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Early View

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Title: The short term bronchodilator effects of the dual PDE3 and PDE4 inhibitor RPL554 in COPD

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Take home message: The dual PDE3 and PDE4 inhibitor RPL554 causes additional bronchodilation when combined with commonly used short or long acting bronchodilators

Abstract

Introduction: We investigated the short term bronchodilator effects of RPL554 (an inhaled dual phosphodiesterase 3 and 4 inhibitor) combined with other bronchodilators in COPD patients with reversibility (>150 mL to short acting bronchodilators).

Methods: Study 1: six way placebo controlled crossover study (n=36) with single doses of RPL554 (6mg), salbutamol (200µg), ipratropium (40µg), RPL554 + salbutamol, RPL554 + ipratropium and placebo. Study 2: three way crossover study (n=30) of tiotropium (18 µg) combined with RPL554 (1.5 mg or 6mg) or placebo for 3 days. FEV_1, lung volumes and sGaw were measured.

Results: Study 1; Peak FEV_1 change compared to placebo was similar with RPL554, ipratropium and salbutamol (means 223, 199 and 187 mL respectively). The peak FEV_1 was higher for RPL554 + ipratropium versus ipratropium (mean difference 94 mL, $p<0.0001$) and RPL554 + salbutamol versus salbutamol (mean difference 108 mL; $p<0.0001$). Study 2 (day 3); both RPL554 doses caused greater peak FEV_1 effects than placebo. The average $FEV_{1\ (0\text{-}12h)}$ increase was greater with RPL554 6mg only versus placebo (mean difference 65 mL $p=0.0009$). In both studies, lung volumes and sGaw showed greater RPL554 combination treatment effects versus monotherapy.

Conclusion: RPL554 combined with standard bronchodilators caused additional bronchodilation and hyperinflation reduction.

Key words: COPD; bronchodilator; phosphodiesterase inhibitor.

Introduction

RPL554 is a first-in-class, dual inhibitor of both PDE3 and PDE4 isoforms (1, 2). PDE3 inhibitors principally target smooth muscle cells to cause bronchodilation (3-5), while PDE4 inhibitors exert anti-inflammatory effects across a range of immune cell types (6, 7); RPL554 therefore represents a novel drug class combining bronchodilator and anti-inflammatory effects in a single molecule. Initial clinical trials showed that inhaled RPL554 caused bronchodilation in patients with asthma and COPD, likely due to PDE3 inhibition, and demonstrated significant anti-inflammatory effects in the healthy volunteer LPS inhalation model of neutrophilic lung disease, likely due to PDE4 inhibition (2). However, cell and animal models have shown that combined PDE3 and PDE4 inhibition causes additive or synergistic anti-inflammatory and bronchodilator effects (8). Inhaled RPL554 delivery minimises systemic exposure, thereby reducing the potential for PDE3 or PDE4 mediated side effects, and has been well tolerated in early phase clinical trials to date (2).

While preclinical data demonstrates that combining RPL554 with other bronchodilators produces additional bronchodilation(9, 10), this concept has not been investigated in COPD clinical trials. The future use of RPL554 in clinical practice is likely to be in conjunction with other bronchodilators. We report two phase 2 clinical trials in COPD patients investigating the bronchodilator effects of RPL554 combined with other bronchodilators. In one study, RPL554 was combined with short-acting bronchodilators, while in another study RPL554 was combined with the long acting muscarinic antagonist tiotropium.

Methods

Subjects

Both studies were performed at the Medicines Evaluation Unit, Manchester, UK (www.clinical trials.gov; NCT02542254 and NCT03028142). Inclusion and exclusion criteria are listed fully in the on-line supplement. For both studies, patients with a diagnosis of COPD and a post-bronchodilator FEV_1 ≥40% and ≤80% predicted were recruited, and COPD patients with significant cardiovascular disease including angina or recent myocardial infarction were excluded. For study 1, FEV_1 reversibility >150 mls after inhalation of salbutamol (200µg) and ipratropium (40µg) together was required. For study 2, FEV_1 reversibility >150 mls after inhalation of salbutamol (400µg) was required. One patient participated in both studies. Ethical approval was obtained, and participants provided written informed consent before screening.

Study Design

Study 1 was a randomised, double blind, placebo controlled, double dummy, complete block six way crossover study to investigate combination treatment with nebulised RPL554 (6mg) and salbutamol (200 µg) or ipratropium (40µg) compared to salbutamol or ipratropium alone (see Figure 1). The salbutamol and ipratropium doses are those approved for COPD patients. Long acting bronchodilator treatment was withdrawn at screening. There were six treatment visits separated by 3 - 14 day washout periods. The pre-dose FEV_1 at treatment visits was required to be within ±15% of the value at the first treatment visit. On each treatment visit, patients received a single dose (two puffs) from a blinded pressurised metered dose inhaler (pMDI) of salbutamol (200µg) or matched placebo followed, within 1 minute, by a single dose (two puffs) from a second blinded pMDI of ipratropium (40µg) or matched placebo. This was followed immediately (within 2 minutes) by a single double blind dose of either RPL554 (6mg) or placebo. Spirometry was performed pre-dose and at various times up to 12 hours post-dose. Whole body plethysmography was performed pre-dose and up to 4 hours post dose to obtain measurements of functional residual capacity (FRC), residual volume (RV), total lung capacity (TLC) and airway conductance (sGaw).

Study 2 was a randomised, double blind, placebo controlled, complete block three way crossover study to investigate combination treatment with nebulised RPL554 (1.5 mg or 6mg) and tiotropium (18 µg using Handihaler) compared to tiotropium alone (see Figure 1).

Long acting bronchodilator treatment was withdrawn at screening. There were three treatment visits separated by 7 - 21 day washout periods. On each treatment visit, patients received one of the following treatments for 2 days and the morning of day 3:

Tiotropium 18µg once daily and RPL554 6 mg twice daily (TIO + RPL554 6 mg)

Tiotropium 18µg once daily and RPL554 1.5 mg twice daily (TIO + RPL554 1.5 mg)

Tiotropium 18µg once daily and placebo twice daily (TIO + placebo)

Tiotropium was administered open label, while RPL554 or placebo was administered double blind. Spirometry was performed on days 1 and 3 at pre-dose and at various times up to 12 hours post-dose, and on day 2 at pre-dose and up to 4 hours post-dose. Whole body plethysmography was performed at pre-dose on day 1 and at pre-dose and 1.25 hrs post-dose on day 2.

For both studies, patients using inhaled corticosteroids were allowed to continue this treatment during the study. Patients were allowed to use short acting bronchodilators throughout, but these were withheld for at least 8 hours prior to spirometry. RPL554 manufacture and administration using a PARI LC Sprint® jet nebuliser is described in the on-line supplement. The RPL554 doses were selected based on the results of a preliminary study(11). Spirometry assessments were performed in accordance with guidelines with three technically acceptable measurements recorded(12); predicted values calculated using European Community of Coal and Steel reference equations (13).

Statistical analysis

Sample size calculations are in the on-line supplement. The study 1 primary endpoints were change from baseline in peak and average FEV_1 over 8 hours comparing RPL554 + salbutamol versus salbutamol and RPL554 + ipratropium versus ipratropium. The study 2 primary endpoints were change from baseline (pre-dose day 1) in peak and average FEV_1 over 12 hours on day 3 comparing RPL554 versus placebo. For both studies, the secondary endpoints included onset of action (defined as time to reach 10% increase in FEV_1 from baseline), FVC, body plethysmography measurements (FRC, RV, sGaw) and safety. Analysis of covariance (ANCOVA) models were used as described in the supplement.

Results

For both studies, flow diagrams showing the number of patients screened, reasons for screen failure and withdrawals are in the on-line supplement.

Study 1

Table 1 lists the characteristics of the 36 enrolled COPD patients; the mean post-bronchodilator FEV_1 was 60.6% predicted with 17.4% reversibility. 6 patients did not complete the study; 2 patients were withdrawn due to chest infections, while 4 patients failed to meet the criteria for pre-dose FEV_1 variability and were withdrawn.

FEV_1

The change from baseline in peak FEV_1 compared to placebo was similar with RPL554 (mean 223 mL), ipratropium bromide (mean 199 mL) and salbutamol (mean 187 mL) (Figure 2), with no differences between treatments ($p>0.05$; see Table 2). The combination of RPL554 with ipratropium bromide caused a greater increase in peak FEV_1 compared to placebo (mean 292 mL; $p<0.0001$) and compared to ipratropium alone (mean 94 mL; $p<0.0001$). RPL554 combined with salbutamol caused a greater peak FEV_1 compared to placebo (mean 295 mL; $p<0.0001$), and compared to salbutamol alone (mean 108 mL; $p<0.0001$).

The average $FEV_{1\ (0\text{-}8h)}$ increase compared to placebo was similar for RPL554 (mean 169 mL) and ipratropium (mean 165 mL), while RPL554 was statistically superior to salbutamol (mean 123 mL; $p=0.014$) (Figure 2 and statistical analysis in Table 2). RPL554 combined with ipratropium caused a mean increase of 229 mL compared to placebo ($p<0.0001$), and 64 mL compared to ipratropium alone ($p=0.0006$). RPL554 combined with salbutamol caused a mean increase of 235 mL compared to placebo ($p<0.0001$), and 112 mL compared to salbutamol alone ($p<0.0001$).

The median time of onset of action ($\geq$ 10% increase from baseline) for RPL554 was 14.6 minutes, while for ipratropium bromide and salbutamol this was 18.4 minutes and 6.0 minutes respectively. RPL554 administered in combination with either salbutamol or ipratropium bromide reduced the median time of onset to 3.6 minutes ($p=0.009$ versus salbutamol alone) and 4.8 minutes ($p<0.0001$ versus ipratropium bromide alone) respectively.

Analysis of peak FVC and average $FVC_{(0\text{-}8h)}$ showed greater effects of RPL554 combined with another bronchodilator compared to a bronchodilator alone (analysis shown in on-line supplement tables S1 and S2).

Body Plethysmography

Each treatment alone reduced RV to a similar extent (Figure 3). The combination of RPL554 with ipratropium bromide caused a 695 mL reduction in RV at 1 hr, which was significantly greater than ipratropium alone (treatment ratio 0.91 95% CI 0.88-0.95; p<0.001). Likewise RPL554 with salbutamol caused a significantly greater reduction in RV than salbutamol alone at both 1 hr and 4 hr. For sGaw, the effects of combination treatment involving RPL554 were significantly greater than ipratropium or salbutamol alone (treatment ratios at 1 hr; 1.18, 95% CI 1.08 – 1.28, p<0.001 and 1.20, 95% CI 1.11 -1.31, p<0.001 respectively; Figure 3). The FRC results are shown in on-line supplement Table S3; there was a greater effect of combination treatment involving RPL554 than ipratropium alone or salbutamol alone (e.g. treatment ratios at 4 hrs respectively; 0.96, 95% CI 0.94 – 0.99; p=0.006 and 0.97, 95% CI 0.94 – 0.99; p=0.01).

Safety

RPL554 was well tolerated alone or in combination with salbutamol or ipratropium with a similar rate of adverse events compared to placebo (Table 3).

Study 2

Table 1 lists the characteristics of the 30 enrolled COPD patients; the mean post-bronchodilator FEV_1 was 60.1% predicted with 19.5% reversibility. 4 patients did not complete the study; 2 patients were withdrawn because of worsening COPD symptoms, 1 patient withdrew with pneumonia, and 1 patient withdrew consent.

FEV_1

The FEV_1 change from baseline (pre-dose on day 1) over 12 hours on day 3 is shown in Fig 4. The peak FEV_1 changes from baseline for tiotropium (TIO) combined with placebo or RPL554 1.5 mg or 6 mg were 373 mls, 477 mls and 500 mls respectively (Figure 4). The effects of TIO + RPL554 1.5 mg and 6 mg were significantly greater than TIO + placebo (p=0.002 and p<0.0001 respectively). The average $FEV_{1\ (0\text{-}12h)}$ increase on day 3 was greater with TIO + RPL554 6mg (331 mls) compared to TIO + placebo (266 mls; p=0.0009); Figure

4. There was no difference for TIO + RPL554 1.5 mg (317 mls) versus TIO + placebo (p=0.09).

The mean change in morning trough FEV_1 on day 3 compared to baseline (day 1 pre-dose) was greater with TIO + RPL554 6mg (230 mls) compared to TIO + placebo (114 mls, p=0.0003) while there was no difference between TIO + RPL554 1.5 mg (168 mls) compared to TIO + placebo (p=0.35).

On day 1, there were significantly greater FEV_1 improvements with TIO + RPL554 6 mg compared to TIO + placebo, while TIO + RPL554 1.5mg was not significantly different to TIO + placebo; these results are shown in the online supplement. Notably, for peak FEV_1 the mean difference between TIO + RPL554 6 mg and TIO + placebo was 95 mls (p=0.0039). The median time of onset of action on day 1 for TIO + RPL554 1.5 mg and 6 mg was 4.2 and 4.6 minutes respectively, compared to 37.6 minutes for TIO + placebo (p<0.0001 for comparisons of RPL554 versus placebo).

Analysis of peak FVC and average $FVC_{(0\text{-}12h)}$ showed a greater effect of RPL554 6 mg compared to placebo, and are shown in the on-line supplement.

Body Plethysmography

On day 2, there was a reduction in RV with all treatments at 1.25 hrs post-dose (Figure 5). TIO + RPL554 6mg caused a significantly greater effect than TIO + placebo (p=0.0048) while the effect of TIO + RPL554 1.5mg was not significant (p=0.071). The improvements in FRC were significantly greater with TIO + RPL554 1.5 mg and 6 mg compared to TIO + placebo (treatment ratios; 0.96, 95% CI 0.94 – 0.99, p=0.027 and 0.97, 95% CI 0.95 – 1.00, p=0.047 respectively). Similarly, sGaw improvements were significantly greater with TIO + RPL554 1.5 mg and 6 mg compared to TIO + placebo (treatment ratios; 1.24, 95% CI 1.14 – 1.35 and 1.31, 95% CI 1.20 – 1.43 respectively, p<0.0001 for both comparisons).

Safety

TIO + RPL554 was well tolerated with a similar rate of adverse events compared to TIO + placebo (Table 3). Withdrawals due to COPD worsening occurred during washout periods, and were not attributed to RPL554.

Discussion

We show that RPL554 combined with short acting bronchodilators or tiotropium caused additional improvements in FEV_1 and hyperinflation in reversible COPD patients. In study 1, a single RPL554 6mg dose in addition to salbutamol or ipratropium caused significantly greater peak FEV_1 improvements compared to either short-acting bronchodilator alone. In study 2, additional improvements in peak, trough and average $_{(0\text{-}12h)}$ FEV_1 were observed when RPL554 6mg was administered with tiotropium for 3 days.

Study 1 was designed to test the mechanistic hypothesis that RPL554 could provide additional bronchodilation in combination with either a β_2 agonist or a muscarinic antagonist. This can be regarded as a "proof of pharmacology" study to investigate effects caused by different mechanisms of action alone or combined. This study was focused on peak FEV_1, and showed similar effects of RPL554, salbutamol and ipratropium administered alone, suggesting that RPL554 causes clinically relevant bronchodilation. The additional effects of RPL554 when combined with short acting bronchodilators provided mechanistic insights that PDE 3/4 inhibition, which increases cyclic AMP levels(7), can cause additional effects when combined with a beta agonist, which also acts through regulation of cyclic AMP levels(10), or a muscarinic antagonist. Study 1 therefore provided mechanistic support to investigate RPL554 further in combination with long acting bronchodilator treatment.

Tiotropium was chosen for study 2 as it is a commonly used long acting bronchodilator. On day 3, the magnitude of additional bronchodilation caused by RPL554 was 116 mL at trough (pre-dose) suggesting that twice a day RPL554 dosing provides sustained additional bronchodilation persisting for 12 hours post-dose. RPL554 6 mg had a greater effect on FEV_1 parameters than the 1.5 mg dose, and appears to be a suitable twice daily dose for further investigation. While study 2 demonstrated an additional effect of RPL554 when administered with a LAMA, it would also be relevant to investigate the effects of RPL554 administered with a LABA or a LAMA/LABA combination.

Hyperinflation and gas trapping are major causes of the sensation of dyspnoea, and improvements in lung volumes can improve dyspnoea and exercise performance(14). RV, a measurement of gas trapping, was reduced by RPL554 in both studies, suggesting a possible

effect of RPL554 on small airway function. The onset of bronchodilator action was also faster in both studies when RPL554 was used in combination. A faster onset of bronchodilation may be important to some patients in terms of providing symptom relief.

Pre-clinical studies using human isolated bronchial smooth muscle preparations have demonstrated that RPL554 added to other bronchodilators caused additional bronchodilation, with some evidence of synergistic effects when combined with a muscarinic antagonist (9, 10, 15). In our clinical trials, additional bronchodilation was observed when using RPL554 in combination with other bronchodilators. Whilst the RPL554 bronchodilator effects are likely to be mainly attributable to PDE3 inhibition, pre-clinical studies have suggested that PDE4 inhibition relaxes inherent tone in isolated human airway tissue (16, 17).

Roflumilast is an orally administered PDE4 inhibitor that reduces exacerbation rates, but the frequency of side effects, including nausea, weight loss and gastro-intestinal disturbance, limit its use in clinical practice (18, 19). RPL554 was well tolerated in these short term studies. Clinical trials with a longer duration and larger sample size are needed for proper safety evaluation. RPL554 has the potential for less side effects compared to orally administered PDE4 inhibitors due to reduced systemic exposure, although there may also be intrinsic differences in the pharmacological potential of these different molecules to cause side effects.

The limitations of the studies reported here include; (1) longer studies are needed to evaluate effects on key clinical endpoints including symptoms and exacerbations, and to properly evaluate safety; (2) the COPD patients included had evidence of reversibility (>150 mL) at screening, and the effects of RPL554 in broader population groups need to be studied (3) the effects of RPL554 in addition to combination treatments that are commonly used in clinical practice remains to be studied, such as LAMA/LABA or triple (inhaled corticosteroid plus LABA plus LAMA) combinations (4) while the anti-inflammatory effects of RPL554 have previously been demonstrated in the LPS challenge model in healthy volunteers (2), further studies of anti-inflammatory effects in COPD patients would be informative.

In conclusion, RPL554 provided additional bronchodilation, reduced gas trapping, improved airway conductance, and a more rapid onset of action when administered in combination with either a β_2 agonist or muscarinic antagonist. These short term bronchodilator studies provide support to further study RPL554 in longer term COPD studies focused on other endpoints including symptoms and exacerbations.

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Table 1. Demographics and baseline characteristics. Data are number or mean (SD). BMI=Body mass index; COPD = chronic obstructive pulmonary disease. FEV_1 = forced expiratory volume in 1s. ***** 30min post salbutamol ****** 30 min post salbutamol and ipratropium

	Study 1 (n=32)	**Study 2 (n=36)**
Male **Female**	21 11	19 17
Age (years)	62.4 (6.7)	61.3 (5.2)
Body-mass index (kg/m^2)	26.5 (3.9)	25.7 (3.1)
FEV_1 (%)	55.3 (8.5)	50.4 (12.2)
FEV_1 (L)	1.59 (0.4)	1.44 (0.5)
Bronchodilator reversibility (%)	16.3 (9.0) *****	** 17.4 (11.1)
Present smokers / ex smokers	14 / 18	24 / 12
Cigarette pack-years	50.6 (28.2)	47.1 (19.3)

Table 2. Statistical analysis of treatment effects in study 1. Diff = mean difference (L)

	Treatment difference; peak FEV_1			Treatment difference; FEV_1 average effect 0-8 hrs		
	Diff	**95% CI**	**p-value**	**Diff**	**95% CI**	**p-value**
Salbutamol vs Placebo	0.187	(0.142-0.232)	<0.001	0.123	(0.087-0.159)	<0.001
Ipratropium vs Placebo	0.199	(0.153-0.244)	<0.001	0.165	(0.128-0.201)	<0.001
RPL554 vs Placebo	0.223	(0.178-0.269)	<0.001	0.165	(0.133-0.206)	<0.001
Salbutamol/RPL554 vs Placebo	0.292	(0.250-0.340)	<0.001	0.235	(0.199-0.271)	<0.001
Ipratropium/RPL vs Placebo	0.292	(0.247-0.337)	<0.001	0.229	(0.192-0.265)	<0.001
RPL554 vs Salbutamol	0.039	(-0.009-0.081)	0.120	0.046	(0.010-0.082)	0.0136
RPL554 vs Ipratropium	0.024	(-0.021-0.070)	0.294	0.004	(-0.032-0.041)	0.8148
Salbutamol vs Ipratropium	-0.012	(-0.057-0.034)	0.614	-0.042	(-0.078-0.005)	0.0245
Salbutamol/RPL554 vs Salbutamol	0.108	(0.063-0.153)	<0.001	0.112	(0.076-0.148)	<0.001
Ipratropium/RPL554 vs Ipratropium	0.094	(0.049-0.139)	<0.001	0.064	(0.028-0.100)	0.0006

Table 3: Adverse events.

Treatment emergent adverse events reported by more than one patient are presented. Patients may have experienced the same adverse event following different study treatments

Study 1						
	Placebo N=31	RPL554 (6mg) N=31	Salbutamol (200µg) N=32	Salbutamol (200µg) + RPL554 (6mg) N=31	Ipratropium (40µg) N=32	Ipratropium (40µg) + RPL554 (6mg) N=33
Cough	4 (12.9%)	4 (12.9%)	7 (21.9%)	5 (16.1%)	2 (6.3%)	8 (24.2%)
Dyspnoea	0	2 (6.5%)	2 (6.3%)	0	1 (3.1%)	1 (3.0%)
Catheter site bruise	0	0	1 (3.1%)	1 (3.2%)	1 (3.1%)	1 (3.0%)
Dizziness	1 (3.2%)	0	2 (6.3%)	0	1 (3.1%)	1 (3.0%)
Headache	1 (3.2%)	1 (3.2%)	2 (6.3%)	0	0	0
Oropharyngeal pain	3 (9.7%)	0	0	1 (3.2%)	0	0
Rash	0	1 (3.2%)	2 (6.3%)	0	0	2 (6.1%)
Confusion	0	1 (3.2%)	1 (3.1%)	0	1 (3.1%)	1 (3.0%)
Back pain	0	0	2 (6.3%)	0	1 (3.1%)	0
Chronic Obstructive Pulmonary Disease	0	1 (3.2%)	0	0	2 (6.3%)	0
Diarrhoea	1 (3.2%)	1 (3.2%)	1 (3.1%)	0	0	0
Chest discomfort	1 (3.2%)	0	0	0	0	1 (3.0%)
Haematoma	1 (3.2%)	0	0	1 (3.2%)	0	0
Migraine	1 (3.2%)	0	0	1 (3.2%)	0	0
Nasopharyngitis	0	0	0	2 (6.5%)	0	0

Study 2						
	RPL554 1.5mg (N=29)		RPL554 6mg (N=27)		Placebo (N=28)	
System organ class preferred term	Events	Patients (%)	Events	Patients (%)	Events	Patients (%)
Total number of TEAEs	16	12 (41.4)	20	12 (44.4)	17	12 (42.9)
General disorders and administration site conditions	5	4 (13.8)	4	4 (14.8)	7	6 (21.4)
Medical device site reaction	4	4 (13.8)	1	1 (3.7)	4	4 (14.3)
Chest Discomfort	1	1 (3.4)	1	1 (3.7)	2	2 (7.1)
Nervous system disorders	2	2 (6.9)	8	5 (18.5)	3	3 (10.7)
Headache	2	2 (6.9)	8	5 (18.5)	3	3 (10.7)
Respiratory, thoracic and mediastinal disorders	3	3 (10.3)	3	3 (11.1)	3	2 (7.1)
Dyspnoea	1	1 (3.4)	1	1 (3.7)	3	2 (7.1)
Infections and infestations	1	1 (3.4)	1	1 (3.7)	3	2 (7.1)
Musculoskeletal and connective tissue disorders	2	2 (6.9)	2	2 (7.4)	0	0

Figure Legends

Figure 1: Overview of study designs

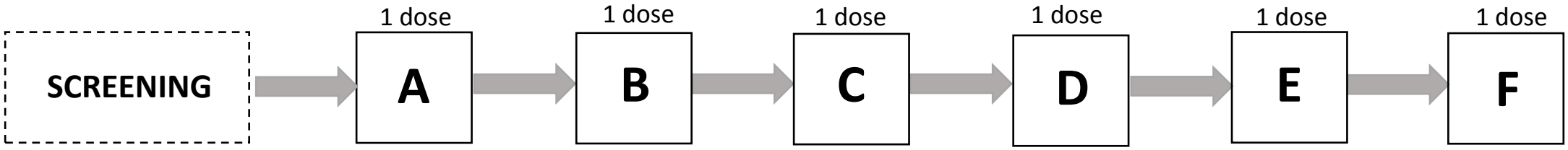
Figure 2: FEV_1 changes caused by RPL554, salbutamol (200µg) and ipratropium (40µg) alone and in combination in study 1 (single doses). (a) Peak FEV_1 (b) average $FEV_{1\ (0\text{-}8h)}$. * denotes $p<0.05$, ** denotes $p<0.01$. Mean and SEM shown

Figure 3: Residual volume (RV) and specific airway conductance (sGaw) changes caused by RPL554, salbutamol (200µg) and ipratropium (40µg) alone and in combination in study 1 (single doses). * denotes $p<0.05$, ** denotes $p<0.01$. Mean and SEM shown

Figure 4: Study 2 lung function on day 3. (a) The FEV_1 change from baseline (pre-dose on day 1) is shown. (b) Peak FEV_1 change from baseline (pre-dose on day 1) caused by tiotropium, tiotropium + RPL554 1.5 mg and tiotropium + RPL554 6 mg (c) The average $FEV_{1\ (0\text{-}12h)}$ change from baseline (pre-dose on day 1) caused by tiotropium, tiotropium + RPL554 1.5 mg and tiotropium + RPL554 6 mg * denotes $p<0.05$, ** denotes $p<0.01$. Mean and SEM shown.

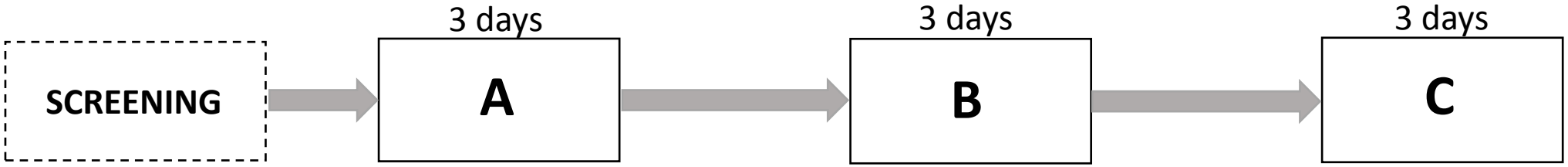
Figure 5: Residual volume (RV) and specific airway conductance (sGaw) changes on day 3 caused by tiotropium, tiotropium + RPL554 1.5 mg and tiotropium + RPL554 6 mg (study 2). The change from baseline (pre-dose on day 1) is shown. * denotes $p<0.05$, ** denotes $p<0.01$. Mean and SEM shown

Study 1

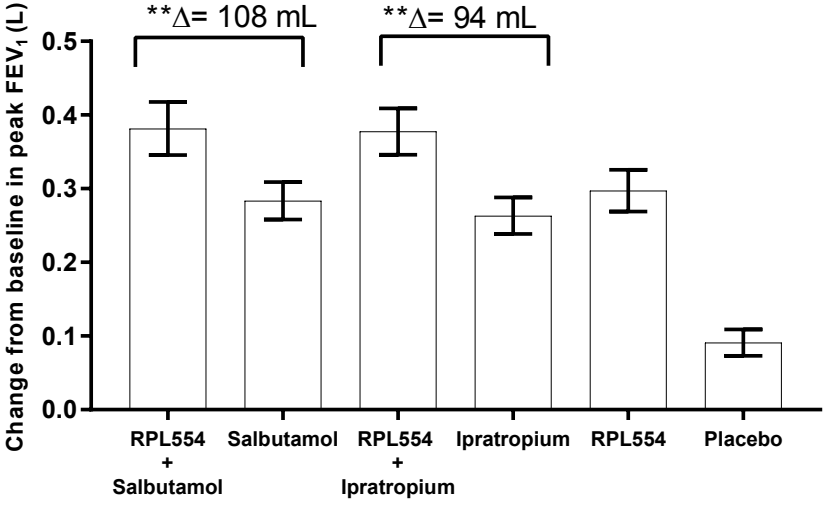


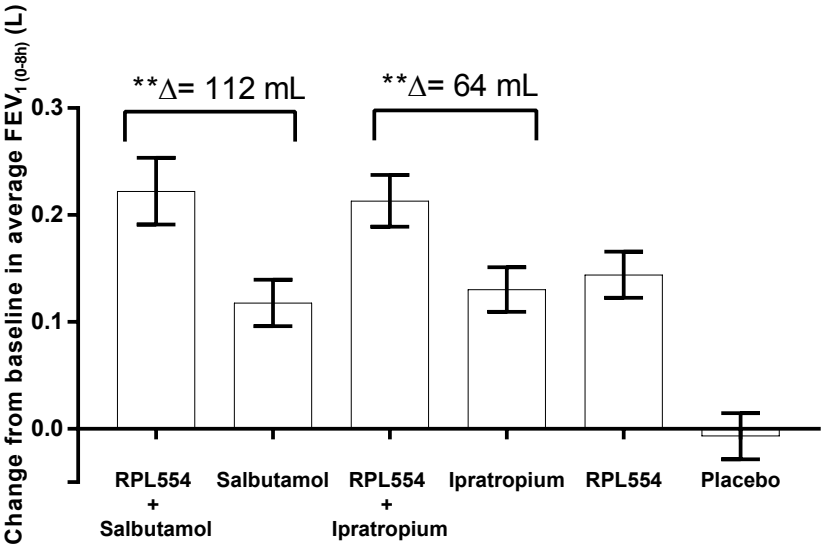
Randomised treatments (A – F) are:-
RPL554 (6mg), Salbutamol, Ipratropium, RPL554 + Salbutamol, RPL554 + Ipratropium, Placebo

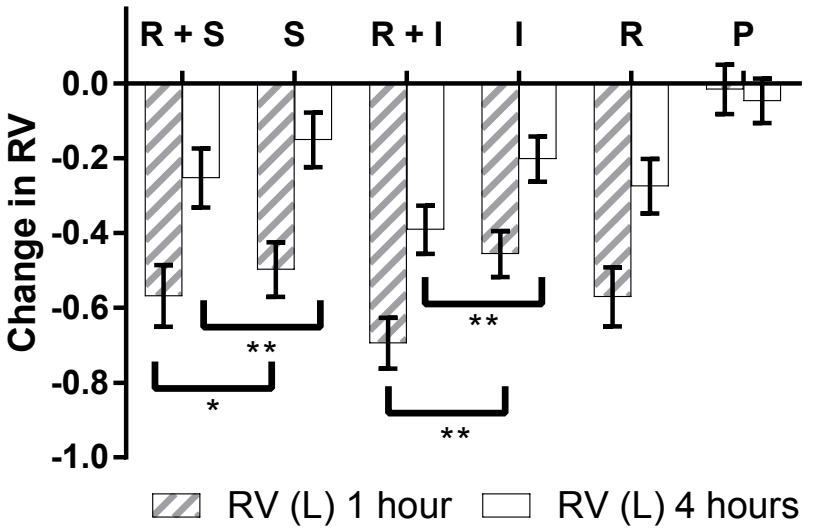
Study 2

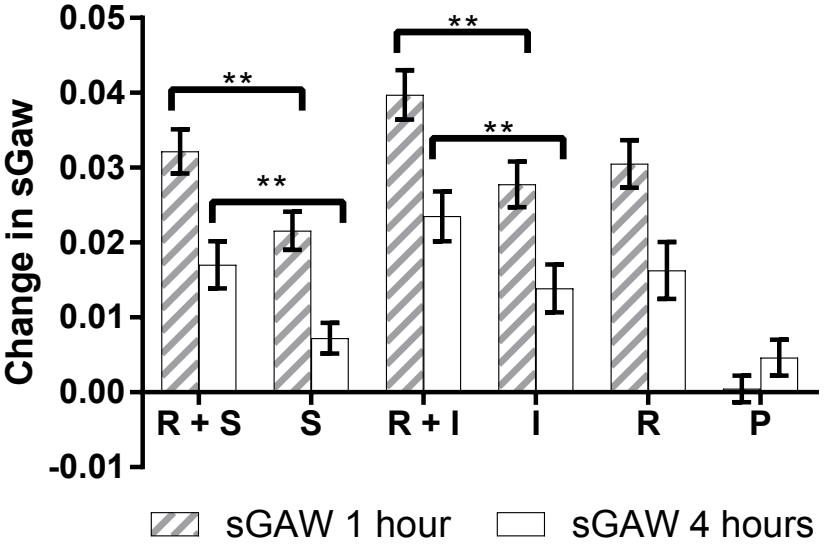


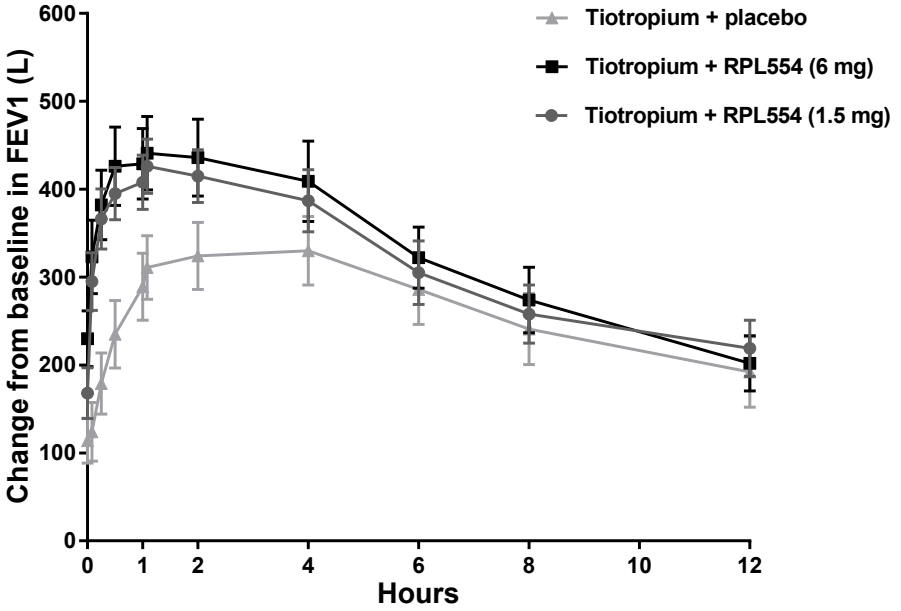
Randomised treatments (A – C) are:-
Once daily Tiotropium 18 µg and twice daily (i) Placebo or (ii) RPL554 or (iii) RPL554 6mg

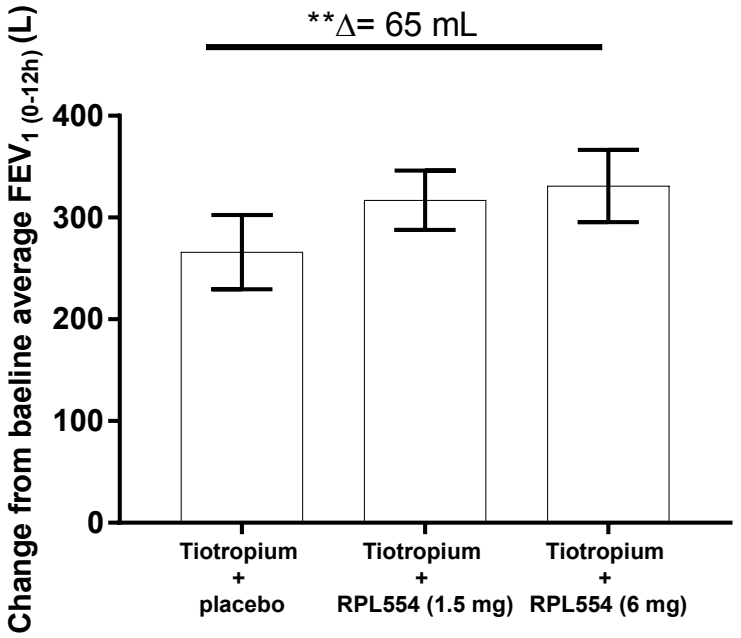

**Δ= 108 mL
**Δ= 94 mL
Change from baseline in peak FEV1 (L)
0.5
0.4
0.3
0.2
0.1
0.0
RPL554 + Salbutamol
Salbutamol
RPL554 + Ipratropium
Ipratropium
RPL554
Placebo

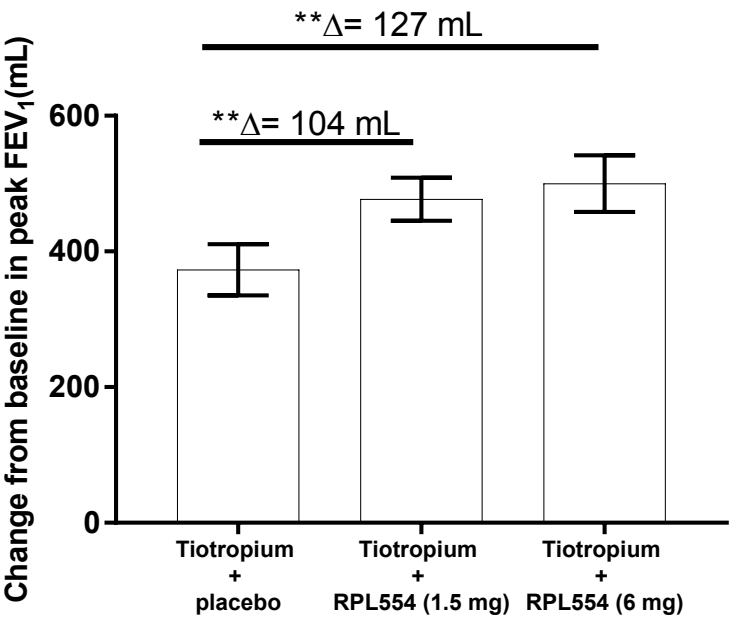

**Δ= 112 mL
**Δ= 64 mL
Change from baseline in average FEV1 (0-8h) (L)
0.3
0.2
0.1
0.0
RPL554 + Salbutamol
Salbutamol
RPL554 + Ipratropium
Ipratropium
RPL554
Placebo

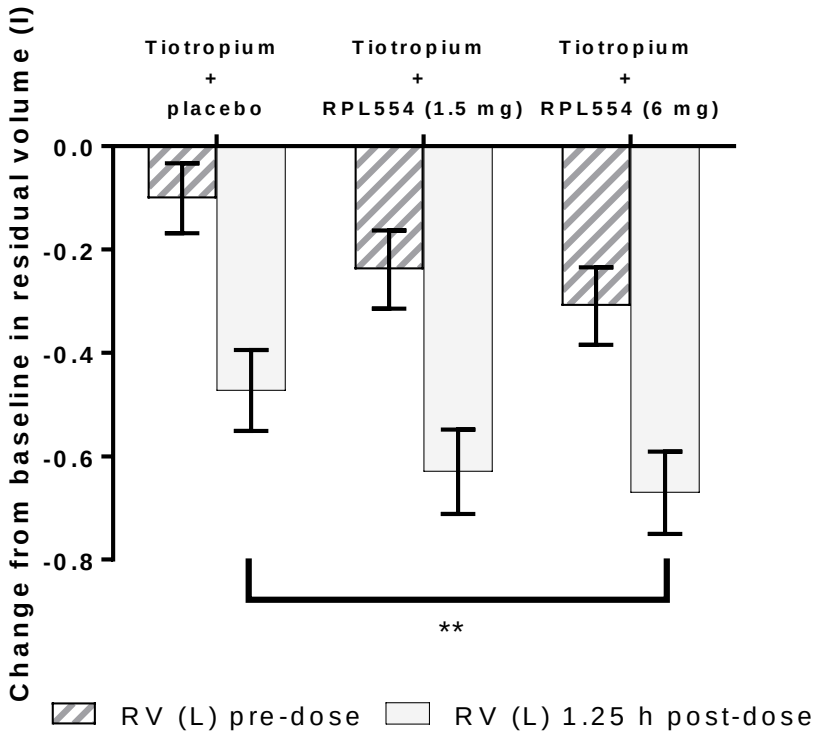

R + S
S
R + I
I
R
P
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Change in RV
**
*
**
**
RV (L) 1 hour
RV (L) 4 hours

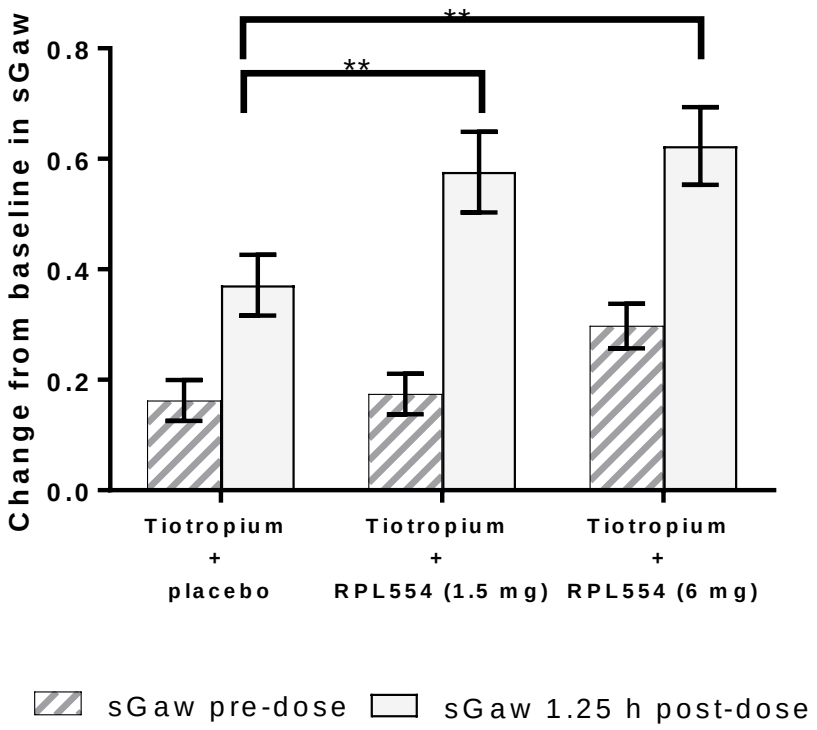

0.05
0.04
0.03
0.02
0.01
0.00
-0.01
Change in sGaw
**
**
**
**
R + S
S
R + I
I
R
P
sGAW 1 hour
sGAW 4 hours


Tiotropium + placebo
Tiotropium + RPL554 (6 mg)
Tiotropium + RPL554 (1.5 mg)
Change from baseline in FEV1 (L)
600
500
400
300
200
100
0
0
1
2
4
6
8
10
12
Hours


**Δ= 65 mL
Change from baeline average FEV1 (0-12h) (L)
400
300
200
100
0
Tiotropium + placebo
Tiotropium + RPL554 (1.5 mg)
Tiotropium + RPL554 (6 mg)


**Δ= 127 mL
**Δ= 104 mL
Change from baseline in peak FEV1(mL)
600
400
200
0
Tiotropium + placebo
Tiotropium + RPL554 (1.5 mg)
Tiotropium + RPL554 (6 mg)


Change from baseline in residual volume (l)
Tiotropium + placebo
Tiotropium + RPL554 (1.5 mg)
Tiotropium + RPL554 (6 mg)
0.0
-0.2
-0.4
-0.6
-0.8
**
RV (L) pre-dose
RV (L) 1.25 h post-dose


Change from baseline in sGaw
0.8
0.6
0.4
0.2
0.0
**
**
Tiotropium + placebo
Tiotropium + RPL554 (1.5 mg)
Tiotropium + RPL554 (6 mg)
sGaw pre-dose
sGaw 1.25 h post-dose

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

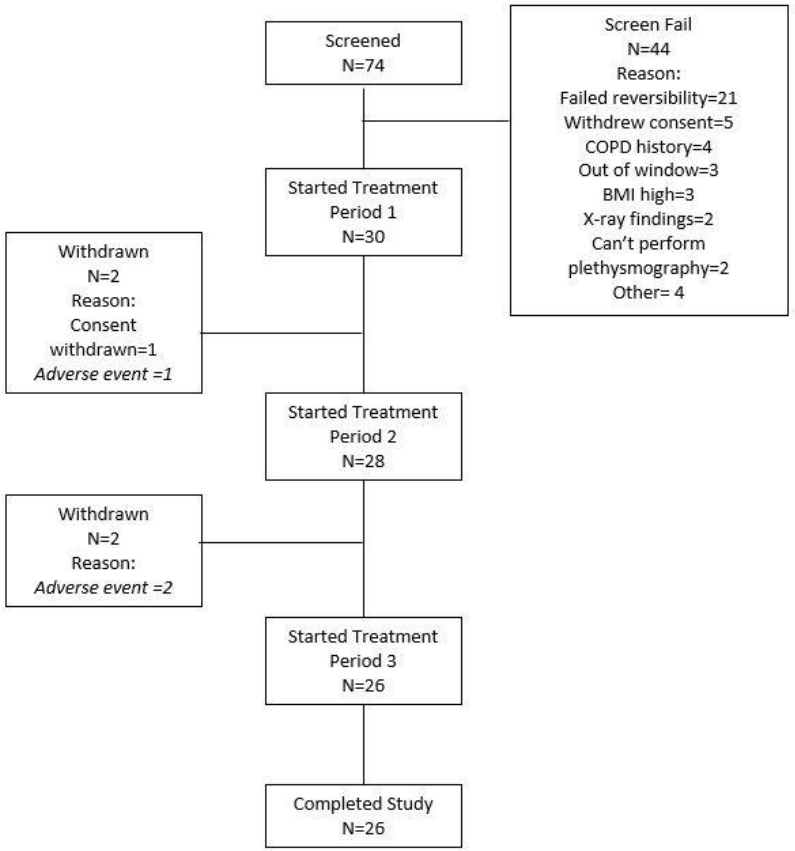
Table S3. Change from Baseline in Total Lung Capacity and Functional Residual Capacity in Study 1.

		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)

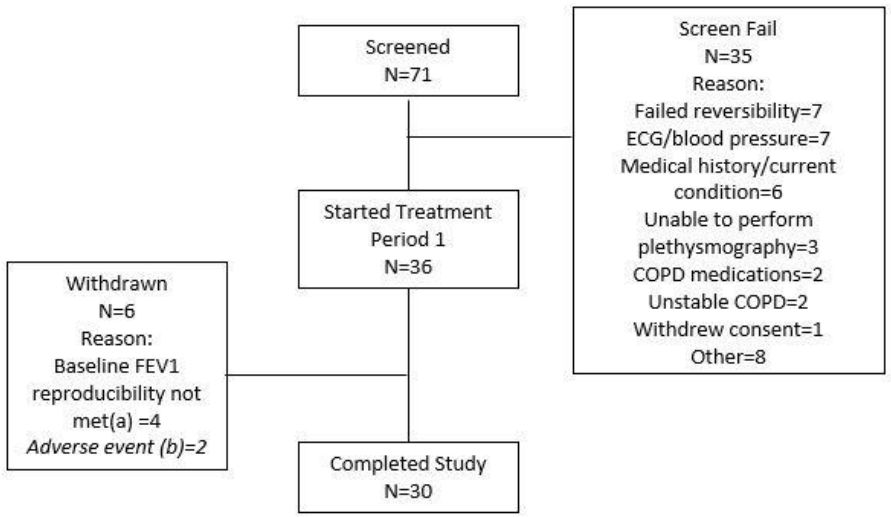
Abbreviations: TLC: Tidal Lung Capacity; FRC Functional Residual Capacity

Study 1: Flow of patients



Abbreviations:
BMI=body mass index
COPD=chronic obstructive pulmonary disorder

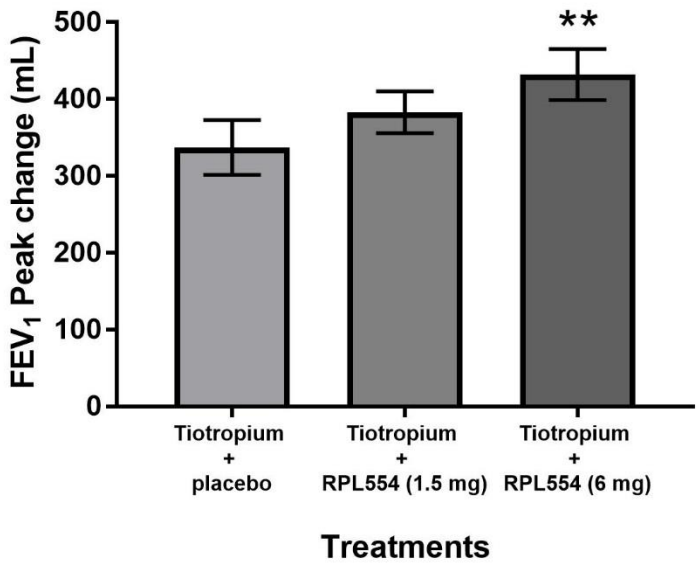
Study 2: Flow of patients

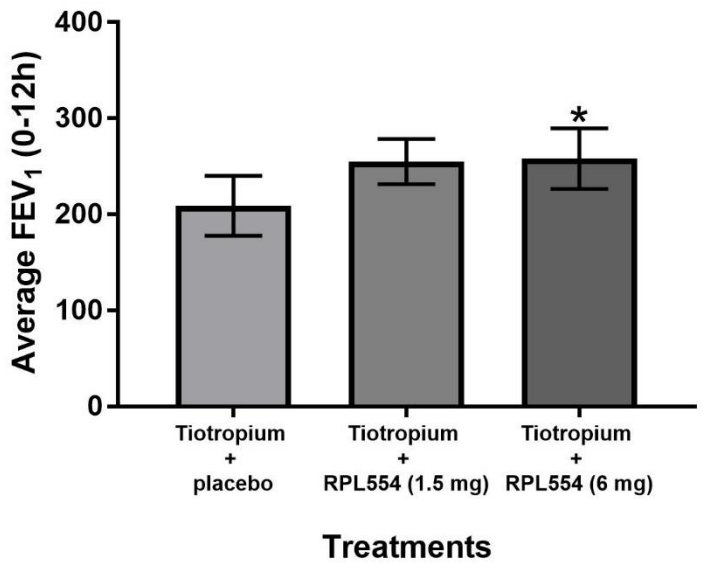


Abbreviations:
COPD=chronic obstructive pulmonary disorder
ECG= electrocardiogram
FEV1=forced expired volume in 1 second

(a) Pre-dose FEV1 not within 15% of the Visit 2 value at Visit 3 to 7 (to ensure consistent baseline for all treatments)
(b) Both were COPD exacerbations

Study 2: Day 1 FEV_1 changes. * denotes p<0.05, ** denotes p<0.01 for comparisons versus placebo.





Study 2: Day 3 FVC changes. * denotes p<0.05, ** denotes p<0.01 for comparisons versus placebo.