomisation [pre-dose in Treatment Period 1])
14. Received an experimental drug within 30 days or five half-lives, whichever is longer.
15. Patients with a history of chronic uncontrolled disease including, but not limited to, endocrine, active hyperthyroidism, neurological, hepatic, gastrointestinal, renal, haematological, urological, immunological, or ophthalmic diseases that the Investigator believes are clinically significant.
16. Documented cardiovascular disease: arrhythmias, angina, recent or suspected myocardial infarction, congestive heart failure, a history of unstable, or uncontrolled hypertension, or has been diagnosed with hypertension in the 3 months prior to screening or randomisation.
17. Concurrent use of non-cardioselective oral beta-blockers.

18. Has had major surgery, (requiring general anaesthesia) in the 6 weeks prior to screening or randomisation (pre-dose in Treatment Period 1), or will not have fully recovered from surgery, or planned surgery through the end of the study.
19. A disclosed history or one known to the Investigator, of significant non-compliance in previous investigational studies or with prescribed medications.
20. Requires oxygen therapy, even on an occasional basis.
21. Clinically significant prostatic hyperplasia (judged by the Investigator) or bladder-neck obstruction or with narrow-angle glaucoma.
22. Any other reason that the Investigator considers makes the patient unsuitable to participate.

Statistics

For both studies, sample sizes were calculated assuming a residual coefficient of variation of 6% for peak FEV_1. For study 1, 30 patients gave 80% power to detect a pairwise difference in maximum FEV_1 of 4.6%. Assuming a mean baseline FEV_1 of 1.5 litres, this corresponded to a difference of approximately 70 mL. For study 2, 24 patients gave 80% power to detect a pairwise difference in maximum FEV_1 of 75 mls. The endpoints were compared between study treatments using analysis of covariance (ANCOVA) models adjusting for treatment, period, patient and baseline as covariate; additive models for FEV_1 and multiplicative models for plethysmography. The full analysis set, including all randomised patients with data from at least two treatment visits, were used for all analysis.

Table S1. Study 1 Peak FVC

Contrast	Treatment difference		
	Diff	95% C.I.	p-value
Salbutamol/RPL vs Placebo	0.468	(0.376 – 0.561)	<0.001
Ipra /RPL vs Placebo	0.481	(0.388 – 0.574)	<0.001
Salbutamol/RPL vs Salbutamol	0.196	(0.103 – 0.288)	<0.001
Ipra /RPL vs Ipra	0.122	(0.030 – 0.215)	0.010
RPL vs Placebo	0.376	(0.282 – 0.470)	<0.001
Salbutamol vs Placebo	0.273	(0.181 – 0.365)	<0.001
Ipra vs Placebo	0.359	(0.266 – 0.452)	<0.001
RPL vs Salbutamol	0.103	(0.009 – 0.197)	0.032
RPL vs Ipra	0.017	(-0.077 – 0.111)	0.720
Salbutamol vs Ipra	-0.086	(-0.179 – 0.007)	0.070

Table S2. Study 1: FVC Average effect over 8 hours:

Contrast	Treatment difference		
	Diff	95% C.I.	p-value
Salbutamol/RPL vs Placebo	0.336	(0.270 – 0.401)	<0.001
Ipra /RPL vs Placebo	0.320	(0.255 – 0.386)	<0.001
Salbutamol/RPL vs Salbutamol	0.179	(0.114 – 0.245)	<0.001
Ipra /RPL vs Ipra	0.097	(0.032 – 0.162)	0.004
RPL vs Placebo	0.252	(0.187 – 0.318)	<0.001
Salbutamol vs Placebo	0.156	(0.091 – 0.221)	<0.001
Ipra vs Placebo	0.224	(0.158– 0.289)	<0.001
RPL vs Salbutamol	0.096	(0.030 – 0.162)	0.005
RPL vs Ipra	0.029	(-0.037 – 0.095)	0.388
Salbutamol vs Ipra	-0.067	(-0.133 – -0.002)	0.043

Table S3. Change from Baseline in Total Lung Capacity and Functional Residual Capacity in Study 1.

		RPL554 (6mg) + Salbutamol (200mg) N=30-31	**Salbutamol (200mg) N=30-31**	**RPL554 (6mg) + Ipratropium (40mg) N=29-31**	**Ipratropium (40mg) N=30-31**	**RPL554 (6mg) N=29-31**	**Placebo N=31**
TLC (L)	1h	-0.078 (0.1834)	-0.152 (0.1659)	-0.122 (0.2933)	-0.059 (0.1914)	-0.134 (0.2551)	-0.040 (0.1797)
	4h	0.029 (0.2713)	-0.025 (0.1952)	-0.024 (0.2302)	-0.043 (0.1734)	-0.061 (0.2535)	-0.003 (0.1657)
TLC (% predicted)	1h	-2.4 (4.40)	-0.8 (3.21)	-2.2 (4.13)	-0.7 (3.13)	-1.3 (3.20)	-2.7 (3.19)
	4h	-0.5 (3.70)	-0.7 (2.81)	-1.1 (4.68)	0.1 (2.96)	0.6 (4.51)	-0.4 (3.64)
FRC (L)	1h	-0.390 (0.3827)	-0.360 (0.2883)	-0.400 (0.2866)	-0.275 (0.2461)	-0.334 (0.2686)	-0.037 (0.2280)
	4h	-0.187 (0.3198)	-0.114 (0.2704)	-0.254 (0.2267)	-0.073 (0.2049)	-0.119 (0.2920)	-0.005 (0.2778)
FRC (% predicted)	1h	-13.3 (8.77)	-9.3 (8.00)	-11.2 (8.84)	-1.3 (7.25)	-11.9 (11.36)	-11.5 (8.63)
	4h	-8.3 (7.62)	-2.3 (6.98)	-4.3 (9.43)	-0.0 (9.14)	-5.5 (10.14)	-3.4 (8.67)

Data are represented as mean (sd)

Abbreviations: TLC: Tidal Lung Capacity; FRC Functional Residual Capacity