CLINICAL STUDY PROTOCOL

Protocol Title:	A Phase IIb, randomized, double-blind, placebo-controlled study to assess the efficacy, safety, and pharmacokinetics of glycopyrrolate inhalation solution over 1 week in subjects with COPD
Protocol Number:	RPL554-CO-211
Version:	3.0
Investigational Product:	glycopyrrolate
Short Title:	A Phase IIb study of glycopyrrolate inhalation solution over 1 week in subjects with COPD
Study Phase:	IIb
Sponsor Name:	Verona Pharma plc
Legal Registered Address:	3 More London Riverside London, SE1 2RE UK
Regulatory Agency Identifying Number(s):	US IND Number: 170330
Date of Protocol:	14 October 2024

Sponsor Signatory:

I have read this protocol in its entirety and agree with the content:

[redacted] **Date**

Medical Monitor name and contact information can be found in Appendix 2.

PROTOCOL AMENDMENT SUMMARY OF CHANGES

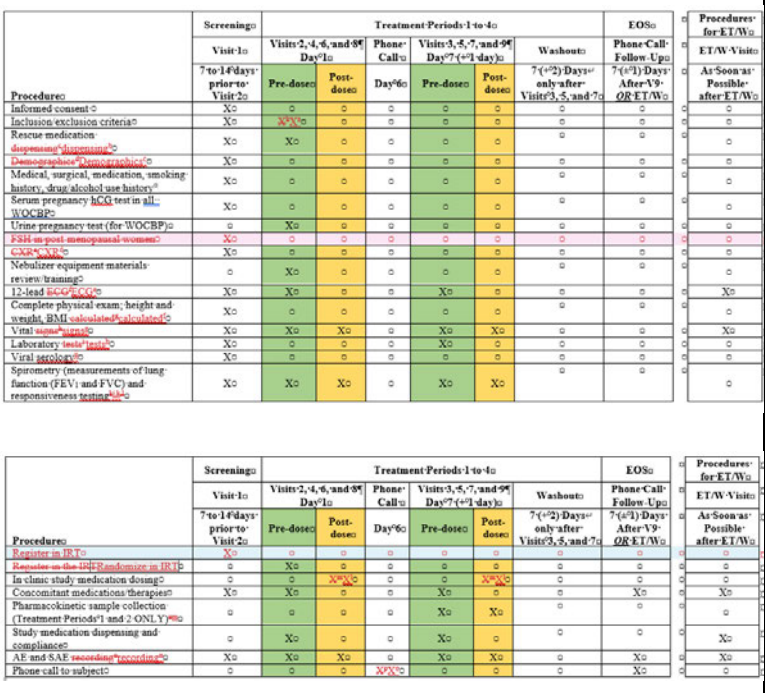
Document History

Protocol Version	Amendment	Date	Substantial – Timing	Region
Version 1.0	Original Protocol	12 March 2024	Original Protocol	Global
Version 2.0	Amendment 1.0	6 June 2024	Yes – prior to initial regulatory submission	Global
Version 3.0	Amendment 2.0	14 October 2024	No	Global

Protocol Version 3.0, Amendment 2.0: 14 October 2024 Overall Rationale for the Amendment

The protocol is amended to address:

1. Minor administrative items (spelling, punctuation, spaces, and table numbering).
2. Clarifications.
3. Clarify procedures to address public emergencies and natural disasters.

Section Number	Change	Brief Rationale
Synopsis: Overall Design & Sec. 4.1	Each subject is expected to complete all 4 Treatment Periods. Subjects who do not complete at least 2 Treatment Periods may be replaced.	Clarifying plan to replace subjects who do not complete at least 2 Treatment Periods.
Synopsis: Overall Design	During the treatment period, subjects will complete all assessments and procedures as outlined in the Schedule of Activities (Section 1.3) for approximately ~~24~~ 10 weeks for each subject.	Corrected study duration.
Synopsis: Number of Investigators and Study Sites	Approximately ~~67~~ Investigators and study centers are expected to participate in this study.	Increased estimated site number.
SoA Sec. 1.3		Updates for consistency and clarity. Added line to clarify timing of registration in IRT versus randomization in IRT. Removed collection of FSH for consistency with Appendix 3 and Appendix 7.

Procedure	Screening	Treatment Periods 1 to 4						EOS	Procedures for ET/W
	Visit 1	Visits 2, 4, 6, and 8 Day 1		Phone Call	Visits 3, 5, 7, and 9 Day 7 (+/-1 day)		Washout	Phone Call Follow-Up	ET/W Visits
	7 to 14 days prior to Visit 2	Pre-dose	Post-dose	Day 6	Pre-dose	Post-dose	7 (+/-2) Days only after Visits 3, 5, and 7	7 (+/-1) Days After V9 OR ET/W	As Soon as Possible after ET/W
Informed consent	X								
Inclusion/exclusion criteria	X	~~X~~							
Rescue medication dispensing/dispensing	X	X							
~~Demographics~~/Demographics	X								
Medical, surgical, medication, smoking history, drug/alcohol use history	X								
Serum pregnancy hCG test in all WOCBP	X								
Urine pregnancy test (for WOCBP)		X							
~~FSH in post-menopausal women~~	~~X~~								
~~CXR~~/CXR	X								
Nebulizer equipment materials review/training		X							
12-lead ~~ECG~~/ECG	X	X			X				X
Complete physical exam; height and weight, BMI ~~calculated~~/calculated	X								
Vital ~~signs~~/signs	X	X	X		X	X			X
Laboratory ~~tests~~/tests	X				X				
Viral serology	X								
Spirometry (measurements of lung function (FEV1 and FVC) and responsiveness testing~~b~~	X	X	X		X	X			

Procedure	Screening	Treatment Periods 1 to 4						EOS	Procedures for ET/W
	Visit 1	Visits 2, 4, 6, and 8 Day 1		Phone Call	Visits 3, 5, 7, and 9 Day 7 (+/-1 day)		Washout	Phone Call Follow-Up	ET/W Visits
	7 to 14 days prior to Visit 2	Pre-dose	Post-dose	Day 6	Pre-dose	Post-dose	7 (+/-2) Days only after Visits 3, 5, and 7	7 (+/-1) Days After V9 OR ET/W	As Soon as Possible after ET/W
~~Register in IRT~~	X								
~~Register in the IRT~~/Randomize in IRT		X							
In-clinic study medication dosing			~~X~~/X			~~X~~/X			
Concomitant medications/therapies	X	X			X			X	X
Pharmacokinetic sample collection (Treatment Periods 1 and 2 ONLY)					X	X			
Study medication dispensing and compliance		X			X				X
AE and SAE ~~recording~~/recording	X	X			X	X		X	X
Phone call to subject				~~X~~/X				X	

Study Treatment Sec. 6.0	Subjects will receive double-blind study medication (either glycopyrrolate or placebo) in the proposed FDC formulation administered by inhalation via a standard jet nebulizer (Section 6.2.2) supplied by the Sponsor. To account for visit windows, sufficient drug supply will be dispensed for up to 11 days of treatment for each treatment period.	Added language to clarify amount of drug dispensed.
Subject Discontinuation Sec. 7.3	Subjects who require an extended washout period > 28 days will be withdrawn from the study.	Added language to clarify withdrawal criteria.
Study Assessments Sec. 8.0	Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct. In the event a public emergency or natural disaster impacts a subject's ability to complete study requirements, refer to Section 8.9.	Added reference to new section for emergencies.
Emergency Procedures Sec. 8.9	Sufficient study drug is provided to enable up to 11 days of dosing in a given treatment period. In the event of a public emergency or natural disaster, the dosing interval may be extended up to 11 days to allow at least 6 consecutive days of dosing prior to the End of Treatment Period Visit (nominal Day 7 Visit) which may occur between Day 7 and Day 11. If a public emergency or natural disaster results in a subject being unable to complete at least 6 consecutive days of dosing prior to the End of Treatment Period Visit (nominal Day 7 visit which may occur between Days 7 and 11), the treatment period should be repeated with the repeat Day 1 visit occurring at least 7 days after the last administered dose of blinded study medication. In the event of a public emergency or natural disaster, the 7-day washout period may be extended up to 28 days. Subjects requiring a washout period > 28 days will be withdrawn from the study.	New section to address public health emergencies and natural disasters.

Protocol Version 2.0, Amendment 1.0: 06 June 2024 Overall Rational for the Amendment

The protocol is amended to address:

1. Minor administrative items (spelling, punctuation, spaces, and Table numbering).
2. Clarifications.
3. Allow use of LABA ± ICS BID as background therapy during the study.

4. Remove requirement to discontinue any COPD medications for study entry.
5. Remove the interim analysis.
6. Remove the inclusion criteria around FEV_1 responsiveness requirements.
7. Add a cap to the proportion of subjects with low responsiveness to albuterol.
8. Change timeframe for the exclusion criteria around COPD symptoms.
9. Add COPD withdrawal criteria.

<table>
<tr><th>Section Number</th><th>Change</th><th>Brief Rationale</th></tr>
<tr><td>Synopsis: Rationale & Sec 2.2.3</td><td>It has ~~similar~~ affinity to the subtypes of muscarinic receptors M1 to M5, and higher affinity for M3 over M2.</td><td>Clarifying data on glycopyrrolate</td></tr>
<tr><td>Synopsis: Objectives & Sec 3.1.2 & multiple other locations</td><td>• To evaluate the bronchodilator effect of BID inhaled glycopyrrolate solution in the proposed FDC formulation on Day 7 peak FEV_1, average FEV_1 area under the curve versus time from time 0 to 4 hours ($AUC_{0\text{-}4h}$), average FEV_1 $AUC_{0\text{-}12h}$, and evening trough FEV_1.</td><td>Clarification</td></tr>
<tr><td>Synopsis: Overall Design & 4.1</td><td><table><tr><th>Dose Level</th><th>Blinded Study Medication</th></tr><tr><td>A</td><td>Glycopyrrolate 85 µg BID</td></tr><tr><td>B</td><td>Glycopyrrolate 42.5 µg BID</td></tr><tr><td>C</td><td>Glycopyrrolate 14 µg BID</td></tr><tr><td>D</td><td>Placebo BID</td></tr></table>~~Dose Arm A: glycopyrrolate (85 µg) BID~~
~~Dose Arm B: glycopyrrolate (42.5 µg) BID~~
~~Dose Arm C: glycopyrrolate (14 µg) BID~~
~~Dose Arm D: placebo BID~~</td><td>Clarification putting text in table</td></tr>
<tr><td>Synopsis: Overall Design & 4.1</td><td>Subjects will be randomly assigned to Treatment Sequences 1 to 4 in a 1:1:1:1 ratio to receive the 4 dose levels in the following sequences:<table><tr><th rowspan="2">Treatment Sequence</th><th colspan="4">Dose Levels Administered in Each Treatment Period</th></tr><tr><th>Treatment Period 1 (N = 40)</th><th>Treatment Period 2 (N = 40)</th><th>Treatment Period 3 (N = 40)</th><th>Treatment Period 4 (N = 40)</th></tr><tr><td>Sequence 1 (N = ~10)</td><td>A</td><td>D</td><td>B</td><td>C</td></tr><tr><td>Sequence 2 (N = ~10)</td><td>B</td><td>A</td><td>C</td><td>D</td></tr><tr><td>Sequence 3 (N = ~10)</td><td>C</td><td>B</td><td>D</td><td>A</td></tr><tr><td>Sequence 4 (N = ~10)</td><td>D</td><td>C</td><td>A</td><td>B</td></tr></table>~~receive the 4 dose levels in 1 of 4 dosing sequences:~~
~~Sequence 1: A:D:B:C~~
~~Sequence 2: B:A:C:D~~
~~Sequence 3: C:B:D:A~~
~~Sequence 4: D:C:A:B~~</td><td>Clarifying text</td></tr>
</table>

Synopsis: Overall Design & 4.1	Screening for eligibility will include a ~~reversibility~~ responsiveness test (spirometry both pre- and post~~--~~4 puffs of albuterol) performed between 7 and 14 days before the first dose of blinded study medication. The proportion of the study population who have a post-albuterol FEV_1 increase of < 12% or < 200 mL will be capped at approximately 50%.~~To be eligible, subjects must have a ≥ 150 mL increase in FEV_1 with albuterol. Rescreening is allowed once for a screen failure due to Inclusion Criterion #6: ≥ 150 mL increase from pre-bronchodilator FEV_1, if the subject that screen failed was within 10% (15 mL) of this threshold.~~	Remove the inclusion criteria around FEV_1 responsiveness requirements & add a cap to the proportion of subjects with low responsiveness to albuterol
Synopsis: Overall Design & 4.1	~~Eligible subjects will have discontinued long-acting Some~~Prohibited COPD medications ~~are prohibited for the time periods~~prior to and during the study are described in Table 8 ~~prior to Visit 2~~. ~~It is not recommended that subjects discontinue~~ Discontinuation of any maintenance COPD therapies for the sole purpose of ~~entering this study~~study eligibility is not recommended.	Remove requirement to discontinue any COPD medications for study entry
Synopsis: Overall Design & 4.1	~~U~~Stable use of BID LABA ± inhaled corticosteroids (ICS) at a maintenance dose is ~~allowed under certain cond~~permitted during the study~~itions~~ (Table 7).	Allow use of LABA ± ICS BID as background therapy during the study
Synopsis: Overall Design & 4.1	At ~~the~~ Screening (Visit 1), all subjects will be dispensed albuterol as rescue medication to be used as needed during the screening, treatment, and washout periods. ~~–~~Subjects who are required to withhold medications prior to Screening (Visit 1) ~~for a washout period~~ will be dispensed albuterol as rescue medication upon signing of the Informed Consent Form (ICF) for use prior to Visit 1.	Remove requirement to discontinue any COPD medications for study entry
Synopsis: Overall Design & 4.1	Subjects ~~will~~ should withhold rescue medication use for ≥ 4 hours and, if applicable, BID LABA ± ICS use for approximately 24~~≥ 12~~ hours prior to spirometry at all required visits. ~~If~~ If the rescue medication ~~this~~ withholding period is not met~~,~~ or if BID LABA ± ICS medications are taken the morning of a clinic visit where spirometry is conducted, the subject will be rescheduled for a repeat visit within 7 or 3 days of Day 1 or Day 7, respectively.	Add withholding requirement for background LABA/ICS prior to spirometry.
Synopsis: Overall Design & 4.1	Subjects will be screened for eligibility during a screening period of 7 to 14 days. ~~Prohibited medications and the prohibited time intervals prior to Screening are described in Section .~~	Remove redundant language
Synopsis: Overall Design & 4.1	~~Subjects will complete all assessments and procedures as outlined in the Schedule of Activities (SoA;).~~ During the treatment period, subjects will complete all assessments and procedures as outlined in the Schedule of Activities (Section 1.3) for 24 weeks. Subjects completing treatment will complete a Follow-Up Phone Call 7 ± 1 days after the last scheduled study visit.	Clarification
Synopsis: Overall Design	Subjects who experience ≥ 1 moderate or severe COPD exacerbation~~s~~ during the study will discontinue study medication and be withdrawn from the study.	Addition of COPD withdrawal criteria

Synopsis: Overall Design & 4.1	Subjects who permanently discontinue double-blind study medication will be withdrawn from the study. Subjects meeting the withdrawal criteria during the study or who withdraw from the study for other reasons will be discontinued and requested to complete the Early Termination/Withdrawal Procedures and complete a Follow-Up Phone Call 7 ± 1 days after the Early Termination/Withdrawal Visit.~~Subjects completing treatment will complete a Termination Visit 7 ± 1 days after the last dose of blinded study medication. Subjects who discontinue blinded study medication, meet the withdrawal criteria during the study, or who withdraw from the study for other reasons will be discontinued from the study and requested to complete Early Withdrawal Visit 7 ± 1 days after the last dose of blinded study medication.~~ ~~Subjects who permanently discontinue double-blind study medication will be withdrawn from the study.~~	Clarification
Synopsis: Inclusion Criteria & Sec 5.1	6. Post-bronchodilator (4 puffs of albuterol) spirometry at Screening demonstrating the following: • FEV_1/forced vital capacity (FVC) ratio of < 0.70 • FEV_1 ≥ ~~40~~ 30 % and ≤ ~~80~~70% of predicted normal (Quanjer et al. 2012) ~~≥ 150 mL increase from pre-bronchodilator FEV_1~~ ***Note:*** *The proportion of the study population who have a post-albuterol FEV_1 increase of < 12% or < 200 mL will be capped at approximately 50%.*	Remove FEV_1 responsiveness criterion, change severity criterion, and add cap on low-responsive subjects.
Synopsis: Inclusion Criteria & Sec 5.1	8.0 Capable of withdrawing from ~~long-acting bronchodilators for the duration of the study and~~ short-acting bronchodilators for 4 hours prior to spirometry testing and from BID LABA ± ICS therapy for 24 hours prior to spirometry.~~.~~	Remove withdrawal of COPD meds and add requirement to withhold LABA/ICS prior to spirometry.
Synopsis: Exclusion Criteria & Sec 5.2	2.0 Within ~~3~~ 6 months prior to Screening: a. COPD exacerbation requiring hospitalization. b. Use of therapies for COPD exacerbation (e.g., oral, intravenous, or intramuscular glucocorticoids).	Change timeframe for the exclusion criteria around COPD symptoms.
Synopsis: Exclusion Criteria & Sec 5.2	~~1.~~18. Severe comorbidities including unstable cardiac, (e.g., myocardial infarction within 1 year prior to screening, unstable angina within 6 months prior to screening, or unstable or life-threatening arrhythmia requiring intervention within 3 months prior to screening, diagnosis of NYHA class III or IV heart disease) or any other clinically significant medical conditions including uncontrolled diseases (e.g., endocrine, neurological, hepatic, gastrointestinal, renal, hematological, urological, immunological, psychiatric or ophthalmic diseases) that would, in the opinion of the Investigator, preclude the subject from safely completing the required tests or the study, or is likely to result in disease progression that would require withdrawal of the subject.	Change the order of the Exclusion Criteria for clarity.
Synopsis: Treatment Groups and Duration	All blinded study medications will be double-blind and administered using the inhaled route via a standard jet nebulizer supplied by the Sponsor. Nebulization time should be approximately 5 to 10 minutes. Subjects taking maintenance BID LABA ± ICS medications should take the LABA ± ICS medication prior to taking blinded study medication when taking the blinded study medication at home.	Provide guidance on when to dose LABA/ICS

<table>
<tr><td>Synopsis: Statistical Methods & Sec 9.6</td><td>~~A Data Assessment Committee (DAC) will perform an unblinded interim analysis either when approximately 18 subjects have completed Visit 9 (Day 7 of Treatment Period 4) OR when approximately 36 subjects have completed Visit 5 (Day 7 of Treatment Period 2), whichever occurs earliest. This interim analysis will analyze available FEV_1, PK, and safety data to inform glycopyrrolate dose selection for a subsequent fixed dose combination study of glycopyrrolate and ensifentrine (Study CO-212). The interim analysis will not impact study integrity, completion, change subject recruitment, or lead to design changes for this study. Unblinded data and interim results will not be shared outside the DAC~~No interim analysis is planned.</td><td>Remove interim analysis</td></tr>
<tr><td>Sec 1.3</td><td><table>
<tr><th></th><th>Screening</th><th colspan="5">Treatment Periods 1 to 4</th><th>EOS</th><th>Procedures for ET/~~E~~W/~~T~~ ET/W/~~T~~ Visit</th></tr>
<tr><th></th><th>Visit 1</th><th colspan="2">Visits 2, 4, 6, and 8 Day 1</th><th>Phone Call</th><th colspan="2">Visits 3, 5, 7, and 9 Day 7 (+ 1 day)</th><th>Washout</th><th>Phone Call Follow-Up</th><th></th></tr>
<tr><th>Procedure</th><th>7 to 10 14 days prior to Visit 2</th><th>Pre-dose</th><th>Post-dose</th><th>Day 6</th><th>Pre-dose</th><th>Post-dose</th><th>7 (+ 2) Days only after Visits 3, 5, and 7</th><th>7 (± 1) Days After V9 OR ET/W</th><th>As Soon as Possible after ET/W/T</th></tr>
<tr><td>Informed consent</td><td>X</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>~~Informed consent,~~ Inclusion/exclusion criteria</td><td>X</td><td>X[b]</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Rescue medication dispensing[g]</td><td>X</td><td>X</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Demographics[f]</td><td>X</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Medical, surgical, medication, smoking history, drug/alcohol use history</td><td>X</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Serum pregnancy hCG test in all WOCBP~~women of child-bearing potential~~</td><td>X</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table></td><td>Edits for Clarity</td></tr>
</table>

Sec 1.3	a. Check subject's use of ~~check~~ concomitant medications, subject's ~~for~~use of prohibited medications, subject's ~~washout~~ withholding compliance, and that there are no clinically relevant changes in health status that, in the opinion of the investigator, would prohibit the subject from completing the ~~treatment~~ Treatment ~~p~~Period. b. Study rescue medication should be dispensed once the subject has signed the ICF for subjects that require a ~~wash out~~withholding period prior to Screening (Visit 1). c. Demographics to include (date of birth, age, gender, race, ethnicity). d. A posterior-anterior CXR at Screening or within 12 months prior to Screening showing no clinically significant abnormalities unrelated to COPD is required. If a CXR within the past 12 months is not available but a CT scan within the same time period is available, the CT scan may be reviewed in place of a CXR. e. ECGs should be obtained before all other simultaneously scheduled procedures are completed. f. Complete physical exam at Screening ~~and on Day 1 pre-dose~~ to ~~include:~~include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (spleen and liver), lymph nodes and extremities. Findings of the physical exam should be recorded in the subjects medical history. g. Vital signs: (pulse rate, blood pressure) will be at ~~screening~~Screening, pre-dose, 1 hour and 4 hours post-dose on Days 1 and~~,~~ Day 7~~,~~ and at Early Withdrawal/Termination Visit ~~(pre-dose)~~. ~~h.~~ The laboratory test to be performed are specified in Table 11.~~Hematology: WBC with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), RBC, Hg, HCT, and platelet count.~~ i. ~~Chemistry: BUN, creatinine, glucose, GGT, AST, ALT, ALP, creatine kinase, magnesium, potassium, sodium, chloride, amylase, total, indirect and direct bilirubin, total protein, phosphate, carbon dioxide (bicarbonate), calcium, lactase dehydrogenase, albumin, uric acid, triglycerides, eGFR (CK/MB fraction and conjugated and unconjugated bilirubin (if clinically indicated).~~ j. The serology test to be performed are specified in Table 11~~Hepatitis B serology (markers include HBsAg, anti-HBs, anti-HBc, and others) and hepatitis C virus antibody.~~. k. On Days 1 and 7 of each Treatment Period, ~~pre-dose~~ spirometry will be ~~to be~~ ceonducted ~~pre-dose at 2 separate time points within 40 min pre-dose on and post-dose spirometry at 30 min, 1 h, 1.5 h, 2 h, and 4 h post dose. On Day 7 of each Treatment Period, pre-dose spirometry to be conducted pre-dose at one pre-dose timepoint approximately 12 hours following the prior evening dose and post-dose spirometry at 30 min, 1, 2, 4, 6, 8, and 12 hours post dose~~ (as specified in Table 2~~)~~. l. Responsiveness testing only conducted at the Screening Visit. m. In clinic dosing performed after pre-dose spirometry and pre-dose PK sampling in the morning~~,. and after completion of the 12-hour spirometry timepoint in the evening.~~ n. PK sample collection times on Day 7 of Treatment Periods 1 and 2 ~~only are as follows: pre-dose (within 30 minutes prior to dosing) and post-dose at 10 ± 4, 20 ± 4, and 40 ± 4 minutes post-dose and at 1, 1.5, 2, 4, 8, and 12 hours (all later timepoints at ± 15 minutes) post-dose~~are specified in ~~(~~Table 3~~)~~. PK samples should be taken after all concomitantly scheduled assessments (e.g., ECG, FEV_1). o. AEs will be collected Day 1 through the EOS. SAE~~'~~s related to study participation will be recorded from the time the subject consents until study discharge. p. Phone call reminder to subjects to remind them to take their evening dose approximately 12 hours prior to the anticipated arrival time to the clinic on Day 7, to note the time of the Day 6 evening dose was taken and to report that time to site staff at the Day 7 visit, to withhold the Day 7 morning dose until at the clinic, to withhold albuterol within 4 hours of their Day 7 visit, to withhold, if applicable, BID LABA ± ICS use within approximately 24 hours of their Day 7 visit, and to bring their unused study medication to the clinic visit the next day.	Remove washout of COPD meds and clarify text.
Sec 1.3	Abbreviations: AE = adverse event; ~~ALT = alanine aminotransferase; ALP = alkaline phosphatase; anti-HBc = total hepatitis B core antibody; anti-HBs = hepatitis B surface antibody; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CK = creatine kinase;~~ COPD = Chronic Obstructive Pulmonary Disease; CT = Computed Tomography; CXR = Chest X-Ray; ECG = Electrocardiogram; ~~eGFR = estimated glomerular filtration rate;~~ EOS = End of Study; ET/W~~T~~ = Early Termination/Withdrawal~~Termination~~; FEV_1 = forced expiratory volume over 1 second; FSH = follicle-stimulating hormone; FVC = forced vital capacity; ~~GGT = gamma-glutamyl transpeptidase; HBsAg = hepatitis B surface antigen;~~ hCG = human chorionic gonadotropin; ICF = informed consent form; IRT = Interactive Response Technology; ~~HCT = hematocrit; Hg = hemoglobin; MB = myocardial band;~~ PK = pharmacokinetics; ~~RBC = red blood cell;~~ SAE = serious adverse events; ~~WBC = white blood cell;~~ WOCBP = women of childbearing potential.	Clarify text.

Sec 1.4	Note: Additional details on information contained in this table can be found in Section 6.0 and Section 8.3.1. Rescue medications should have been withheld for ≥ 4 hours prior to spirometry and, if applicable, BID LABA ± ICS medications should have been withheld for approximately 24 hours prior to spirometry.	Add withholding periods for meds prior to spirometry
Sec 2.3	~~Serious adverse events (SAEs) have not been reported in product labels for nebulized glycopyrrolate products.~~ No required dose adjustment in renal or hepatic impairment COPD patients has been required for nebulized glycopyrrolate,	Remove incorrect statement
Sec 3.2.5	Glycopyrronium free base multiple dose PK parameters ($AUC_{0\text{-}12h}$, maximum ~~serum~~ plasma drug concentration [C_{max}], time to C_{max} [t_{max}]) post-morning dose on Day 7.	Change serum to plasma
Sec 4.2	Study Design Justification The purpose of this study is to inform the dose selection of glycopyrrolate for the planned Phase 2b Study CO-212. Study CO-212 is planned as a 4-week parallel group study to compare multiple doses of glycopyrrolate combined with 3 mg of ensifentrine as a FDC to the glycopyrrolate and ensifentrine individual components and placebo. This study will use pulmonary function test results and PK analyses to perform an initial evaluation of ~~the potential~~ glycopyrrolate doses over a dose range. The study intends to enroll a population of subjects with stable, moderate to severe COPD. COPD patients can exhibit varying degrees of responsiveness to bronchodilators such as albuterol, with approximately 20- to 50% of clinical trial populations displaying ≥ 12% and ≥ 200- mL increases in FEV_1 following dosing with albuterol (Hanania et al. 2011; Anzueto et al. 2023). Given that this study is intended to inform on dose-responsiveness to a bronchodilator (glycopyrrolate), entry criteria is planned to enroll approximately 50% of COPD subjects with ≥ 12% and ≥ 200 mL increase in FEV_1 following albuterol, in order to optimally assess differences in dose levels. -Incorporation of a placebo arm into the study design will allow accurate interpretation of ~~these results~~treatment effect for each dose level and clear identification of the proper glycopyrrolate doses for use in Study CO-212. The use of a placebo arm in the study is ~~considered safe for the subjects~~justified for the following reasons: • Subjects will be permitted to be on BID LABA ± ICS background therapy to treat their COPD throughout the study. • Subjects with unstable COPD (i.e., any severe COPD exacerbations within 6 months of Screening) are excluded. • Discontinuation of any maintenance COPD therapies for the sole purpose of study eligibility is not recommended. • The maximum continuous period that a subject could be on placebo and no study medication is 3 weeks (1 week of placebo therapy between two 1-week wash-out periods). • The maximum continuous period that a subject could be on placebo, low-dose glycopyrrolate, and no active study medication is 5 weeks (i.e., 1 week of placebo therapy and 1 week of low-dose glycopyrrolate, each between two 1-week wash-out periods).	Add further design justification

Sec 5.3	3. Significantly abnormal ECG finding, as defined in Appendix 4~~.~~, on the 12-lead ECG obtained pre-dose at Visit 2~~at Screening~~ as assessed by the investigator or site medical doctor/medically qualified person ~~or on the pre-dose ECG obtained at Visit 1~~ before the subject has been randomized. ~~After a subject has been randomized, ECG withdrawal criteria () will apply. In the event that the central ECG reviewer discovers a significant ECG abnormality meeting an ECG withdrawal criterion on the Visit 1 ECG in 2 of 3 triplicate measurements, the subject may be discontinued.~~ 4. Did not meet ≥ 1 of the Inclusion Criteria (Section 5.1) or met ≥ 1 of the Exclusion Criteria (Section 5.2) as assessed ~~at Screening (e.g., including~~through ~~overreads or lab values obtained after the day of Screening~~data	Clarifying text
Sec 5.3	5. At Screening the subject's albuterol responsiveness test had an FEV_1 increase of < 12% or < 200 mL after 4 puffs of albuterol AND the randomization cap of approximately 50% of subjects in that responsiveness category has been met.	Add criterion for cap of low-responsive subjects
Sec 5.5.1	Subjects may *not* be rescreened if their post-albuterol spirometry at Screening demonstrated any of the following: • FEV_1/FVC ratio ≥ 0.7 OR • FEV_1 < 30% of predicted normal OR • FEV_1 > 70% of predicted normal. Rescreening is allowed only once for a screen failure due to both of following: • A subject's Screening albuterol responsiveness test had an FEV_1 increase of < 12% or < 200 mL after 4 puffs of albuterol AND • The randomization cap of approximately 50% of subjects in that responsiveness category has been met. S~~S~~ubjects who are screen failures for other reason (Section 5.6) may be rescreened with approval from the Medical Monitor.~~.~~ Rescreened subjects should be assigned a new subject number different from the initial Screening event. ~~Rescreening is allowed once for a screen failure due to Inclusion Criterion #6: ≥ 150 mL increase from pre-bronchodilator FEV_1, if the subject that screen failed was within 10% (15 mL) of this threshold. Rescreening due to failure to meet other lung function criteria within Criterion #6 is not allowed.~~	Clarification and add cap of low-responsive subjects
Sec 6.0	In this protocol the terms 'investigational product', 'double-blind study medication', and 'blinded study medication' are the same and refer to the blinded nebulized study medication. Subjects taking maintenance BID LABA ± ICS medications should take the LABA ± ICS medication prior to taking blinded study medication when taking the blinded study medication at home.	Add guidance on timing of LABA/ICS dosing
Sec 6.1.1	• If the rescue medications are not withheld for ~~the time intervals defined in Section~~ 4 hours prior to spirometry.~~.~~ • If the pre-dose FEV_1 on Day 1 of the current Treatment Period is not within ± 20% of the pre dose FEV_1 at Day 1 of the first Treatment Period • For a subject on a maintenance BID LABA ± ICS therapy, if the BID LABA ± ICS are not withheld for approximately 12 hours (e.g. ~~≥ 12 hours~~LABA maintenance therapy taken the morning of the clinic visit) prior to spirometry.	Clarification and add withholding requirements for LABA/ICS

Table 7		Must be withheld for at least 4 hours prior to spirometry. Should be withheld for at least 4 hours prior to ECGs.	Allow use of LABA/ICS
	BID LABA ± ICS	Stable use of BID LABA ± ICS is permitted ONLY IF the subject: 1. Has been taking LABA ± ICS at any dosing frequency at a stable dose for at least 4 weeks prior to Screening AND 2. If on a non-BID LABA ± ICS therapy, can transition, to a BID LABA ± ICS ≥ 7 days prior to randomization for use for remaining duration of the study AND 3. Can withhold use of the BID LABA ± ICS for approximately 24 h prior to any clinic visit where spirometry will be performed (i.e., Visits 1 through 9). BID LABA ± ICS use should not be initiated, dose modified, or discontinued during the study after randomization. ICS monotherapy and high dose ICS (e.g., > 1000 µg of fluticasone propionate or equivalent) is not allowed.	
	~~ICS~~	~~Stable use of ICS is permitted IF the subject has been taking the ICS at least 4 weeks prior to Screening. ICS should not be initiated, dose modified, or discontinued during the study.~~ ~~ICS monotherapy and high dose ICS (e.g., > 1000 µg of fluticasone propionate or equivalent) is not allowed.~~	
Sec 6.6.3	Prohibited medications and therapies are provided in Table 8. All prohibited medications are not to be taken during the time periods described~~study conduct, including screening periods, treatment periods, washout periods, and the follow-up period~~. Discontinuation of any maintenance COPD therapies for the sole purpose of study eligibility is not recommended.		Remove washout of COPD meds.
Table 8	~~3~~ 6 months prior to Screening and prohibited during the study.		
Table 8	~~1 day~~24 hours prior to Screening and prohibited during the study.		Clarification
Table 8	LAMAs 1. Once-daily LAMAs 2. BID LAMAs	1. 48 hours prior to Screening and prohibited during the study 2. 24 hours prior to Screening and prohibited during the study	Add LAMA restrictions
Table 8	Starting or stopping ICS is not allowed during the study. ICS monotherapy and high dose ICS (e.g., > 1000 µg of fluticasone propionate or equivalent) is not allowed.		Clarify ICS restrictions
Sec 8.3.1.2	○ *Blinded Study Medication*: Blinded study medication must be withheld until pre-dose spirometry is completed. ~~o~~ *Rescue Medication and~~: Albuterol must be withheld for ≥ 4 hours.~~ BID LABA ± ICS*: Must be~~24~~12 ○ ~~*If the withholding periods above are not met, the visit should be rescheduled~~ withheld per Section 6.1.1.		Add withholding for LABA/ICS
Sec 8.9	~~8.9 Data Assessment Committee~~ ~~A Data Assessment Committee (DAC) will be established for this study for the sole purpose of interpreting the planned interim analysis to inform on dose selection for fixed dose combination Study CO 212 (Section). The DAC will not be involved in study conduct or decisions relating to Study CO 211. Further details as to the composition, conduct, and handling of unblinded data can be found in the DAC Charter.~~		Remove interim analysis

Appendix 7	~~**Male Subjects**~~ • ~~Male subjects with female partners of childbearing potential are eligible to participate if they agree to ONE of the following from the first dose up to 30 days after the last dose of study medication:~~ – ~~Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long-term and persistent basis) and agree to remain abstinent.~~ – ~~Agree to use a male condom plus partner use of a contraceptive method with a failure rate of < 1% per year as described in when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.~~ • ~~In addition, male subjects must refrain from donating sperm for the duration of the study and for 30 days after the last dose of study medication.~~ • ~~Male subjects with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration during the study and for 30 days after the last dose of study medication.~~	Remove Male contraception Guidance not required for glycopyrrolate
Appendix 7	~~***Male subjects with partners who become pregnant***~~ ~~The Investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in the study. This applies only to male subjects who receive glycopyrrolate.~~ ~~After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy.~~ ~~Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.~~	Remove Male contraception Guidance not required for glycopyrrolate
Appendix 7	The subject will be approached and asked to consent to be followed to determine the outcome of the pregnancy.	Clarify procedure for female subjects who become pregnant
Table 11	Neutrophils, absolute | ~~COVID-19 testing[2]~~	Remove COVID-19 testing
Table 11	~~[2]COVID-19 test (optional) may be performed locally.~~	Remove COVID-19 testing

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1.0 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title

A Phase IIb, randomized, double-blind, placebo-controlled study to assess the efficacy, safety, and pharmacokinetics of glycopyrrolate inhalation solution over 1 week in subjects with COPD.

Short Title

A Phase IIb study of glycopyrrolate inhalation solution over 1 week in subjects with COPD.

Rationale

Glycopyrrolate is a long-acting muscarinic antagonist (LAMA) which is often referred to as an anticholinergic. It has affinity to the subtypes of muscarinic receptors M1 to M5, and higher affinity for M3 over M2. In the airways, glycopyrrolate exhibits pharmacological effects through inhibition of M3 receptor in smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism has been shown with human and animal origin receptors and isolated organ preparations.

Glycopyrrolate has been formulated in multiple inhaled approved products, both alone and in combination with other chronic obstructive pulmonary disease (COPD) maintenance therapies, for the maintenance treatment of COPD as a twice-daily (BID) treatment in the United States (US).

Ensifentrine is a novel, small molecule, potent, selective dual inhibitor of Phosphodiesterase (PDE)3 and PDE4 being developed as treatment of COPD and other conditions.

A fixed dose combination (FDC) product with ensifentrine and glycopyrrolate could provide a single nebulized product with pharmacology equivalent to 2 bronchodilator mechanisms and a non-steroidal anti-inflammatory mechanism.

The new FDC product is proposed to contain ensifentrine in suspension and glycopyrrolate in solution in a citric acid-buffered formulation at pH 4.5 (FDC formulation) for delivery via a standard jet nebulizer.

This dose-ranging trial (RPL554-CO-211) with glycopyrrolate solution delivered via standard jet nebulizer in the proposed FDC formulation will support the selection of the glycopyrrolate dose for further development.

Objectives

Primary Objective

- The primary objective of this study is to evaluate the bronchodilator effect of BID inhaled glycopyrrolate solution in the proposed FDC formulation over a dose range administered by standard jet nebulizer in subjects with COPD in terms of morning trough FEV_1 on Day 7.

Secondary Objectives

- To evaluate the bronchodilator effect of BID inhaled glycopyrrolate solution in the proposed FDC formulation on Day 7 peak FEV_1, average FEV_1 area under the curve versus time from time 0 to 4 hours (AUC_{0-4h}), average FEV_1 AUC_{0-12h}, and evening trough FEV_1.
- To evaluate the bronchodilator effect of a single dose of inhaled glycopyrrolate solution in the proposed FDC formulation on Day 1 peak FEV_1 and FEV_1 AUC_{0-4h}.

Exploratory Objective

- To assess the dose response of BID inhaled glycopyrrolate solution in the proposed FDC formulation on peak FEV_1, average FEV_1 AUC_{0-12h}, and morning trough FEV_1 on Day 7.

Safety Objective

- To evaluate the safety and tolerability of inhaled glycopyrrolate solution in the proposed FDC formulation in subjects with COPD.

Pharmacokinetic Objective

- To assess the PK profile of multiple doses of inhaled glycopyrrolate solution in the proposed FDC formulation in subjects with COPD on Day 7.

Endpoints

Primary Endpoint

- Change from average baseline FEV_1 to morning trough FEV_1 measured on Day 7.

Secondary Endpoints

- Change from average baseline FEV_1 to average peak FEV_1 measured over 4 hours post-dose on Day 7.
- Change from average baseline FEV_1 to average FEV_1 AUC_{0-4h} on Day 7.
- Change from average baseline FEV_1 to average FEV_1 AUC_{0-12h} on Day 7.
- Change from average baseline FEV_1 to evening trough FEV_1 on Day 7.
- Change from average baseline FEV_1 to peak FEV_1 measured over 4 hours after first dose on Day 1.
- Change from average baseline FEV_1 to average FEV_1 AUC_{0-4h} measured after first dose on Day 1.

Exploratory Endpoint

- Association between glycopyrrolate dose and peak FEV_1, average FEV_1 AUC_{0-12h} after morning dose on Day 7, and morning trough FEV_1 prior to the last dose on Day 7.

Safety Endpoints

- Incidence of treatment emergent adverse events (AEs).
- Change from baseline in laboratory safety tests (hematology and blood chemistry).
- Change from baseline to markedly abnormal in laboratory safety tests (hematology and blood chemistry).
- Shifts in laboratory safety tests (hematology and blood chemistry) classified as marked abnormalities.
- Change from baseline in 12-lead ECG (including QTcF and heart rate).
- Change from baseline to markedly abnormal 12-lead ECG (including QTcF and heart rate).
- Shifts in 12-lead ECG (including QTcF and heart rate) from normal to abnormal.
- Change from baseline in vital signs (blood pressure and pulse rate).
- Change from baseline to markedly abnormal in vital signs (blood pressure and pulse rate).

Pharmacokinetic Endpoint

- Glycopyrronium free base multiple dose PK parameters (AUC_{0-12h}, C_{max}, t_{max}) post-morning dose on Day 7.

Overall Design

RPL554-CO-211 is a multicenter, randomized, double blind, placebo-controlled, 4-period cross-over study to evaluate glycopyrrolate dose-levels administered BID by a standard jet nebulizer in the proposed FDC formulation: 0 (placebo), 14 µg, 42.5 µg, and 85 µg glycopyrrolate.

Approximately 40 eligible subjects meeting all inclusion and no exclusion criteria will be dosed for 4 consecutive 7-day Treatment Periods. The last dose in each Treatment Period will be the morning of the Day 7 clinic visit, and the Treatment Periods will be separated by a 7-day washout period (Figure 1).

Each subject will take 4 dose levels of blinded study medication, with 1 dose level taken during each Treatment Period:

Dose Level	Blinded Study Medication
A	Glycopyrrolate 85 µg BID
B	Glycopyrrolate 42.5 µg BID
C	Glycopyrrolate 14 µg BID
D	Placebo BID

Subjects will be randomly assigned to Treatment Sequences 1 to 4 in a 1:1:1:1 ratio to receive the 4 dose levels in the following sequences:

Treatment Sequence	Dose Levels Administer in Each Treatment Period			
	Treatment Period 1 (N = 40)	**Treatment Period 2 (N = 40)**	**Treatment Period 3 (N = 40)**	**Treatment Period 4 (N = 40)**
Sequence 1 (N = 10)	A	D	B	C
Sequence 2 (N = 10)	B	A	C	D
Sequence 3 (N = 10)	C	B	D	A
Sequence 4 (N = 10)	D	C	A	B

Each subject is expected to complete all 4 Treatment Periods. Subjects who do not complete at least 2 Treatment Periods may be replaced.

Spirometry will be assessed pre- and post-dose on Days 1 and 7, as described in Table 2.

PK sampling will take place on Day 7 in Treatment Periods 1 and 2 only following any ECG or lung function assessments, as described in Table 3.

The pre-dose FEV_1 on Day 1 of Treatment Periods 2 to 4 (i.e., Visits 4, 6, and 8) should be within ± 20% of the pre-dose FEV_1 at Day 1 of Treatment Period 1 (Visit 2) to ensure a consistent baseline FEV_1 for each Treatment Period. If the FEV_1 varies by more than 20%, the start of the Treatment Period must be rescheduled to occur within 7 days.

Screening for eligibility will include a responsiveness test (spirometry both pre- and post-4 puffs of albuterol) performed between 7 and 14 days before the first dose of blinded study medication. The proportion of the study population who have a post-albuterol FEV_1 increase of < 12% or < 200 mL will be capped at approximately 50%.

Prohibited COPD medications prior to and during the study are described in Table 8.

Discontinuation of any maintenance COPD therapies for the sole purpose of study eligibility is not recommended.

Stable use of BID LABA ± inhaled corticosteroids (ICS) at a maintenance dose is permitted during the study (Table 7).

At Screening (Visit 1), all subjects will be dispensed albuterol as rescue medication to be used as needed during the screening, treatment, and washout periods. Subjects who are required to withhold medications prior to Screening (Visit 1) will be dispensed albuterol as rescue medication upon signing of the Informed Consent Form (ICF) for use prior to Visit 1.

Subjects should withhold rescue medication use for ≥ 4 hours and, if applicable, BID LABA ± ICS use for approximately 24 hours prior to spirometry at all required visits. If the rescue medication withholding period is not met or if BID LABA ± ICS medications are taken the morning of a clinic visit where spirometry is conducted, the subject will be rescheduled for a repeat visit within 7 or 3 days of Day 1 or Day 7, respectively.

All sites will use standardized spirometry and electrocardiogram (ECG) equipment provided by a central vendor, and all spirometry and ECGs will be over-read by the blinded central vendor.

Subjects will be screened for eligibility during a screening period of 7 to 14 days.

During the treatment period, subjects will complete all assessments and procedures as outlined in the Schedule of Activities (Section 1.3) for approximately 10 weeks for each subject.

Subjects completing treatment will complete a Follow-Up Phone Call 7 ± 1 days after the last scheduled study visit.

Subjects who experience ≥ 1 moderate or severe COPD exacerbation during the study will discontinue study medication and be withdrawn from the study.

Subjects who permanently discontinue double-blind study medication will be withdrawn from the study.

Subjects meeting the withdrawal criteria during the study or who withdraw from the study for other reasons will be discontinued and requested to complete the Early Termination/ Withdrawal Procedures and complete a Follow-Up Phone Call 7 ± 1 days after the Early Termination/Withdrawal Visit.

Number of Investigators and Study Centers

Approximately 7 Investigators and study centers are expected to participate in this study.

Number of Subjects

Approximately 40 randomized subjects are planned.

Inclusion Criteria

1. Capable of giving informed consent indicating that they understand the purpose of the study and study procedures and agree to comply with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.
2. Age: Subject must be 40 to 80 years of age inclusive, at the time of Screening.
3. Sex:

   - Males are eligible to participate if they agree to use contraception as described in the contraceptive guidance (Appendix 7) from Screening and throughout the study and for at least 30 days after the last dose of blinded study medication.
   - Females are eligible to participate if they are not pregnant, not breastfeeding, and ≥ 1 of the following conditions apply:

     a) Not a woman of childbearing potential (WOCBP) as defined in Appendix 7.

     OR

     b) A WOCBP who agrees to follow the contraceptive guidance in Appendix 7 from Screening and throughout the study and for at least 30 days after the last dose of blinded study medication.

4. Smoking History: Current or former cigarette smokers with a history of cigarette smoking ≥ 10 pack years at Screening [number of pack years = (number of cigarettes per day / 20) × number of years smoked (e.g., 20 cigarettes per day for 10 years, or 10 cigarettes per day for 20 years)]. Pipe and/or cigar use cannot be used to calculate pack-year history. Former smokers are defined as those who have stopped smoking for at least 6 months prior to Screening. Smoking cessation programs are permitted during the study.
5. COPD Diagnosis: Subjects with an established clinical history of COPD as defined by the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines (Celli et al. 2004) with symptoms compatible with COPD.
6. Post-bronchodilator (4 puffs of salbutamol) spirometry at Screening demonstrating both the following:

   a. FEV_1/forced vital capacity (FVC) ratio of < 0.70 AND
   b. FEV_1 ≥ 30 % and ≤ 70% of predicted normal (Quanjer et al. 2012).

   Note: The proportion of the study population who have a post-albuterol FEV_1 increase of < 12% or < 200 mL will be capped at approximately 50%.

7. A posterior-anterior chest x-ray (CXR) at Screening or within 12 months prior to Screening showing no clinically significant abnormalities unrelated to COPD. If a CXR within the past 12 months is not available but a computed tomography (CT) scan within the same time period is available, the CT scan may be reviewed in place of a CXR.
8. Capable of withdrawing from short-acting bronchodilators for 4 hours prior to spirometry testing and from BID LABA ± ICS therapy for 24 hours prior to spirometry.
9. Capable of using the study nebulizer correctly.
10. Ability to perform acceptable spirometry in accordance with ATS/ERS guidelines (Graham et al. 2019).
11. Willing and able to attend all study visits and adhere to all study assessments and procedures.

Exclusion Criteria

1. Concomitant clinically significant pulmonary disease other than COPD (i.e., asthma, tuberculosis, lung cancer, bronchiectasis, sarcoidosis, lung fibrosis, interstitial lung diseases, sleep apnea (unless controlled with stable continuous positive airway pressure [CPAP] use), known alpha-1 antitrypsin deficiency, core pulmonale or other non-specific pulmonary disease).
2. Within 6 months prior to Screening:
    a. COPD exacerbation requiring hospitalization.
    b. Use of therapies for COPD exacerbation (e.g., oral, intravenous, or intramuscular glucocorticoids).
3. Lower respiratory tract infection within 6 weeks of Screening or an active infection at Screening.
4. History of life-threatening COPD, including Intensive Care Unit admission and/or requiring intubation.
5. Previous lung resection or lung reduction surgery within 1-year of Screening.
6. Long term oxygen use defined as oxygen therapy prescribed for greater than 12 hours per day. As needed oxygen use (≤ 12 hours per day) is not exclusionary.
7. Severe comorbidities including unstable cardiac, (e.g., myocardial infarction within 1 year prior to screening, unstable angina within 6 months prior to screening, or unstable or life-threatening arrhythmia requiring intervention within

3 months prior to screening, diagnosis of New York Heart Association (NYHA) class III or IV heart disease) or any other clinically significant medical conditions including uncontrolled diseases (e.g., endocrine, neurological, hepatic, gastrointestinal, renal, hematological, urological, immunological, psychiatric or ophthalmic diseases) that would, in the opinion of the Investigator, preclude the subject from safely completing the required tests or the study, or is likely to result in disease progression that would require withdrawal of the subject.

8. History of or clinically significant on-going bladder outflow obstruction or history of catheterization for relief of bladder outflow obstruction within the previous 6 months.
9. History of narrow angle glaucoma.
10. History of hypersensitivity or intolerance to aerosol medications, salbutamol or glycopyrrolate or any of its excipients/components, anticholinergic agents, or sympathomimetic amines.
11. Pulmonary rehabilitation unless such treatment has been stable from 4 weeks prior to Screening (Visit 1) and remains stable during the study. Pulmonary rehabilitation programs should not be started or completed during participation in the study.
12. Major surgery (requiring general anesthesia) in the 6 weeks prior to Screening, lack of full recovery from surgery at Screening, or planned surgery through the end of the study.
13. History of or current malignancy of any organ system, treated or untreated within the past 5 years, except for localized basal or squamous cell carcinoma of the skin.
14. Significant psychiatric disease that would likely result in the subject not being able to complete the study, in the opinion of the Investigator.
15. Findings on physical examination that an investigator considers to be clinically significant at Screening.
16. Use of prohibited medications within the time intervals defined in Section 6.6.3.
17. Current or history of drug or alcohol abuse within the past 5 years.
18. Estimated glomerular filtration rate (eGFR) < 30 mL/min. The Chronic Kidney Disease Epidemiology Collaboration Creatinine (2021) calculation will be used (Inker et al. 2021).

19. Alanine aminotransferase (ALT) ≥ 2 × upper limit of normal (ULN), alkaline phosphatase and/or bilirubin > 1.5 × ULN (isolated bilirubin > 1.5 × ULN is acceptable if fractionated bilirubin < 35%).
20. Any other abnormal hematology, biochemistry, or viral serology deemed by the Investigator to be clinically significant. Abnormal chemistry and/or hematology may be repeated during Screening.
21. Abnormal ECG (Appendix 4) at the screening visit that is clinically significant.
22. Women who are breast feeding.
23. Use of an experimental drug within 30 days or 5 half-lives of Screening, whichever is longer, and/or participation in a study treatment-free follow-up phase of a clinical study within 30 days prior to Screening.
24. Use of an experimental medical device or participation in a follow-up phase of an experimental medical device clinical study within 30 days prior to Screening.
25. Affiliation with the investigator site, including an Investigator, Sub-Investigator, study coordinator, study nurse, other employee of participating investigator or study site or a family member of the aforementioned.
26. A disclosed history or one known to the Investigator of significant noncompliance in previous investigational studies or with prescribed medications.
27. Any other reason that the Investigator considers makes the subject unsuitable to participate.

Treatment Groups and Duration

Randomization will assign each subject in a crossover fashion to one of 4 treatment sequences (see definitions above), representing a unique combination of four 1-week treatment periods covering each treatment arm of the study, Periods 1 through 4 will be separated by a 7-day washout period.

All blinded study medications will be double-blind and administered using the inhaled route via a standard jet nebulizer supplied by the Sponsor. Nebulization time should be approximately 5 to 10 minutes. Subjects taking maintenance BID LABA ± ICS medications should take the LABA ± ICS medication prior to taking blinded study medication when taking the blinded study medication at home.

Following Visit 2, subjects will self-administer their daily morning and evening doses at home at approximately the same times, approximately 12 hours apart, each day for the duration of the Treatment Period, except for clinic visits on Days 1 and 7 of each Treatment Period when subjects will have their morning doses administered in the clinic.

Statistical Methods

The aim of this study is to quantify the benefit of each glycopyrrolate dose over placebo in efficacy as mean change from baseline in FEV_1. The null hypotheses to be tested for each efficacy endpoint will be that there is no difference in change from baseline between glycopyrrolate and placebo for each glycopyrrolate dose, against the alternative hypothesis that change differs between that glycopyrrolate dose and placebo. All tests will be two-sided at a 5% significance level. Primary and secondary endpoints will be evaluated at a nominal Type 1 error level of 0.05, and Type 1 error will not be adjusted for multiplicity.

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

Treatments will be characterized and comparisons, when performed, will be made from estimates obtained from analysis of covariance models.

Spirometry parameters will be analyzed as change from average baseline FEV_1 on study Day 1 and, separately, on Day 7, and will not be transformed prior to analysis. Models will include terms for treatment, period, and subject as fixed effects, and baseline as a covariate. A separate model will be fit for each efficacy parameter from Day 1 and from Day 7.

Each efficacy parameter will be summarized both for the absolute value and for change from baseline using descriptive statistics (N, mean, median, SD, minimum, and maximum) for each treatment, generated separately for each Period as well as overall. Average baseline will represent the average of the two pre-treatment FEV_1 values prior to the first dose of double-blind medication in each Treatment Period.

Safety analysis will be descriptive and based on the safety set including all randomized and treated subjects. Safety analysis will be by actual treatment received.

The following PK parameters will be calculated from plasma concentrations of glycopyrronium on Day 7.

- $AUC_{0\text{-}12h}$ denotes the area under the plasma concentration curve versus time over the 12-hour dosing interval following the morning dose.
- C_{max} denotes the highest plasma concentration measured over the dosing interval.
- C_{trough} denotes the plasma concentration measured prior to dose administration.
- C_{avg} denotes the plasma average concentration measured over the dosing interval, calculated as $AUC_{0\text{-}12h}$ divided by the dosing interval.
- t_{max} denotes the time point corresponding to C_{max}.

Other PK parameters may also be calculated.

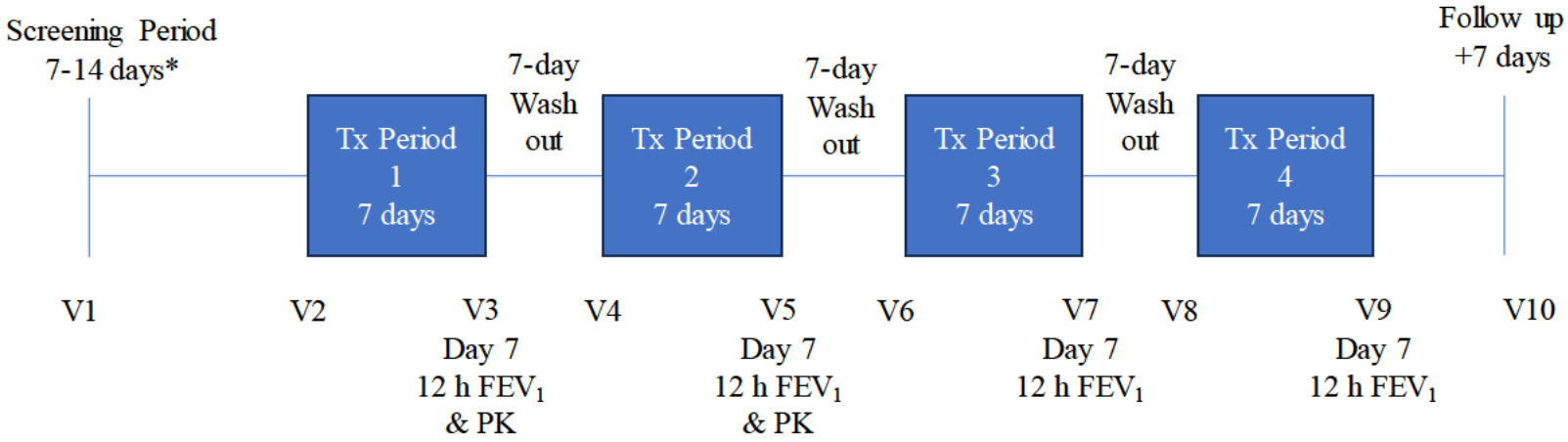
No interim analysis is planned.

Sample Size

Estimates obtained from a 6-period multi-dose crossover study of BID nebulized glycopyrrolate at Day 7 for change from baseline in trough FEV_1 (Saluja et al. 2017). Assuming the standard deviation for the differences (SDD) equal to 0.153 L, a sample size of 36 subjects provides 85% statistical power to detect a between-group difference with placebo in trough FEV_1 at Day 7 of at least 81 mL. The sample size provides adequate statistical power for statistical comparisons of each secondary endpoint. Subjects who withdraw prior to completing 2 treatment periods may be replaced. Assuming approximately 10% of early withdrawals, approximately 40 subjects will be enrolled.

1.2 Schema

Figure 1: Study Schematic



*All long-acting bronchodilators should be withheld out for 24 or 48h prior to screening spirometry. Consent must be obtained prior to any study procedures including withholding.

Abbreviations: FEV_1 = forced expiratory volume over 1 second; h = hour; PK = pharmacokinetic; Tx = treatment; V = visit.

1.3 Schedule of Activities

Table 1: Schedule of Assessments and Procedures

Procedure	Screening	Treatment Periods 1 to 4						EOS	Procedures for ET/W
	Visit 1	Visits 2, 4, 6, and 8 Day 1		Phone Call	Visits 3, 5, 7, and 9 Day 7 (+ 1 day)		Washout	Phone Call Follow-Up	ET/W Visit
	7 to 14 days prior to Visit 2	Pre-dose	Post-dose	Day 6	Pre-dose	Post-dose	7 (+ 2) Days only after Visits 3, 5, and 7	7 (± 1) Days After V9 *OR* ET/W	As Soon as Possible after ET/W
Informed consent	X								
Inclusion/exclusion criteria	X	X[a]							
Rescue medication dispensing[b]	X	X							
Demographics[c]	X								
Medical, surgical, medication, smoking history, drug/alcohol use history	X								
Serum pregnancy hCG test in all WOCBP	X								
Urine pregnancy test (for WOCBP)		X							
CXR[d]	X								
Nebulizer equipment materials review/training		X							
12-lead ECG[e]	X	X			X				X
Complete physical exam; height and weight, BMI calculated[f]	X								
Vital signs[g]	X	X	X		X	X			X
Laboratory tests[h]	X				X				
Viral serology[i]	X								
Spirometry (measurements of lung function (FEV_1 and FVC) and responsiveness testing[j,k]	X	X	X		X	X			
Register in IRT	X								
Randomize in IRT		X							

Procedure	Screening	Treatment Periods 1 to 4						EOS	Procedures for ET/W
	Visit 1	Visits 2, 4, 6, and 8 Day 1		Phone Call	Visits 3, 5, 7, and 9 Day 7 (+ 1 day)		Washout	Phone Call Follow-Up	ET/W Visit
	7 to 14 days prior to Visit 2	Pre-dose	Post-dose	Day 6	Pre-dose	Post-dose	7 (+ 2) Days only after Visits 3, 5, and 7	7 (± 1) Days After V9 ***OR*** ET/W	As Soon as Possible after ET/W
In clinic study medication dosing			X[l]			X[l]			
Concomitant medications/therapies	X	X			X			X	X
Pharmacokinetic sample collection (Treatment Periods 1 and 2 ONLY)[m]					X	X			
Study medication dispensing and compliance		X			X				X
AE and SAE recording[n]	X	X	X		X	X		X	X
Phone call to subject				X[o]				X	

a. Check subject's use of concomitant medications, subject's use of prohibited medications, subject's withholding compliance, and that there are no clinically relevant changes in health status that, in the opinion of the investigator, would prohibit the subject from completing the Treatment Period.
b. Study rescue medication should be dispensed once the subject has signed the ICF for subjects that require a withholding period prior to Screening (Visit 1).
c. Demographics to include (date of birth, age, gender, race, ethnicity).
d. A posterior-anterior CXR at Screening or within 12 months prior to Screening showing no clinically significant abnormalities unrelated to COPD is required. If a CXR within the past 12 months is not available but a CT scan within the same time period is available, the CT scan may be reviewed in place of a CXR.
e. ECGs should be obtained before all other simultaneously scheduled procedures are completed.
f. Complete physical exam at Screening to include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (spleen and liver), lymph nodes and extremities. Findings of the physical exam should be recorded in the subjects medical history.
g. Vital signs: (pulse rate, blood pressure) will be at Screening, pre-dose, 1 hour ± 15 min and 4 hours ± 30 min post-dose on Days 1 and Day 7, and at Early Withdrawal/Termination Visit.
h. The laboratory test to be performed are specified in Table 11.
i. The serology test to be performed are specified in Table 11.
j. On Days 1 and 7 of each Treatment Period, spirometry will be conducted as specified in Table 2.
k. Responsiveness testing only conducted at the Screening Visit.
l. In clinic dosing performed after pre-dose spirometry and pre-dose PK sampling in the morning.
m. PK sample collection times on Day 7 of Treatment Periods 1 and 2 are specified in Table 3. PK samples should be taken after all concomitantly scheduled assessments (e.g., ECG, FEV_1).
n. AEs will be collected Day 1 through the EOS. SAEs related to study participation will be recorded from the time the subject consents until study discharge.
o. Phone call reminder to subjects to remind them to take their evening dose approximately 12 hours prior to the anticipated arrival time to the clinic on Day 7, to note the time of the Day 6 evening dose was taken and to report that time to site staff at the Day 7 visit, to withhold the Day 7 morning dose until at the clinic, to

withhold albuterol within 4 hours of their Day 7 visit, to withhold, if applicable, BID LABA ± ICS use within approximately 24 hours of their Day 7 visit, and to bring their unused study medication to the clinic visit the next day.

Abbreviations: AE = adverse event; COPD = Chronic Obstructive Pulmonary Disease; CT = Computed Tomography; CXR = Chest X-Ray; ECG = Electrocardiogram; EOS = End of Study; ET/W = Early Termination/Withdrawal; FEV_1 = forced expiratory volume over 1 second; FVC = forced vital capacity; hCG = human chorionic gonadotropin; ICF = informed consent form; IRT = Interactive Response Technology; PK = pharmacokinetics; SAE = serious adverse events; WOCBP = women of childbearing potential.

1.4 Schedule of Spirometry, PK, and In-Clinic Dosing with Blinded Study Medication

Table 2: Schedule of Spirometry and In-Clinic Dosing with Blinded Study Medication

Every effort should be made to initiate spirometry according to these timings:	**Day 1** **For Each Treatment Period (Visits 2, 4, 6, and 8)**	**Day 7** **For Each Treatment Period (Visits 3, 5, 7, and 9)**
Pre-Dose Spirometry	Two separate timepoints within 40 min prior to dosing.	One timepoint approximately 12 hours following the prior evening dose and prior to Day 7 dosing.
Morning Blinded Study Medication Dosing in the Clinic	Time: Between 6 AM and 10 AM.	Time: Between 6 AM and 10 AM.
Post-Dose Serial Spirometry	30 min, 1-, 2-, and 4-hours post-dose.	30 min, 1-, 2-, 4-, 6-, 8-, and 12-hours post-dose. The 12-hour post-dose spirometry should not start prior to 11.5 hours post-dose.

Note: Additional details on information contained in this table can be found in Section 6.0 and Section 8.3.1. Rescue medications should have been withheld for ≥ 4 hours prior to spirometry and, if applicable, BID LABA ± ICS medications should have been withheld for approximately 24 hours prior to spirometry.

Table 3: Schedule of PK and In-Clinic Dosing with Blinded Study Medication

Every effort should be made to collect PK samples according to these timings:	**Day 7** **For Treatment Period 1 (Visit 3) and Treatment Period 2 (Visit 5) Only**
Pre-Dose PK: 1 sample	One timepoint within 30 minutes prior to Day 7 dosing.
Morning Blinded Study Medication Dosing in the Clinic	Time: Between 6 AM and 10 AM.
Post-Dose Serial PK: 9 samples	10 ± 4, 20 ± 4, and 40 ± 4 minutes post-dose and at 1, 1.5, 2, 4, 8, and 12 hours (all later timepoints at ± 15 minutes) post-dose.

Note: PK samples should be taken after all concomitantly scheduled assessments (e.g., ECG, FEV_1).
Abbreviations: ECG = electrocardiogram; FEV_1 = forced expiratory volume in 1 second; PK = pharmacokinetic.

2.0 INTRODUCTION

2.1 Study Rationale

Verona Pharma plc. (Verona) is developing inhaled nebulized ensifentrine and glycopyrrolate fixed dose combination (FDC) as a novel therapy for the maintenance treatment of chronic obstructive pulmonary disease (COPD). RPL554-CO-211 (Study CO-211) is the first of 2 planned Phase II studies investigating the ensifentrine and glycopyrrolate combination. Study CO-211 will assess a dose-range for glycopyrrolate solution delivered via standard jet nebulizer in the proposed FDC formulation. The results from this study will inform the dose selection for the second Phase II study, RPL554-CO-212 (Study CO-212), which will assess combinations of ensifentrine and glycopyrrolate, with planned comparisons to individual components, ensifentrine and glycopyrrolate.

2.2 Background

2.2.1 Chronic Obstructive Pulmonary Disease

COPD is characterized by progressive airflow obstruction which is largely irreversible. Chronic inflammation of the respiratory tract, acute exacerbations primarily caused by viral and/or bacterial infections, airway remodeling, and excessive mucus production are believed to contribute to the airflow obstruction and lung parenchymal destruction. COPD is predicted to be the third leading cause of death and fourth most common cause of disability worldwide by 2030, and chronic tobacco smoke exposure is believed to be a key etiological factor (STUCKLER 2008). Current standard-of-care treatments include inhaled short- and long-acting bronchodilators (i.e., muscarinic antagonists and β_2 agonists) and inhaled corticosteroids (ICS). One oral phosphodiesterase (PDE) 4 inhibitor, roflumilast, has been approved for the reduction in risk of COPD exacerbations in patients with severe COPD associated with chronic bronchitis and a history of exacerbations. These therapies have little or no effect on disease progression or mortality, although there is evidence that they reduce exacerbation rates and improve quality of life (Rabe et al. 2005; Calverley et al. 2007; Rennard et al. 2011; Singh 2015).

Current therapies in the COPD market (e.g., long-acting muscarinic antagonists [LAMAs], long-acting β_2-agonists [LABAs], ICS, Daliresp [PDE4 inhibitor]) are typically used in combination to optimize treatment effects in patients. In the United States (US), > 80% of COPD patients are currently treated with combination products (Mannino et al. 2022). This is also further emphasized in the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2024 update, which recommends LAMA/LABA combination therapy as the first treatment option for patients with COPD (GOLD 2024). Several fixed-dose combination products have been approved for the treatment of COPD. DuoNeb® (short acting) is the only nebulized FDC product, while all the other FDC

products are formulated for dry powder inhaler (DPI) or pressurized metered dose inhaler (pMDI) delivery.

There is a need for *nebulized* combination maintenance therapies for COPD patients, as none are currently available or in development. Nebulized therapies offer ease of use compared to current handheld delivery systems and are often used and preferred by patients. Nebulizers can be ideal delivery devices for any COPD patient, particularly patients with low inspiratory flow levels and/or cognitive and dexterity issues which limit overall effectiveness of DPI or pMDI delivery devices. In fact, up to 90% of COPD patients do not use inhalers correctly (Dijk et al. 2023), which can lead to continued symptoms and future exacerbations.

2.2.2 Ensifentrine

Ensifentrine is a novel, small molecule, potent, and selective dual inhibitor of PDE3 and PDE4. This dual mechanism of action has the potential to offer an important maintenance treatment option for the treatment of obstructive and inflammatory diseases of the respiratory tract, such as COPD, non-cystic fibrosis bronchiectasis (NCFBE), cystic fibrosis, and asthma.

Ensifentrine has been evaluated in 22 completed clinical studies involving approximately 3,000 subjects, of which 16 studies involving over 2,800 subjects comprise the COPD development program including pharmacokinetic (PK), pharmacodynamic (PD), Phase IIb, and Phase III studies. One additional PK study in Chinese healthy volunteers has been completed. In the COPD clinical program, ensifentrine has demonstrated pronounced and consistent bronchodilator effects as well as substantial reductions in moderate and severe COPD exacerbation rate and risk, as measured by time to first event. In addition, subjects treated with ensifentrine experienced improvements in symptoms and quality of life. Overall, ensifentrine was well-tolerated with a low incidence of adverse events (AEs), with rates generally similar to placebo (please refer to the Investigator's Brochure [IB] for further detail).

A New Drug Application for ensifentrine (3 mg) inhalation suspension for the maintenance treatment of COPD was submitted to the US Food and Drug Administration (FDA) on 26 June 2023 and is currently under review by the FDA with a Prescription Drug User Fee Act target action date of 26 June 2024.

2.2.3 Glycopyrrolate

Glycopyrrolate is a LAMA which is often referred to as an anticholinergic (Chabicovsky et al. 2019). It has affinity to the subtypes of muscarinic receptors M1 to M5, and higher affinity for M3 over M2. In the airways, glycopyrrolate exhibits pharmacological effects through inhibition of M3 receptor at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations.

Glycopyrrolate has been formulated in multiple approved inhaled products for the maintenance treatment of COPD as a twice-daily (BID) treatment in the US (Table 4), including Lonhala Magnair (nebulized glycopyrrolate solution) (Sunovion 2019).

Table 4: Glycopyrrolate-containing Products for COPD Treatment Approved in the US

Drug Name	Active Ingredients	Strength/Dose	Dosage Form	Action Date	Marketing Status
UTIBRON™ NEOHALER®	Glycopyrrolate; Indacaterol Maleate	15.6 µg/inh; 27.5 µg/inh, BID	Powder, oral inhalation	29-Oct-2015	Discontinued
SEEBRI™ NEOHALER®	Glycopyrrolate	15.6 µg/inh, BID	Powder, oral inhalation	29-Oct-2015	Discontinued
BEVESPI AEROSPHERE™	Formoterol Fumarate; Glycopyrrolate	4.8 µg/inh; 9 µg/inh Total dose = 2 inh, BID	Pressurized metered dose inhaler	25-Apr-2016	Prescription
LONHALA™ MAGNAIR™	Glycopyrrolate	25 µg in 1 mL, BID	Solution, inhalation	5-Dec-2017	Discontinued
BREZTRI AEROSPHERE™	Budesonide; Formoterol Fumarate; Glycopyrrolate	160 µg/inh; 4.8 µg/inh; 9 µg/inh Total dose = 2 inh, BID	Pressurized metered dose inhaler	23-Jul-2020	Prescription

Abbreviations: BID = twice daily; inh = inhaled; US = United States.

2.2.4 Ensifentrine and Glycopyrrolate Combination

An FDC product with ensifentrine and another bronchodilator could provide a single nebulized product with pharmacology equivalent to 2 bronchodilator mechanisms and a non-steroidal anti-inflammatory mechanism. Clinical data with ensifentrine added on to LAMAs compared with placebo added onto LAMAs demonstrate complementary efficacy in terms of lung function improvement, symptoms, quality of life, and exacerbation reduction (see the IB for details). The pharmacological profile of ensifentrine added to a LAMA strongly supports development of a FDC product. As clinical data support BID inhaled dosing via nebulizer for both ensifentrine and glycopyrrolate and with nonclinical evidence of a synergistic effect, glycopyrrolate has been selected as the combination partner.

The new FDC product is proposed to contain ensifentrine in suspension and glycopyrrolate in solution in a citric acid-buffered formulation at pH 4.5 for delivery via a standard jet nebulizer. The proposed formulation for the FDC of glycopyrrolate and ensifentrine has not been evaluated in humans to date with either medicine.

2.3 Benefit/Risk Assessment

Risks for nebulized glycopyrrolate include paradoxical bronchospasm, immediate hypersensitivity reactions, worsening of narrow-angle glaucoma, and worsening of urinary retention (Sunovion 2019). Data from clinical studies of nebulized glycopyrrolate suggest a potential for dyspnea and urinary tract infection with short-term (12-week) treatment. Although the glycopyrrolate formulation nebulized in this study has not been administered previously, nebulized glycopyrrolate in a citric acid buffered solution at pH 4 (Lonhala) has been well tolerated in moderate-severe COPD patients (Sunovion 2019). No required dose adjustment in renal or hepatic impairment COPD patients has been required for nebulized glycopyrrolate, and no clinically relevant changes in cardiac electrophysiology have been observed. Nebulized glycopyrrolate has not been associated with teratogenic effects or effects on embryo-fetal survival and development in the rat or rabbit. Glycopyrronium has been detected in the milk of lactating rats.

Significant improvement in morning trough forced expiratory volume in 1 second (FEV_1) and FEV_1 area under the curve versus time from time 0 to 12 hours ($AUC_{0\text{-}12h}$) have been demonstrated following treatment with nebulized glycopyrrolate after 7 days of treatment at doses of ≥ 6.25 µg BID. Glycopyrrolate is to be administered for approximately 21 days (42 doses) in this study; any benefit to study subjects will be limited to the duration of the study.

3.0 OBJECTIVES AND ENDPOINTS

3.1 Objectives

3.1.1 Primary Objective

- To evaluate the bronchodilator effect of BID inhaled glycopyrrolate solution in the proposed FDC formulation over a dose range administered by standard jet nebulizer in subjects with COPD in terms of morning trough FEV_1 on Day 7.

3.1.2 Secondary Objectives

- To evaluate the bronchodilator effect of BID inhaled glycopyrrolate solution in the proposed FDC formulation on Day 7 peak FEV_1, average FEV_1 area under the curve versus time from time 0 to 4 hours (AUC_{0-4h}), average FEV_1 AUC_{0-12h}, and evening trough FEV_1.
- To evaluate the bronchodilator effect of a single dose of inhaled glycopyrrolate solution in the proposed FDC formulation on Day 1 peak FEV_1 and FEV_1 AUC_{0-4h}.

3.1.3 Exploratory Objective

- To assess the dose response of BID inhaled glycopyrrolate solution in the proposed FDC formulation on peak FEV_1, average FEV_1 AUC_{0-12h}, and morning trough FEV_1 on Day 7.

3.1.4 Safety Objective

- To evaluate the safety and tolerability of inhaled glycopyrrolate solution in the proposed FDC formulation in subjects with COPD.

3.1.5 Pharmacokinetics Objective

- To assess the PK profile of multiple doses of inhaled glycopyrrolate solution in the proposed FDC formulation in subjects with COPD on Day 7.

3.2 Endpoints

3.2.1 Primary Endpoint

- Change from average baseline in morning trough FEV_1 measured on Day 7.

3.2.2 Secondary Endpoints

- Change from average baseline FEV_1 to average peak FEV_1 measured over 4 hours -post dose on Day 7.
- Change from average baseline FEV_1 to average FEV_1 AUC_{0-4h} on Day 7.
- Change from average baseline FEV_1 to average FEV_1 AUC_{0-12h} on Day 7.
- Change from average baseline FEV_1 to evening trough FEV_1 on Day 7.

- Change from average baseline FEV_1 to peak FEV_1 measured over 4 hours after first dose on Day 1.
- Change from average baseline FEV_1 to average FEV_1 AUC_{0-4h} measured after first dose on Day 1.

3.2.3 Exploratory Endpoint

- Association between glycopyrrolate dose and peak FEV_1, average FEV_1 AUC_{0-12h} after morning dose on Day 7, and morning trough FEV_1 prior to the last dose on Day 7.

3.2.4 Safety Endpoints

- Incidence of AEs.
- Change from baseline in laboratory safety tests (hematology and blood chemistry).
- Markedly abnormal changes in laboratory safety tests (hematology and blood chemistry).
- Shifts in laboratory safety tests (hematology and blood chemistry) classified as marked abnormalities.
- Change from baseline in 12-lead electrocardiogram (ECG) (including QT interval corrected for heart rate using Fridericia's formula [QTcF] and heart rate).
- Shifts in 12-lead ECG (including QTcF and heart rate) from normal to abnormal.
- Change from baseline in vital signs (blood pressure and pulse rate).
- Shifts in vital signs (blood pressure and pulse rate) classified as marked abnormalities.

3.2.5 Pharmacokinetic Endpoints

- Glycopyrronium free base multiple dose PK parameters (AUC_{0-12h}, maximum plasma drug concentration [C_{max}], time to C_{max} [t_{max}]) post-morning dose on Day 7.

4.0 Study Design

4.1 Overall Design

Study CO-211 is a multicenter, randomized, double blind, placebo-controlled, 4-period cross-over study to evaluate glycopyrrolate dose-levels administered BID by a standard jet nebulizer in the proposed FDC formulation: 0 (placebo), 14 µg, 42.5 µg, and 85 µg glycopyrrolate.

Approximately 40 eligible subjects meeting all inclusion and no exclusion criteria will be dosed for 4 consecutive 7-day Treatment Periods. The last dose in each Treatment Period will be the morning of the Day 7 clinic visit, and the Treatment Periods will be separated by a 7-day washout period (Figure 1).

Each subject will take 4 dose levels of blinded study medication, with one dose level taken during each of the Treatment Periods (Table 5).

Table 5: Dose Levels

Dose Level	Treatment
A	glycopyrrolate (85 µg) BID
B	glycopyrrolate (42.5 µg) BID
C	glycopyrrolate (14 µg) BID
D	placebo BID

Subjects will be randomly assigned to Treatment Sequences 1 to 4 in a 1:1:1:1 ratio to receive the 4 dose levels in the following sequences:

Treatment Sequence	Dose Levels Administer in Each Treatment Period			
	Treatment Period 1 (N = 40)	**Treatment Period 2 (N = 40)**	**Treatment Period 3 (N = 40)**	**Treatment Period 4 (N = 40)**
Sequence 1 (N = 10)	A	D	B	C
Sequence 2 (N = 10)	B	A	C	D
Sequence 3 (N = 10)	C	B	D	A
Sequence 4 (N = 10)	D	C	A	B

Each subject is expected to complete all 4 Treatment Periods. Subjects who do not complete at least 2 Treatment Periods may be replaced.

Spirometry will be assessed pre- and post-dose on Days 1 and 7, as described in Table 2.

PK sampling will take place on Day 7 in Treatment Periods 1 and 2 only following any ECG or lung function assessments, as described in Table 3.

The pre-dose FEV_1 at on Day 1 of Treatment Periods 2 to 4 (i.e., Visits 4, 6, and 8) should be within ± 20% of the pre-dose- FEV_1 at Day 1 of Treatment Period 1 (Visit 2) to ensure a consistent baseline FEV_1 for each Treatment Period. If the FEV_1 varies by more than 20%, the start of the Treatment Period must be rescheduled to occur within 7 days (Section 6.1.1).

Screening for eligibility will include a responsiveness test (spirometry both pre- and post-4 puffs of albuterol) between 7 and 14 days before the first dose of blinded study medication at Visit 2. The proportion of the study population who have a post-albuterol FEV_1 increase of < 12% or < 200 mL will be capped at approximately 50%.

Prohibited medications and the prohibited time intervals prior to Screening are described in Section 6.6.3. Discontinuation of any maintenance COPD therapies for the sole purpose of study eligibility is not recommended.

Stable use of BID LABA ± ICS at a maintenance dose is permitted during the study (Table 7).

At the Screening (Visit 1), all subjects will be dispensed albuterol as rescue medication to be used as needed during the screening, treatment, and washout periods. Subjects who are required to withhold medications prior to Screening (Visit 1) will be dispensed albuterol as rescue medication upon signing of the Informed Consent Form (ICF) for use prior to Visit 1.

Subjects should withhold rescue medication use for ≥ 4 hours and, if applicable, BID LABA ± ICS use for approximately 24 hours prior to spirometry at all required visits. If the rescue medication withholding period is not met or if BID LABA ± ICS medications are taken the morning of a clinic visit where spirometry is conducted, the subject will be rescheduled for a repeat visit within 7 or 3 days of Day 1 or Day 7, respectively (Section 6.1.1).

All sites will use standardized spirometry and ECG equipment provided by a central vendor, and all spirometry and ECGs will be over-read by the blinded central vendor.

Subjects will be screened for eligibility during a screening period of 7 to 14 days.

During each Treatment Period, subjects will complete all assessments and procedures as outlined in the Schedule of Activities (SoA; Table 1). Subjects will receive a phone call reminder on Day 6 of each Treatment Period to remind them to take their evening dose of blinded study medication approximately 12 hours prior to the anticipated arrival time to the clinic on Day 7, to note the time of the Day 6 evening dose was taken, to report that time to site staff at the Day 7 visit, to withhold the Day 7 morning dose until at the clinic, to withhold albuterol within 4 hours of their Day 7 visit, if applicable, to withhold BID

LABA ± ICS within approximately 24 hours of their Day 7 visit, and to bring their unused study medication to the clinic visit the next day.

Subjects completing treatment will complete a Follow-Up Phone Call 7 ± 1 days after the last scheduled study visit.

Subjects who experience ≥ 1 moderate or severe COPD exacerbation during the study will discontinue study medication and be withdrawn from the study.

Subjects who permanently discontinue double-blind study medication will be withdrawn from the study.

Subjects meeting the withdrawal criteria during the study or who withdraw from the study for other reasons will be discontinued and requested to complete the Early Termination/Withdrawal Procedures and complete a Follow-Up Phone Call 7 ± 1 days after the Early Termination/Withdrawal Visit.

4.2 Study Design Justification

The purpose of this study is to inform the dose selection of glycopyrrolate for the planned Phase 2 Study CO-212. Study CO-212 is planned as a 4-week parallel group study to compare multiple doses of glycopyrrolate combined with 3 mg of ensifentrine as a FDC to the glycopyrrolate and ensifentrine individual components and placebo.

This study will use pulmonary function test results and PK analyses to perform an initial evaluation of glycopyrrolate doses over a dose range. The study intends to enroll a population of subjects with stable, moderate to severe COPD. COPD patients can exhibit varying degrees of responsiveness to bronchodilators such as albuterol, with approximately 20 to 50% of clinical trial populations displaying ≥ 12% and ≥ 200 mL increases in FEV_1 following dosing with albuterol (Hanania et al. 2011; Anzueto et al. 2023). Given that this study is intended to inform on dose-responsiveness to a bronchodilator (glycopyrrolate), entry criteria is planned to enroll approximately 50% of COPD subjects with ≥ 12% and ≥ 200 mL increase in FEV_1 following albuterol in order to optimally assess differences in dose levels.

Incorporation of a placebo arm into the study design will allow accurate interpretation of treatment effect for each dose level and clear identification of the proper glycopyrrolate doses for use in Study CO-212.

The use of a placebo arm in the study is justified for the following reasons:

- Subjects will be permitted to be on BID LABA ± ICS background therapy to treat their COPD throughout the study.
- Subjects with unstable COPD (i.e., any severe COPD exacerbations within 6 months of Screening) are excluded.

- Discontinuation of any maintenance COPD therapies for the sole purpose of study eligibility is not recommended.
- The maximum continuous period that a subject could be on placebo and no study medication is 3 weeks (1 week of placebo therapy between two 1-week wash-out periods).
- The maximum continuous period that a subject could be on placebo, low-dose glycopyrrolate, and no active study medication is 5 weeks (i.e., 1 week of placebo therapy and 1 week of low-dose glycopyrrolate, each between two 1-week wash-out periods).

4.2.1 Justification for Dose

The Lonhala Magnair (glycopyrrolate) inhalation solution (25 µg/1 mL) aerosol performance has been reported in the literature (Pleasants and II 2019; Sunovion 2019). An appropriate dose range for glycopyrrolate solution in the proposed FDC formulation delivered via standard jet nebulizer (Pari Sprint) was determined based on performance characteristics of Lonhala Magnair. Based on the fine particle dose and delivered dose comparison to Lonhala 25 µg (the approved BID dose), it is expected that a 42.5 µg BID dose level will approximate the aerosol characteristics of the Lonhala Magnair (eFlow) glycopyrrolate 25 µg dose. Additionally, a higher dose of 85 µg (equivalent to 50 µg Lonhala dose) and lower dose of 14 µg (equivalent to 6.25 µg Lonhala dose, which produced a suboptimal FEV_1 response in subjects with COPD) are planned in order to characterize the dose-response and confirm appropriate glycopyrrolate dose selection in subjects with COPD when delivered via standard jet nebulizer in the proposed FDC formulation.

4.2.2 Justification for Duration

The Lonhala Phase II development program confirmed that PK and pharmacodynamic steady state was achieved within 7 days of BID glycopyrrolate inhalation solution dosing (Pleasants and II 2019; Sunovion 2019). Regarding PK, the mean $AUC_{0\text{-}t}$ for 12.5 µg and 50 µg glycopyrrolate were 89 and 255 pg·hr/mL at Day 7 (BID dosing) with corresponding accumulation indices of 2.051 and 1.398, respectively (Saluja et al. 2017). Assuming t = 12 hours and linear pharmacokinetics, elimination rate constants of 0.0557 and 0.1047, respectively, were derived. These parameters were used to estimate that greater than 99% steady state was reached after the seventh and fourth dose (respectively) of glycopyrrolate BID treatment. In this study, we anticipate PK sampling on Day 7 will represent glycopyrrolate steady state. In Lonhala Phase IIb program, a similar magnitude of effect in morning trough FEV_1 was shown with a 50 µg BID dose after 1 week of dosing compared with 4 weeks of dosing (placebo-corrected change from baseline 146 mL) (Karimi-Shah 2017), supporting that a 1-week duration is sufficient to inform on lung function effects of BID inhaled glycopyrrolate solution in the proposed

FDC formulation. A 7-day washout is planned to ensure that all subjects exceed 5 elimination half-lives for glycopyrrolate between treatment periods.

4.3 End of Study Definition

A subject is considered to have completed the study if he/she has successfully completed all scheduled visits and completed follow-up contact. With no rescheduled visits, the study should last approximately 10 weeks for each subject.

The end of the study is defined as the date of the last follow-up contact of the last subject in the study.

5.0 STUDY POPULATION

Prospective approvals of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, are not permitted.

5.1 Inclusion Criteria

Informed Consent

1. Capable of giving informed consent indicating that they understand the purpose of the study and study procedures and agree to comply with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

Age and Sex

2. Age: Subject must be 40 to 80 years of age inclusive, at the time of Screening.
3. Sex:

- Males are eligible to participate if they agree to use contraception as described in the contraceptive guidance (Appendix 7) from Screening and throughout the study and for at least 30 days after the last dose of blinded study medication.
- Females are eligible to participate if they are not pregnant, not breastfeeding, and ≥ 1 of the following conditions apply:

    a) Not a woman of childbearing potential (WOCBP) as defined in Appendix 7.

    OR

    b) A WOCBP who agrees to follow the contraceptive guidance in Appendix 7 from Screening and throughout the study and for at least 30 days after the last dose of blinded study medication.

Smoking History

4. Smoking History: Current or former cigarette smokers with a history of cigarette smoking ≥ 10 pack years at Screening [number of pack years = (number of cigarettes per day / 20) × number of years smoked (e.g., 20 cigarettes per day for 10 years, or 10 cigarettes per day for 20 years)]. Pipe and/or cigar use cannot be used to calculate pack-year history. Former smokers are defined as those who have stopped smoking for at least 6 months prior to Screening. Smoking cessation programs are permitted during the study.

COPD Diagnosis, Symptoms, Severity and Maintenance Therapy

5. COPD Diagnosis: Subjects with an established clinical history of COPD as defined by the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines (Celli et al. 2004) with symptoms compatible with COPD.
6. Post-bronchodilator (4 puffs of salbutamol) spirometry at Screening demonstrating both the following:
   a. FEV_1/forced vital capacity (FVC) ratio of < 0.70 AND
   b. $FEV_1 \geq 30$ % and ≤ 70% of predicted normal (Quanjer et al. 2012).

   Note: The proportion of the study population who have a post-albuterol FEV_1 increase of < 12% or < 200 mL will be capped at approximately 50%.
7. A posterior-anterior chest x-ray (CXR) at Screening or within 12 months prior to Screening showing no clinically significant abnormalities unrelated to COPD. If a CXR within the past 12 months is not available but a computed tomography (CT) scan within the same time period is available, the CT scan may be reviewed in place of a CXR.
8. Capable of withdrawing from short-acting bronchodilators for 4 hours prior to spirometry testing and from BID LABA ± ICS therapy for 24 hours prior to spirometry.

Other Requirements for Inclusion

9. Capable of using the study nebulizer correctly.
10. Ability to perform acceptable spirometry in accordance with ATS/ERS guidelines (Graham et al. 2019).
11. Willing and able to attend all study visits and adhere to all study assessments and procedures.

5.2 Exclusion Criteria

Current Condition or Medical History

1. Concomitant clinically significant pulmonary disease other than COPD (i.e., asthma, tuberculosis, lung cancer, bronchiectasis, sarcoidosis, lung fibrosis, interstitial lung diseases, sleep apnea (unless controlled with stable continuous positive airway pressure [CPAP] use), known alpha-1 antitrypsin deficiency, core pulmonale or other non-specific pulmonary disease).

2. Within 6 months prior to Screening:
    a. COPD exacerbation requiring hospitalization.
    b. Use of therapies for COPD exacerbation (e.g., oral, intravenous, or intramuscular glucocorticoids).
3. Lower respiratory tract infection within 6 weeks of Screening or an active infection at Screening.
4. History of life-threatening COPD, including Intensive Care Unit admission and/or requiring intubation.
5. Previous lung resection or lung reduction surgery within 1-year of Screening.
6. Long term oxygen use defined as oxygen therapy prescribed for greater than 12 hours per day. As needed oxygen use (≤ 12 hours per day) is not exclusionary.
7. Severe comorbidities including unstable cardiac, (e.g., myocardial infarction within 1 year prior to screening, unstable angina within 6 months prior to screening, or unstable or life-threatening arrhythmia requiring intervention within 3 months prior to screening, diagnosis of New York Heart Association (NYHA) class III or IV heart disease) or any other clinically significant medical conditions including uncontrolled diseases (e.g., endocrine, neurological, hepatic, gastrointestinal, renal, hematological, urological, immunological, psychiatric or ophthalmic diseases) that would, in the opinion of the Investigator, preclude the subject from safely completing the required tests or the study, or is likely to result in disease progression that would require withdrawal of the subject.
8. History of or clinically significant on-going bladder outflow obstruction or history of catheterization for relief of bladder outflow obstruction within the previous 6 months.
9. History of narrow angle glaucoma.
10. History of hypersensitivity or intolerance to aerosol medications, salbutamol or glycopyrrolate or any of its excipients/components, anticholinergic agents, or sympathomimetic amines.
11. Pulmonary rehabilitation unless such treatment has been stable from 4 weeks prior to Screening (Visit 1) and remains stable during the study. Pulmonary rehabilitation programs should not be started or completed during participation in the study.

12. Major surgery (requiring general anesthesia) in the 6 weeks prior to Screening, lack of full recovery from surgery at Screening, or planned surgery through the end of the study.
13. History of or current malignancy of any organ system, treated or untreated within the past 5 years, except for localized basal or squamous cell carcinoma of the skin.
14. Significant psychiatric disease that would likely result in the subject not being able to complete the study, in the opinion of the Investigator.
15. Findings on physical examination that an investigator considers to be clinically significant at Screening.

Prior/Concomitant Therapy

16. Use of prohibited medications within the time intervals defined in Section 6.6.3.

History of Drug or Alcohol Abuse

17. Current or history of drug or alcohol abuse within the past 5 years.

Laboratory and Other Diagnostic Parameters

18. Estimated glomerular filtration rate (eGFR) < 30 mL/min. The Chronic Kidney Disease Epidemiology Collaboration Creatinine (2021) calculation will be used (Inker et al. 2021).
19. Alanine aminotransferase (ALT) ≥ 2 × upper limit of normal (ULN), alkaline phosphatase and/or bilirubin > 1.5 × ULN (isolated bilirubin > 1.5 × ULN is acceptable if fractionated bilirubin < 35%).
20. Any other abnormal hematology, biochemistry, or viral serology deemed by the Investigator to be clinically significant. Abnormal chemistry and/or hematology may be repeated during Screening.
21. Abnormal ECG (Appendix 4) at the screening visit that is clinically significant.

Other Exclusions

22. Women who are breast feeding.
23. Use of an experimental drug within 30 days or 5 half-lives of Screening, whichever is longer, and/or participation in a study treatment-free follow-up phase of a clinical study within 30 days prior to Screening.
24. Use of an experimental medical device or participation in a follow-up phase of an experimental medical device clinical study within 30 days prior to Screening.

25. Affiliation with the investigator site, including an Investigator, Sub-Investigator, study coordinator, study nurse, other employee of participating investigator or study site or a family member of the aforementioned.
26. A disclosed history or one known to the Investigator of significant noncompliance in previous investigational studies or with prescribed medications.
27. Any other reason that the Investigator considers makes the subject unsuitable to participate.

5.3 Randomization Criteria

If a subject does not meet all inclusion criteria, meets any exclusion criteria, and meets one of the following criteria at Visit 2 prior to randomization, the subject will be considered a screen failure. Subjects discontinued prior to randomization may be replaced. The discontinued subject may be rescreened if the procedure outlined in Section 5.5.1 is followed.

Criteria for Exclusion from Randomization

1. COPD exacerbation or lower respiratory tract infection between Screening and Randomization (defined as use of any additional treatment other than current treatment and rescue medication and/or emergency department or hospital visit). Subjects with a severe COPD exacerbation that requires hospitalization may not be rescreened.
2. Prohibited medication use between Screening and Visit 2.
3. Significantly abnormal ECG finding, as defined in Appendix 4, on the 12-lead ECG obtained pre-dose at Visit 2 as assessed by the investigator or site medical doctor/medically qualified person before the subject has been randomized.
4. Did not meet ≥ 1 of the Inclusion Criteria (Section 5.1) or met ≥ 1 of the Exclusion Criteria (Section 5.2) as assessed through data or analyses produced between Screening and Randomization.
5. At Screening the subject's albuterol responsiveness test had an FEV_1 increase of < 12% or < 200 mL after 4 puffs of albuterol AND the randomization cap of approximately 50% of subjects in that responsiveness category has been met.

5.4 Lifestyle Considerations

5.4.1 Caffeine and Tobacco

Recommendation/request: Subjects should refrain from smoking and/or caffeinated beverages prior to Spirometry and ECGs for the withholding periods below:

- Smoking: No smoking for at least 1-hour prior to each spirometry and ECG assessment.
- Caffeinated Beverages: Abstain from drinking caffeinated beverages (e.g., tea, coffee) for 2 hours prior to each spirometry and ECG assessment.

5.5 Rescreening

Clinically significant abnormal chemistry and/or hematology may be repeated during Screening.

5.5.1 Rescreening of Subjects

Subjects may *not* be rescreened if their post-albuterol spirometry at Screening demonstrated any of the following:

- FEV_1/FVC ratio $\geq$ 0.7 OR
- FEV_1 < 30% of predicted normal OR
- FEV_1 > 70% of predicted normal.

Rescreening is allowed only once for a screen failure due to both of following:

- A subject's Screening albuterol responsiveness test had an FEV_1 increase of < 12% or < 200 mL after 4 puffs of albuterol AND
- The randomization cap of approximately 50% of subjects in that responsiveness category has been met.

Subjects who are screen failures for other reason (Section 5.6) may be rescreened with approval from the Medical Monitor.

Rescreened subjects should be assigned a new subject number different from the initial Screening event.

5.6 Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but were not randomized to receive blinded study medication. See Section 7.1.1 for instructions on managing subjects who did not meet the criteria for randomization but were randomized in error.

A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials publishing

requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAEs.

6.0 STUDY TREATMENT

Subjects will be randomly assigned to 1 of 4 dosing sequences (Section 4.1) which prescribe the specific order for treatment with each of the glycopyrrolate doses and placebo (Table 5). Subjects will receive double-blind study medication (either glycopyrrolate or placebo) in the proposed FDC formulation administered by inhalation via a standard jet nebulizer (Section 6.2.2) supplied by the Sponsor. To account for visit windows, sufficient drug supply will be dispensed for up to 11 days of treatment for each treatment period.

In this protocol the terms 'investigational product', 'double-blind study medication', and 'blinded study medication' are the same and refer to the blinded nebulized study medication.

Subjects taking maintenance BID LABA ± ICS medications should take the LABA ± ICS medication prior to taking blinded study medication when taking the blinded study medication at home.

6.1 Administration of Blinded Study Medication

6.1.1 Dosing in the Clinic

The Day 1 Clinic Visit *MUST BE RESCHEDULED* to occur within 7 days of the scheduled visit if any of the following apply:

- If the rescue medications are not withheld for 4 hours prior to spirometry.
- If the pre-dose FEV_1 on Day 1 of the current Treatment Period is not within ± 20% of the pre dose FEV_1 at Day 1 of the first Treatment Period
- For a subject on a maintenance BID LABA ± ICS therapy, if the BID LABA ± ICS are not withheld for approximately 12 hours (e.g. LABA maintenance therapy taken the morning of the clinic visit) prior to spirometry.

The Day 7 Clinic Visit *MUST BE RESCHEDULED* to occur within 3 days of the scheduled visit if any of the following apply:

- If the rescue medications are not withheld for 4 hours prior to spirometry.
- For a subject on a maintenance BID LABA ± ICS therapy, if the BID LABA ± ICS are not withheld for approximately 12 hours (e.g. LABA maintenance therapy taken the morning of the clinic visit) prior to spirometry.
- If the morning dose of blinded study medication is not withheld.
- If the subject has not brought the unused ampule for the Day 7 morning dose to the clinic.

Dosing Visit Schedule and Time(s) of Day

Coordination of the timing of spirometry and in-clinic dosing with blinded study medication is critical. **See Section 1.4, for the Schedule of Spirometry and In-Clinic Dosing with Blinded Study Medication.**

Blinded Study Medication Administration

A PARI LC Sprint® jet nebulizer (study supplied nebulizer), with a PARI PRONEB® Max Aerosol Delivery System or equivalent (study supplied compressor) will be dispensed to the subject on Visit 2 and used to dose the blinded study medication in the clinic (Visit 2 only) and at home. A new PARI LC Sprint® jet nebulizer will be supplied at each Day 1 of a treatment period (i.e., Visits, 4, 6, and 8).

- Blinded study medication will be administered by inhalation of an aerosol generated by the reusable study supplied nebulizer attached to a study supplied compressor.
- Site staff must place blinded study medication at room temperature for approximately 15 minutes prior to shaking and opening the ampule.
- The site staff member will need to follow the nebulizer Set-Up instructions provided with the nebulizer and the Pharmacy Manual to prepare and administer the blinded study medication.
- Administration of nebulized blinded study medication will be observed by the site staff member from start of nebulization until end time of nebulization. The site staff member MUST administer and observe blinded study medication at all in-clinic dosing times.
- Blinded study medication nebulization time should be approximately 5 to 10 minutes.
- The end time of blinded study medication nebulization is when a slight sputtering sound from the nebulizer is heard. This will be considered Time 0 for the purposes of scheduling all post-dose study procedures.
- The date, the start time, and the end time of blinded study medication nebulization will be recorded by the site staff member in the subject source document and the electronic case report form (eCRF).

Subjects will take their study supplied nebulizer and study supplied compressor as well as instructions home for use during the study. Only a study supplied nebulizer and a study supplied compressor may be used to administer blinded study medication during the study. The study supplied nebulizer and a study supplied compressor should be used only for the administration of blinded study medication.

6.1.2 Dosing at Home

Following Visit 2, subjects will self-administer their daily morning and evening doses at home at approximately the same times, approximately 12 hours apart, each day for the duration of the Treatment Period, except for clinic visits on Days 1 and 7 of each Treatment Period when subjects will have their morning doses administered in the clinic.

Subjects should note the time of their evening dose on Day 6 for each Treatment Period and report that time to the clinic site staff at the Day 7 Visit.

Blinded Study Medication Administration

- Each subject will follow the Nebulizer Set-Up instructions and blinded study medication label to prepare and self-administer the blinded study medication by inhalation using the reusable study supplied nebulizer attached to the study supplied compressor.
- Subjects must place blinded study medication at room temperature for approximately 15 minutes prior to shaking and opening the ampule.
- Blinded study medication nebulization time should be approximately 5 to 10 minutes.
- The end time of blinded study medication nebulization is considered to be when a slight sputtering sound from the nebulizer is heard.

Used Blinded Study Medication

- Subjects will be instructed to discard all used ampules and open foil pouches of blinded study medication at home.

Unused Blinded Study Medication

- Subjects should be instructed to ***bring all unused*** blinded study medication to the clinic at each clinic visit for Day 7 morning dosing, collection, and assessment of medication compliance by a site staff member.

6.2 Investigational Product/Blinded Study Medication

Investigational product/blinded study medication is described in Table 6.

Table 6: Investigational Product/Blinded Study Medication Details

Name	**Glycopyrrolate**	**Placebo**
Dosage Formulation:	Nebulizer solution	Nebulizer solution
Unit Dose:	14, 42.5, or 85 µg	0 mg
Volume of dose	2.5 mL	2.5 mL
Route of Administration	Inhalation	Inhalation
Dosing Instructions:	1 ampule (2.5 mL) per dose	1 ampule (2.5 mL) per dose
Manufacturer	The Ritedose Corporation, Columbia, SC, USA	The Ritedose Corporation, Columbia, SC, USA

The glycopyrrolate formulation is a sterile solution of dissolved glycopyrrolate in [redacted] saline and is supplied as a 2.5 mL nominal fill in single unit-dose -low-density polyethylene (LDPE) translucent ampule overwrapped in a foil pouch.

The placebo is the same as the glycopyrrolate solution except that the active glycopyrrolate ingredient is omitted, (i.e., it consists of [redacted]) and is also supplied as a single unit-dose LDPE translucent ampule overwrapped in a foil pouch.

Both glycopyrrolate and placebo are manufactured using aseptic manufacturing techniques in accordance with Good Manufacturing Practice guidelines and will be provided to the sites as subject kits.

6.2.1 Blinded Study Medication Dispensing Information

Subjects will be dispensed blinded study medication kit(s) as per the schedule outlined in the SoA (Table 1).

6.2.2 Medical Devices

No Sponsor manufactured medical device is used in this study.

Other medical devices provided for use in this study are:

- PARI LC ®Sprint nebulizer
- PARI PRONEB ®Max Aerosol Delivery System or equivalent

Instructions for medical device use are provided in the package insert.

The blinded study medication should only be administered with the study provided nebulizer. No medications other than the blinded study medication should be administered with the study provided nebulizer.

6.3 Preparation/Handling/Storage/Accountability

Blinded study medication should be stored in a refrigerator between 2°C and 8°C (36°F and 46°F) and should not be frozen.

An investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all blinded study medication received and any discrepancies are reported and resolved before use of the blinded study medication.

All blinded study medication must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized study center staff.

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

Blinded study medication should be placed at room temperature for approximately 15 minutes prior to shaking and opening the ampule.

Only subjects meeting the Randomization Criteria in the study may receive blinded study medication, and only authorized study center staff may supply or administer blinded study medication.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for blinded study medication accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused blinded study medication are provided in the Pharmacy Manual.

The Investigator, a member of the study center staff, the site pharmacist, or pharmacy team member must maintain an adequate record of the receipt and distribution of all blinded study medication using the Drug Accountability Form. These forms must be available for inspection at any time.

6.4 Randomization and Blinding

Both glycopyrrolate and placebo study medication and pouches and packaging are identical in appearance.

Packaging of both glycopyrrolate and placebo will have a blinded description applied and all packs will have a unique kit number applied for identification.

Deliveries of both glycopyrrolate and placebo will not indicate the actual product but will instead indicate a blinded description.

Subjects will be randomized to a sequence which prescribes the order they will receive each glycopyrrolate dose and placebo dose over each treatment period using an electronic Interactive Response Technology (IRT). The IRT will not indicate the treatment sequence during the randomization process but will indicate the unique kit numbers to be dispensed to the subject at Visits 2, 4, 6, and 8.

Site staff will only have access to blinded reports within the IRT.

6.5 Emergency Unblinding

The blind will be broken only if specific emergency treatment would require knowing the treatment status of the subject. If the blind needs to be broken, the Investigator will contact the Sponsor or Medical Monitor as soon as feasible. The Investigator may unblind the blinded study medication immediately if he/she feels it is necessary prior to contacting the Sponsor or Medical Monitor. However, the Investigator should promptly document and explain any premature unblinding to the Sponsor and Medical Monitor.

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

Unblinding that occurs for any other reason, including unintentional unblinding, will be documented and promptly reported to the Sponsor and Medical Monitor.

6.6 Concomitant Therapy

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy (Table 10).

6.6.1 Rescue Medication

All subjects will be dispensed albuterol as rescue medication to be used as needed during the screening, treatment, and washout periods at Screening (Visit 1). Subjects who are required to withhold medications prior to Screening (Visit 1) will be dispensed albuterol as rescue medication upon signing of the ICF for use prior to Visit 1.

6.6.1.1 Documentation of Rescue Medication Use

Documentation of albuterol during the study should be recorded on the concomitant medication page of the eCRF along with reason for use, dates of administration (start and end dates), and dosing information (dose and frequency). End date should be documented as "Ongoing."

Rescue albuterol used during clinic visits where spirometry will be conducted should be recorded in a module provided in the eCRF for each specific spirometry visit. Details (e.g., dose [number of puffs], date, and time of administration) will be recorded.

6.6.2 Permitted Rescue and Background Medications

Permitted rescue and background medications are provided in Table 7.

Any vaccine that is recommended by the subject's healthcare provider is permitted during the study and should be recorded in the eCRF.

Table 7: Permitted Rescue and Background Medications

Medication	Condition
Albuterol	Used as rescue medication as needed during the study. Must be withheld for at least 4 hours prior to spirometry.[1] Should be withheld for at least 4 hours prior to ECGs.
BID LABA ± ICS	Stable use of BID LABA ± ICS is permitted ONLY IF the subject: 1. Has been taking LABA ± ICS at any dosing frequency at a stable dose for at least 4 weeks prior to Screening AND 2. If on a non-BID LABA ± ICS therapy, can transition, to a BID LABA ± ICS ≥ 7 days prior to randomization for use for remaining duration of the study AND 3. Can withhold use of the BID LABA ± ICS for approximately 24 h prior to any clinic visit where spirometry will be performed (i.e., Visits 1 through 9).[1] BID LABA ± ICS use should not be initiated, dose modified, or discontinued during the study after randomization. ICS monotherapy and high dose ICS (e.g., > 1000 µg of fluticasone propionate or equivalent) is not allowed.
[1]If the withholding periods are not met the visit should be rescheduled (Section 6.1.1). Abbreviations: ECG = electrocardiogram; ICS = inhaled corticosteroids.	

6.6.3 Prohibited Medications/Therapy

Prohibited medications and therapies are provided in Table 8. All prohibited medications are not to be taken during the time periods described.

Discontinuation of any maintenance COPD therapies for the sole purpose of study eligibility is not recommended.

Table 8: Prohibited Medications/Therapy

Medication	Time Interval
Oral Therapies for COPD (e.g., oral steroids, theophylline, and roflumilast). Oral mucolytics are allowed.	6 months prior to Screening and prohibited during the study.
Terbutaline	24 hours prior to Screening and prohibited during the study.
LAMAs 1. Once-daily LAMAs 2. BID LAMAs	 1. 48 hours prior to Screening and prohibited during the study 2. 24 hours prior to Screening and prohibited during the study
Non-BID LABAs	Any LABAs that are not dosed BID (e.g., once daily) must be discontinued ≥ 7 days prior to Randomization and are prohibited during the study.

Medication	Time Interval
SAMA, including SAMA/SABA combination therapy (i.e., ipratropium/albuterol combination products)	24 hours prior to Screening and prohibited during the study.
Oral or systemic muscarinic antagonists (e.g., darifenacin, fesoterodine, imidafenacin, oxybutynin, and solifenacin, benztropine and trihexyphenidyl, atropine)	7 days prior to Screening and prohibited during the study.
Non-study provided nebulized therapies	4 weeks prior to Screening and prohibited during the study.
ICS	Starting or stopping ICS is not allowed during the study. ICS monotherapy and high dose ICS (e.g., > 1000 µg of fluticasone propionate or equivalent) is not allowed.
Abbreviations: BID = twice daily; COPD = chronic obstructive pulmonary disease; ICS = inhaled corticosteroids; LAMA = long-acting muscarinic antagonist; LABA = long-acting β2 agonist; SAMA = short-acting muscarinic antagonists; SABA = short-acting β2 agonist.	

6.6.4 Recording Use of Concomitant Medications

6.6.4.1 Recording of Rescue Medication During the Study

Rescue medication (albuterol) use during the study should be recorded as described in Section 6.6.1.

6.6.4.2 Recording of COPD Medications

All COPD medication use during the 6 months prior to Screening and during the study should be recorded on the concomitant medication page of the eCRF along with reason for use, dates of administration (start and end dates), and dosing information (dose and frequency).

6.6.4.3 Recording of Medications other than Rescue Medication and COPD Medications During the Study

All non-COPD medications will be recorded in the eCRF starting at Screening and changes recorded during the study. Information recorded will include, but may not be limited to items such as:

- Reason for use.
- Dates of administration including start and end dates.
- Dosage information including route, dose, and frequency.

6.7 End of Study Procedures

Seven ± 1 days after either the Study Visit 9 or after the Early Termination/Withdrawal Visit (Table 1), the site should contact the subject by telephone to complete the following assessments:

- Concomitant medications
- AEs and SAEs

6.8 Treatment After the End of Study

There are no plans to provide post-study treatment, including study medication for compassionate use, following study completion.

After the End of Study Follow-up Phone Call (Table 1), subjects may resume conventional COPD therapy as prescribed by the investigator or other physician.

Medications initiated after the end of study should not be entered into the eCRF except for those given for a SAE.

7.0 Discontinuation of Blinded Study Medication and Subject Discontinuation/Withdrawal

7.1 Discontinuation of Blinded Study Medication

Subjects may permanently discontinue blinded study medication before the end of the study if they choose to or at the Investigator's discretion. Subjects who permanently discontinue blinded study medication are required to withdraw from the study after completion of the Early Withdrawal/Termination procedures described in Table 1 and Section 7.3 as soon as possible.

Circumstances potentially requiring or actually resulting in discontinuation of blinded study medication are described below.

7.1.1 Does Not Meet Criteria for Randomization

A subject who does not meet the Randomization Criteria (Section 5.3) should not be randomized, will be considered a screen failure (Section 5.6), and may be eligible for rescreening (Section 5.5.1).

If a subject who does not meet the Randomization Criteria (Section 5.3) is inadvertently randomized and receives blinded study medication, the Medical Monitor must be contacted and will consult with the Sponsor to determine if the subject may continue, must be discontinued from blinded study medication, and/or discontinued from the study. Such inadvertently randomized subjects, if discontinued, may be replaced, but are not eligible for rescreening (Section 5.5.1).

7.1.2 Liver Chemistry Stopping Criteria

Discontinuation of blinded study medication for abnormal liver function should be considered by the Investigator when a subject meets 1 of the conditions outlined in Appendix 8 or if the Investigator believes that it is in best interest of the subject.

7.1.3 Electrocardiogram Withdrawal Criteria

All ECGs conducted or overread after a subject has been randomized will be subject to ECG Withdrawal Criteria (Appendix 5). Subjects may be discontinued from blinded study medication if they experience a significant abnormality(ies) on their ECG including, but not limited to those listed in the ECG Withdrawal Criteria, and in consideration with the subject's medical history and baseline ECG measurements. Clinically significant abnormalities as determined by the Investigator may also result in subject discontinuation from study medication per the Investigator's judgement. Triplicate ECGs (over a brief period of time) should be performed on subjects experiencing a significant abnormality on their ECG or other clinically significant abnormality as determined by the Investigator. The intent of triplicate ECG measurements

is to differentiate significant abnormalities from artifact. Abnormalities should be confirmed in 2 of 3 triplicate ECG measurements.

7.1.4 Positive Pregnancy Test in Females

Women who are pregnant or breastfeeding are not eligible to participate. Pregnancy testing will be conducted in women of childbearing potential at Screening and at other times specified in the SoA (Table 1).

Women exhibiting a positive pregnancy test (Appendix 3) during the study will be discontinued from blinded study medication and followed-up per the Collection of Pregnancy Information guidelines in Section 8.7.5 and Appendix 7.

7.1.5 COPD Withdrawal Criteria

Subjects who experience ≥ 1 moderate (i.e., requiring at least 3 days oral/systemic corticosteroids or antibiotics) or severe COPD exacerbation (i.e., requiring hospitalization) during the study will be discontinued from the blinded study medication and be withdrawn from the study.

7.1.6 Other Criteria

Other criteria that may or may not require permanent discontinuation of blinded study medication include but are not limited to AE, lack of efficacy, protocol deviation, non-compliance, study or site closed/terminated, investigator discretion, or subject withdrawal of consent.

7.2 Blinded Study Medication Compliance

The prescribed dosage, timing, and mode of administration may not be changed. Any departures from the intended regimen must be recorded in the eCRFs.

At the Day 7 visit, previously dispensed unused blinded study medication (i.e., unopened, or unused blinded study medication in taped foil pouches) will be retrieved by the site staff and compliance will be assessed. Subjects exhibiting poor compliance as assessed by the number of ampule/foil pouch counts dispensed and returned should be counseled on the importance of good compliance to the study dosing regimen and this counseling should be documented in the subject source document.

Noncompliance is defined as taking less than 70% of blinded study medication during any evaluation period (visit to visit).

7.2.1 Discontinuation of the Study

If the Sponsor discontinues development of the FDC product or terminates the study, all subjects will be permanently discontinued from blinded study medication and should

complete the Early Withdrawal/Termination procedures described in Table 1 and Section 7.3 as soon as possible.

7.3 Subject Discontinuation/Withdrawal from the Study

A subject may withdraw from the study at any time at his/her own request or may be withdrawn from administration of blinded study medication and from all study participation at the discretion of the Investigator at any time for safety, behavioral, compliance, or administrative reasons. See the SoA (Table 1) for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

- If the subject withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a subject withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the study center study records.

Subjects who permanently discontinue the blinded study medication will be withdrawn from the study.

Subjects who require an extended washout period > 28 days will be withdrawn from the study.

Subjects who withdraw prior to completing 2 treatment periods may be replaced.

7.3.1 Early Withdrawal/Termination Procedures

If a subject is withdrawn prior to completion of the study, every attempt should be made to have the subject complete the Early Withdrawal/Termination procedures in the SoA (Table 1) as soon as possible and the End of Study follow-up phone call (Section 6.7).

7.4 Lost to Follow-up

A subject will be considered lost to follow-up if he or she fails to return for scheduled visits and is unable to be contacted by the study center.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The study center must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address

or local equivalent methods). These contact attempts should be documented in the subject's medical record.

- Should the subject continue to be unreachable, he/she will be considered to be lost to follow-up and will be discontinued from the study.

8.0 STUDY ASSESSMENTS AND PROCEDURES

Subjects may not complete any Screening Visit Procedures including the Spirometry Assessment unless all prohibited medications/therapy described in Section 6.6.3 have been withheld for the defined time interval.

Study procedures and their timing are summarized in the SoA (Table 1).

Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the subject should continue or discontinue blinded study medication.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct. In the event a public emergency or natural disaster impacts a subject's ability to complete study requirements, refer to Section 8.9.

All Screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The Investigator will maintain a Screening log to record details of all subjects screened and to CONFIRM eligibility or record reasons for Screening failure, as applicable.

Procedures conducted as part of the subject's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be utilized for Screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA (Table 1).

The maximum amount of blood collected from each subject over the duration of the study, including any extra assessments that may be required, will not exceed 300 mL. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

See Section 5.6 for specific information to be collected for screen failures.

8.1 Screening Assessments and Procedures

8.1.1 Informed Consent

Informed consent must be obtained according to the informed consent process described in Appendix 2.

8.1.2 Demographic Variables

Demographic variables will include items such as age, sex, ethnicity, and race.

8.1.3 Medical, Surgical, COPD, and Smoking History

A history of relevant current or past medical conditions, surgical history, COPD history, and smoking history (current or former [yes or no]) will be obtained:

- Minor surgical procedures (e.g., tonsillectomy, appendectomy) performed more than 5 years prior to Screening do not need to be recorded.
- COPD history will include COPD exacerbation history and COPD type (emphysema and/or chronic bronchitis), as assessed by the investigator or medical professional). Chronic bronchitis is defined as "regular production of sputum for 3 or more months in 2 consecutive years (in the absence of other conditions that may explain it)" (GOLD 2024).
- Former smokers are defined as those who have stopped smoking for at least 6 months prior to Screening.

Medical and COPD condition(s) identified during the Screening Visit will be documented as medical history and not as an AE(s) unless the condition worsens during the study and meets the definition of an AE.

8.1.4 Prior/Concomitant Medications/Therapies

A history of prior medications used will be obtained and recorded as described in Section 6.6.4.

8.1.5 Vital Signs

Vital signs will be completed at Screening per the instructions in Section 8.5.1. In addition, height in centimeters (cm), weight in kilograms (kg), and body mass index will also be measured and recorded at Screening.

8.1.6 Electrocardiogram

12-Lead ECGs will be completed per the instructions in Section 8.5.2.

8.1.7 Chest X-Ray

A CXR (posterior-anterior) will be obtained at Screening or obtained within 12 months prior to Screening. If a CXR within the past 12 months is not available but a CT scan within the same time period is available, the CT scan can be reviewed in place of a CXR.

8.1.8 Spirometry Assessment

Spirometry at Screening will be completed per the instructions in Section 8.3.1.

8.1.9 Clinical Laboratory Assessments

Clinical laboratory tests will be conducted per the instructions in Section 8.5.3. Clinically significant abnormal chemistry and/or hematology may be repeated during Screening.

8.1.10 Physical Examination

A complete physical examination will include, at a minimum, assessments of the Cardiovascular, Respiratory, Gastrointestinal, and Neurological systems.

Investigators should pay special attention to clinical signs to ensure subjects have had no previous serious physical or mental illness(es).

8.1.11 Inclusion/Exclusion Criteria

Subject eligibility will be assessed using the inclusion criteria (Section 5.1) and exclusion criteria (Section 5.2).

8.1.12 Interactive Response Technology

Subjects will be registered in the IRT system.

8.1.13 Nebulizer Equipment Training

See Section 8.6.4 for details on nebulizer equipment training. At the Screening Visit 1, site staff will review the information and instructions provided by the manufacturer with the subject (e.g., Instructions for Use, located in the Pharmacy Manual). Hands-on training with the nebulizer equipment will not occur until the Randomization Visit (Visit 2).

8.1.14 Dispense Rescue Medication

Rescue medication (albuterol) will be dispensed for use as needed during the study but must be withheld for at least 4 hours prior to spirometry and should be withheld for at least 4 hours prior to ECGs. See Section 6.6.2 for additional details.

8.2 Randomization Criteria Assessment – Visit 2

The criteria for Randomization are described in Section 5.3.

8.3 Efficacy Assessments

Planned timepoints for all efficacy assessments are provided in the SoA (Table 1).

8.3.1 Spirometry

Spirometry will be obtained using spirometry equipment provided by the central spirometry vendor that meets or exceeds the ATS minimal performance recommendations (Graham et al. 2019).

The central spirometry vendor will provide a spirometry manual with additional instructions and details.

All Spirometry will be reviewed and over-read by the central spirometry vendor and the spirometry vendor's determination of acceptable endpoint measurements will override determination by the investigative site.

- Acceptable spirometry efforts should have a satisfactory start of test and end of forced expiration (plateau in the volume-time curve) and be free from artifacts due to cough, glottic closure, early termination, poor effort, obstructed mouthpiece, equipment malfunction, or other reason (Graham et al. 2019).
- For FEV_1 and FVC determinations, at least 3 but no more than 8 acceptable spirometry efforts should be obtained.
- The largest FEV_1 and FVC from the 3 acceptable efforts should be recorded, even if they do not come from the same effort.

8.3.1.1 Spirometry Training

Prior to the first spirometry measurement at each clinic visit requiring Spirometry, the site staff will train each subject on how to perform acceptable spirometry maneuvers according to instructions provided by the central spirometry vendor.

8.3.1.2 Spirometry must be performed as follows:

- *Coordination of the timing of spirometry and in-clinic dosing with blinded study medication is critical.* See Section 1.4 for the Schedule of Spirometry and In-Clinic Dosing with Blinded Study Medication.
- Spirometry Start Time: between 06:00 AM and 10:00 AM.
- Withhold the Following or Reschedule the Visit:
  - *Blinded Study Medication*: Blinded study medication must be withheld until pre-dose spirometry is completed.
  - *Rescue Medication and BID LABA ± ICS*: Must be withheld per Section 6.1.1.

Recommendation/Request: Subjects should refrain from smoking and/or caffeinated beverages prior to Spirometry for the withholding periods described in Section 5.4.1.

8.4 Pharmacokinetic Assessments

At both Visits 3 and 5, blood samples of approximately 6 mL per time point will be collected at the 10 time points specified in Table 3 by venipuncture or via indwelling cannula in the forearm into lithium heparin tubes and will be immediately chilled (ice bath). The exact date/time of each blood sample collection will be recorded in the subject's eCRF. Please refer to the Laboratory Manual for details of PK sample handling.

8.5 Safety Assessments

Planned timepoints for all safety assessments are provided in the SoA (Table 1).

8.5.1 Vital Signs

Vital signs should be obtained prior to blinded study medication dosing at all visits.

Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.

Supine blood pressure and pulse measurements should be obtained after the subject has been at rest for at least 5 minutes in the supine position and located in a quiet setting without distractions (e.g., television, cell phones).

8.5.2 Electrocardiogram

12-lead ECG will be obtained using standardized ECG equipment provided by the centralized vendor. Albuterol should be withheld for at least 4 hours prior to ECG assessments. It is recommended that ECG assessments should be obtained after subjects have rested for approximately 5 minutes.

The central ECG vendor will provide an ECG manual with additional instructions and details.

All ECGs will be reviewed and over-read by the central ECG vendor and the ECG vendor's interpretation of the measurement(s) will override the interpretation by the investigative site if consensus is not reached.

8.5.2.1 Randomization/Visit 2: ECGs at Pre-Dose

At Day 1/Randomization/Visit 2 prior to dosing (pre-dose), an ECG must be performed.

8.5.2.2 Clinically Significant Abnormality

Review the ECG Withdrawal Criteria. If a clinically significant ECG abnormality is identified post-randomization, even if the assessment was pre-dose, the ECG Withdrawal Criteria in Appendix 5 should be reviewed and triplicate ECGs (over a brief period of time) should be performed to confirm the abnormality in 2 of 3 measurements.

8.5.2.3 Recommendation/Request

Subjects should refrain from smoking and/or caffeinated beverages prior to ECGs for the withholding periods described in Section 5.4.1.

8.5.3 Clinical Laboratory Assessments

See Appendix 3 for the list of clinical laboratory tests to be performed and additional instructions.

8.5.4 Adverse Event Assessment

The method of detecting AEs will be performed as described in Section 8.7 and Appendix 6.

8.6 Other Assessments and Procedures

Planned timepoints for all other assessments and procedures are provided in the SoA, Table 1.

8.6.1 Concomitant Medication Assessment

Subjects must be queried for concomitant medication use at each contact (clinic visit or phone contact). Concomitant medications must be documented in the eCRF. Concomitant medications/therapy and documentation are described in Section 6.6. The list of permitted rescue and background medications is provided in Section 6.6.2. The list of prohibited medications/therapy is provided in Section 6.6.3.

8.6.2 Interactive Response Technology

Sites will enter information into the IRT at specified visits. All subjects consented will be assigned a subject identification number upon signing of the ICF.

8.6.3 Randomization

Subjects meeting all inclusion criteria (Section 5.1) and none of the Randomization Criteria (Section 5.3) and exclusion criteria (Section 5.2) will be randomly assigned to a dosing sequence (Section 4.1) prescribing the order of blinded study medication use for each of the 4 treatment Periods via the IRT (Section 8.6.2) at Visit 2.

8.6.4 Nebulizer Equipment Training

Subjects and site staff will be trained in the use of the PARI LC Sprint nebulizer and PARI PRONEB Max Aerosol Delivery System, which will be used for inhalation of blinded study medication during the study period. Once the site staff have been trained, they will review the information and instructions provided by the manufacturer with the subject (e.g., Instructions for Use, located in the product box and also in the Pharmacy Manual). Hands on training with the nebulizer will occur at randomization with administration of the first dose of blinded study medication.

8.6.5 Training for Spirometry

Training for spirometry maneuvers will be conducted by the site staff as per the guidelines in the spirometry manual provided by the central spirometry vendor.

8.6.6 Blinded Study Medication Dosing in the Clinic

Blinded study medication dosing in the clinic will be conducted as described in Section 6.1.1.

8.6.7 Dispense Rescue Albuterol

Albuterol will be dispensed at specified study visits for use as needed. See Section 6.6.1 for additional detail.

8.6.8 Dispense/Collect Blinded Study Medication

Blinded study medication will be dispensed/collected at Days 1 and 7 of each Treatment Period (Table 1).

8.7 Adverse Events

The definitions of an AE or SAE can be found in Appendix 6.

AEs will be reported by the subject.

The Investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the blinded study medication or study procedures, or that caused the subject to discontinue the study (Appendix 6).

As stated in Section 8.1.3, medical and COPD condition(s) identified during the Screening Visit will be documented as medical history and not as an AE unless the condition worsens during the study and meets the definition of an AE.

8.7.1 Time Period and Frequency for Collecting AE and SAE Information

AEs and SAEs will be collected from time of first dose of blinded study medication through the follow-up contact with 1 exception. SAEs assessed as related to study participation (e.g., study treatment, protocol-mandated procedures, invasive tests or change in existing therapy) will be collected from the time of consent through the follow-up contact.

All SAEs will be recorded and reported to the Sponsor or designee within 24 hours, as indicated in Appendix 6. The Investigator will submit any updated SAE data to the Sponsor designee within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the blinded study medication or study participation, the Investigator must promptly notify the Sponsor.

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 6.

8.7.2 Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

8.7.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, through the follow-up contact, or the subject is lost to follow-up (as defined in Section 7.4). Further information on follow-up of AEs and SAEs is given in Appendix 6.

8.7.4 Regulatory Reporting Requirements for SAEs

Prompt notification by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a blinded study medication under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study medication under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and Investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and Sponsor policy and forwarded to Investigators, as necessary.

An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

8.7.5 Pregnancy

Details of all pregnancies in female subjects will be collected after the start of blinded study medication and until 30 days after the last dose of blinded study medication.

If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 7.

Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

8.7.6 Adverse Events of Special Interest

AEs of special interest that have been identified for glycopyrrolate:

- Paradoxical bronchospasm
    - As with other inhaled medicines, inhaled glycopyrrolate can produce paradoxical bronchospasm that may be life-threatening. If paradoxical bronchospasm occurs following dosing, it should be treated immediately with an inhaled, short-acting bronchodilator; blinded study medication should be discontinued.
- Immediate hypersensitivity reactions
    - Immediate hypersensitivity reactions may occur after administration of inhaled glycopyrrolate. If signs suggesting allergic reactions occur, in particular, angioedema (including difficulties in breathing or swallowing, swelling of the tongue, lips, and face), urticaria, or skin rash, blinded study medication should be discontinued.
- Worsening of narrow-angle glaucoma
    - Subjects with narrow-angle glaucoma are excluded from this study (Section 5.2). Subjects and investigators should be alert for signs and symptoms of acute narrow-angle glaucoma (e.g., eye pain or discomfort, blurred vision, visual halos or colored images in association with red eyes from conjunctival congestion and corneal edema).
- Worsening of urinary retention
    - Subjects with obstructed urine flow are excluded from this study (Section 5.2). Subjects and investigators should be alert for signs and symptoms of urinary retention (e.g., difficulty passing urine, painful urination), especially in subjects with prostatic hyperplasia or bladder-neck obstruction. Instruct subjects to consult a physician immediately should any of these signs or symptoms develop.

8.7.7 Disease-related Events and/or Disease-related Outcomes Not Qualifying as AEs or SAEs

COPD exacerbations are an expected disease-related outcome. COPD exacerbations including daily expected COPD symptoms (e.g., dyspnea, chest tightness, wheezing, cough, sputum, that are not associated with the dosing event) will not be collected as an AE unless they meet the definition of an SAE.

8.8 Treatment of Overdose

An overdose is defined as a dose greater than the total daily doses prescribed in this study which results in clinical signs and symptoms. These should be recorded by the investigator on the AE/SAE pages.

In the event of an overdose the investigator should use clinical judgement in treating the overdose and contact the study Medical Monitor. Verona Pharma is not recommending specific treatment guidance for overdose and toxicity management.

8.9 Emergency Procedures

Sufficient study drug is provided to enable up to 11 days of dosing in a given treatment period. In the event of a public emergency or natural disaster, the dosing interval may be extended up to 11 days to allow at least 6 consecutive days of dosing prior to the End of Treatment Period Visit (nominal Day 7 Visit) which may occur between Day 7 and Day 11. If a public emergency or natural disaster results in a subject being unable to complete at least 6 consecutive days of dosing prior to the End of Treatment Period Visit (nominal Day 7 visit which may occur between Days 7 and 11), the treatment period should be repeated with the repeat Day 1 visit occurring at least 7 days after the last administered dose of blinded study medication. In the event of a public emergency or natural disaster, the 7-day washout period may be extended up to 28 days. Subjects requiring a washout period > 28 days will be withdrawn from the study.

9.0 STATISTICAL CONSIDERATIONS

Further details of the analyses to be performed can be found in the Statistical Analysis Plan (SAP).

9.1 Statistical Hypotheses

The aim of this study is to quantify the benefit of each glycopyrrolate dose over placebo in efficacy as mean change from baseline for spirometry. The null hypotheses to be tested for each efficacy endpoint will be that there is no difference in change from baseline between glycopyrrolate and placebo for each glycopyrrolate dose, against the alternative hypothesis that change differs between that glycopyrrolate dose and placebo. All tests will be two-sided at a 5% significance level. Primary and secondary endpoints will be evaluated at a nominal Type 1 error level of 0.05, and Type 1 error will not be adjusted for multiplicity.

9.2 Sample Size Determination

Estimates obtained from a 6-period multi-dose crossover study of BID nebulized glycopyrrolate at Day 7 for change from baseline in trough FEV_1 (Saluja et al. 2017). Assuming the standard deviation for the differences (SDD) equal to 0.153 L, a sample size of 36 subjects provides 85% statistical power to detect a between-group difference with placebo in trough FEV_1 at Day 7 of at least 81 mL. The sample size provides adequate statistical power for statistical comparisons of each secondary endpoint. Subjects who withdraw prior to completing 2 treatment periods may be replaced. Assuming approximately 10% of early withdrawals, approximately 40 subjects will be enrolled.

9.3 Populations for Analyses

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

The randomized set will consist of all randomized subjects.

The Full Analysis Set (FAS) will consist of all randomized subjects that took at least 1 dose of double-blind study treatment with a non-missing baseline FEV_1, that have non-missing post-dose spirometry parameter values from at least two periods. Analyses will be by actual treatment received.

The Completers Analysis Set (CAS) will consist of all subjects in the FAS that complete all visits and that do not have missing values for spirometry parameters.

The Per Protocol Set (PPS) will consist of all subjects in the CAS set that did not deviate significantly from the terms and conditions in the study protocol.

The Safety Analysis Set (SAS) will consist of all randomized subjects who took at least one dose of double-blind study treatment. Safety analysis will be by actual treatment received.

The PK analysis set (PKAS) will consist of all randomized subjects with blood sampling performed after at least one dose of glycopyrrolate and have at least 1 quantifiable glycopyrronium free base concentrations without important protocol deviations and/or events with the potential to affect the PK concentrations.

The PK parameter analysis set (PKPAS) will consist of all subjects in the PK analysis set with data sufficient to calculate at least 1 PK parameter without important protocol deviations and/or events with the potential to affect the PK results.

9.4 Statistical Methods

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

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

9.5 Statistical Analyses

Treatments will be characterized and, when compared, comparisons will be made using estimates obtained from analysis of covariance models.

Spirometry parameters will be analyzed as change from average baseline FEV_1 on study Day 1 and, separately, on Day 7, and will not be transformed prior to analysis. Models will include terms for treatment, period, and subject as fixed effects, and baseline as a covariate. A separate model will be fit for each efficacy parameter from Day 1 and from Day 7.

9.5.1 Efficacy Analyses

Primary efficacy analysis will be based on the FAS. Analyses will be repeated for the CAS and the PPS set.

Each efficacy parameter will be summarized both for the absolute value and for change from baseline using descriptive statistics (N, mean, median, SD, minimum, and maximum) for each treatment, generated separately for each Period as well as overall. Average baseline will represent the average of the two pre-treatment FEV_1 values prior to the first dose of double-blind medication in each Treatment Period.

Results will provide the number of subjects included in each analysis, estimated least square means and corresponding standard errors and 95% confidence intervals (CIs). CIs

will not be adjusted for multiplicity. Least square mean differences between each dose and placebo will be provided along with standard errors, 95% CIs, and p-values. CIs and p-values will not be adjusted for multiplicity.

Exploratory efficacy analyses will assess the incremental relationship between the glycopyrrolate dose and the corresponding difference in the least square means. Additional details will be provided in the Statistical Analysis Plan.

9.5.2 Safety Analyses

Safety analysis will be descriptive and based on the SAS including all randomized and treated subjects. Safety analyses will be by actual treatment received.

AEs will be analyzed using quantitative and qualitative measures. Treatment emergent adverse events (TEAEs) will be summarized by treatment for all AEs, related AEs, SAEs, deaths, AEs leading to discontinuation of blinded study medication and, separately, to withdrawal from study, AEs of different severity and AEs of different chronicity.

TEAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by system organ class (SOC) and preferred term (PT) for each treatment.

Laboratory data will be summarized by each dose including change from baseline by treatment. Changes which are markedly abnormal for each parameter will be summarized by treatment. The number of normal and markedly abnormal for each parameter will be summarized for each treatment using shift tables.

Vital signs and continuous parameters from the ECGs will be summarized for each dose, including change from baseline. Values outside pre-specified ranges (low, high) or changes exceeding pre-specified limits will be summarized as change from baseline values and using shift tables for each treatment and each parameter.

The number of normal, abnormal not clinically significant and abnormal clinically significant values on the ECG overall evaluation will be summarized for each dose using shift tables.

All laboratory, vital sign, and ECG data will be listed and values outside reference ranges will be highlighted in the listings.

9.5.3 Pharmacokinetic Analyses

The following PK parameters will be calculated from plasma concentrations of glycopyrronium on Day 7.

- $AUC_{0\text{-}12h}$ denotes the area under the plasma concentration curve versus time over the 12-hour dosing interval following the morning dose.
- C_{max} denotes the highest plasma concentration measured over the dosing interval.
- C_{trough} denotes the plasma concentration measured prior to dose administration.

- C_{avg} denotes the plasma average concentration measured over the dosing interval, calculated as $AUC_{0\text{-}12h}$ divided by the dosing interval.
- t_{max} denotes the time point corresponding to C_{max}
- $C_{L/F}$ denotes the apparent clearance from plasma following glycopyrrolate inhalation.

All final PK parameter calculations will be performed by using actual elapsed time from the start of the inhalation. Dose-normalized (DN) exposure parameters (DN-C_{max} and DN-$AUC_{0\text{-}12h}$) will also be calculated. Additional PK parameters may be calculated as warranted by the data.

Glycopyrronium free base concentrations will be summarized by treatment using the PKAS.

Subject and arithmetic mean (± standard deviation [SD]) profiles of glycopyrronium concentrations at each timepoint over 12 hours will be generated for each glycopyrrolate dose on both original and semi-logarithmic scales.

PK parameters will be analyzed using the PKPAS. Glycopyrronium free base PK parameters will be summarized using descriptive statistics (N, mean, median, SD, geometric mean, geometric CV%, minimum, and maximum) for each treatment, generated separately for each Treatment Period as well as overall for Treatment Periods 1 and 2 combined. Only median, minimum, and maximum will be presented for t_{max}.

Scatter plots of individual and geometric mean PK parameters [C_{max}, $AUC_{0\text{-}12h}$, C_{trough}] versus glycopyrrolate will be presented with data for both treatment periods combined. A similar presentation will be prepared for DN-C_{max} and DN-$AUC_{0\text{-}12h}$.

The dose proportionality of the exposure parameters, C_{max}, $AUC_{0\text{-}12h}$, over the administered dose range will be investigated using the following power model:

log (parameter) = a + b * log (dose)

where ‘a’ is the intercept and ‘b’ is the slope.

The analysis will be performed by including all available exposure parameters for both periods combined. If warranted, this analysis may be repeated utilizing Treatment Period 1 data only.

Further details regarding the pharmacokinetic analysis will be provided in the SAP.

9.5.4 Other Analyses

The subject flow including total number of screened subjects, number of randomized subjects, completers, withdrawn subjects (including reason for withdrawal) and subjects included in each of the analysis sets will be summarized by sequence and overall, and by treatment and overall.

The number of subjects with major protocol deviations will be summarized by category of violation by sequence and overall, and by dose and overall including subset of major protocol deviation leading to exclusion from the per-protocol set.

Demographic and baseline characteristics will be summarized using descriptive statistics for by sequence and overall, and by treatment and overall.

Medical history will be coded using MedDRA and summarized by SOC and PT for each sequence and overall.

Prior medications will denote medications used prior to the first dose of study drug independent of whether the medication was stopped at randomization or not. Prior medications will be summarized by sequence and provided in subject listings.

Concomitant medications will denote medications started prior to but continuing after randomization or medications with a start date at or after the randomization date. Concomitant medications will be summarized separately by Anatomical Therapeutic Chemical (ATC) levels 2 and 4. Concomitant medication will be summarized by sequence and by treatment.

Compliance to blinded study medication will be computed based on the number of ampules/foil pouch count dispensed and unused ampules/foil pouches returned. Non-compliance will be defined as a compliance value less than 70% over the full study period. Compliance will be summarized by sequence and overall and by treatment and overall.

9.5.5 Missing Data

As discontinuation of study medication will result in subject withdrawal, data collected after treatment withdrawal will not be included in the analyses, and missing data will not be imputed.

9.6 Interim Analyses

No interim analysis is planned.

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Appendix 1 Abbreviations

AE	Adverse event
ALT	Alanine aminotransferase
ATC	Anatomical Therapeutic Chemical
ATS	American Thoracic Society
AUC_{0-4h}	Area under the curve versus time from time 0 to 4 hours
AUC_{0-12h}	Area under the curve versus time from time 0 to 12 hours
BID	Twice daily
CAS	Completers analysis set
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
C_{max}	Maximum plasma drug concentration
CPAP	Continuous positive airway pressure
CXR	Chest x-ray
CT	Computed tomography
CV	Coefficient of variation
DAC	Data Assessment Committee
DN	Dose normalized
DPI	Dry powder inhaler
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
ERS	European Respiratory Society
FAS	Full analysis set
FDA	Food and Drug Administration
FDC	Fixed dose combination
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
IB	Investigator's brochure
ICF	Informed consent form
ICS	Inhaled corticosteroid(s)
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	Interactive Response Technology
LABA	Long-acting β2-agonist

LAMA	Long-acting muscarinic antagonist
LDPE	Low-density polyethylene
MedDRA	Medical Dictionary for Regulatory Activities
NCFBE	Non-cystic fibrosis bronchiectasis
NYHA	New York Heart Association
PD	Pharmacodynamic(s)
PDE	Phosphodiesterase
PK	Pharmacokinetic(s)
PKAS	Pharmacokinetic analysis set
PKPAS	Pharmacokinetic parameter analysis set
pMDI	Pressurized metered dose inhaler
PPS	Per-protocol set
PT	Preferred term
QTcF	QT interval corrected for heart rate using Fridericia's formula
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety analysis set
SD	Standard deviation
SoA	Schedule of Activities
SOC	System organ class
Study CO-211	RPL554-CO-211
Study CO-212	RPL554-CO-212
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment-emergent adverse event
t_{max}	Time to maximum plasma drug concentration
ULN	Upper limit of normal
US	United States
Verona	Verona Pharma plc.
WOCBP	Women of Childbearing Potential

DEFINITION OF TERMS

QT interval	The portion of an electrocardiogram between the onset of the Q wave and the end of the T wave.

Appendix 2 Regulatory, Ethical, and Study Oversight Consideration

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
  - Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines.
  - Applicable laws and regulations.
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC and regulatory authority approval, when applicable, before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to subjects.
- The Investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.
  - Providing oversight of the conduct of the study at the study center and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, EU Clinical Trials Directive 2001/20/EC (if applicable), and all other applicable local regulations.
- After reading the protocol, the Principal Investigator will sign the protocol signature page and send a copy of the signed page to the Sponsor or designee (Appendix 9). The study will not start at any study center at which the Investigator has not signed the protocol.

Adequate Resources

The Investigator is responsible for supervising any individual or party to whom the Investigator delegates study-related duties and functions conducted at the study center.

If the Investigator/institution retains the services of any individual or party to perform study related duties and functions, the Investigator/institution should ensure this individual or party is qualified to perform those study-related duties and functions and should implement procedures to ensure the integrity of the study-related duties and functions performed and any data generated.

Financial Disclosure

Investigators and sub-Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

Insurance

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

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

Informed Consent Process

- The Investigator or his/her representative will explain the nature of the study to the subject and/or the subject's legally authorized representative and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary. Subjects will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the subject was entered in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- A copy of the original ICF(s) must be provided to the subject or the subject's legally authorized representative.
- ICF New Information: New information since the time of the original consent can be presented to subjects in format(s) or method(s) including, but not limited to those listed below unless excluded by local requirements:
  - Revised consent document

- Addendum to consent
- Memo or other communication to subjects
- Orally by phone or in person

Documentation of the method the new information was presented to the subject along with the name of the site staff member and date the new information was presented to the subject must be documented in the subject's source document.

Data Protection

- Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.
- The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject.
- The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
- The ICF will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

Administrative Structure

The study administration structure is in Table 9 and the Medical Monitor and 24-hour urgent medical contact information is in Table 10.

Table 9: Study Administrative Structure

Function	Responsible Organization
Study Operations Management	PPD 929 North Front Street Wilmington, NC 28401-3331, US
Medical Monitoring	
Study Master File	
Randomization Code	
Data Management	
Quality Assurance Auditing	Verona Pharma plc 3 More London Riverside; London, SE1 2RE; UK
Biostatistics	Verona Pharma plc and PPD
Clinical Supply Management	
Medical Writing	
Safety Reporting	
Laboratory Assessments	PPD and Q2 Solutions 2400 Ellis Road; Durham NC 27703, US
Spirometry and Electrocardiogram Collection, Review, and Analysis	Clario 818 Market St; Suite 2600; Philadelphia, PA 19103, US

Table 10: Medical Monitor

Primary Medical Monitor:	[redacted]
Secondary Medical Monitor:	[redacted]
24-hour Urgent Medical Contact	Please use this number below and you will be directed to one of the PPD Medical Monitors. PPD Medical Monitoring Hotline: US Number: + 1-888-483-7729

Dissemination of Clinical Study Data

For studies conducted in the United States, the results of the study are required to be reported on clinicaltrials.gov no later than 1 year after the primary completion date of the clinical study, which is defined as the date of final data collection for the primary outcome measure.

Data Quality Assurance

- All subject data relating to the study will be recorded on printed or eCRFs unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The

Investigator is responsible for verifying that data entries are accurate and correct and will need to confirm that the blinding procedures have or have not been maintained for each subject by physically or electronically signing the eCRF.

- The Investigator must maintain accurate documentation (source data) that supports the information entered into the eCRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized study center personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Source Documents

The Investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the study center's subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g., via an audit trail).

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the Investigator's study center.
- Data reported on the eCRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in ICH E6(R2) Section 1.51.

Study and Study Center Closure

The Sponsor reserves the right to close the study center or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study centers will be closed upon study completion. A study center is considered closed when all required documents and study supplies have been collected and a study center closure visit has been performed.

The Investigator may initiate study center closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study center by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of subjects by the Investigator.
- Discontinuation of further study medication development.

Publication Policy

The data generated by this study are the confidential information of the Sponsor.

Appendix 3 Clinical Laboratory Tests

The tests detailed in Table 11 will be performed by the central laboratory.

- Fasting is not required prior to laboratory testing.
- Laboratory tests should be collected prior to dosing with blinded study medication.

Table 11: Protocol Required Laboratory Assessments

Chemistry	**Hematology**	**Viral Serology**
Albumin	Hemoglobin	Hepatitis B Serology (Markers include HBsAg, anti-HBs, anti-HBc, and others)
Alkaline phosphatase	Hematocrit	Hepatitis C virus antibody
Alanine amino transferase (ALT or SGPT)	Platelet count	
Aspartate amino transferase (AST or SGOT)	WBC count	**Other Laboratory Tests**
Bilirubin, direct	Leukocyte differential count	Pregnancy test for WOCBP[1]
Bilirubin, indirect	Neutrophils, absolute	
Bilirubin, total	Neutrophils, segs (%)	
Calcium	Neutrophils, bands (%)	
Chloride	Basophils (%)	
CO_2 content/Bicarbonate	Eosinophils (%)	
Creatinine	Eosinophils, absolute	
Creatine phosphokinase (CPK), total	Lymphocytes (%)	
Gamma glutamyl transferase (GGT)	Monocytes (%)	
Glucose	RBC count	
Phosphorus		
Potassium		
Protein, total serum		
Sodium		
Urea nitrogen (BUN)		
Uric Acid		

[1] Pregnancy test for women of childbearing potential. A serum pregnancy test will be conducted at Visit 1. A urine pregnancy test will be conducted at the Day 1 visit for each of the Treatment Periods.

Abbreviations: anti-HBc = total hepatitis B core antibody; anti-HBs = hepatitis B surface antibody; HBsAg = hepatitis B surface antigen; WOCBP = women of child-bearing potential (Appendix 7).

Additional Instructions:

- The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF.
- If additional tests are required to be completed urgently by a local lab to assess an AE or for any other reason, these labs should also be completed by the central laboratory for this study using the unscheduled laboratory forms and procedures provided in the laboratory manual. The values obtained by the central laboratory will override the values obtained by the local laboratory.
- The laboratory reports must be signed by the investigator who reviewed the report and filed with the source documents.
- Clinically significant abnormal laboratory findings are those that are not associated with the subject's health status and judged by the Investigator to be more severe than expected.
- All abnormal laboratory tests with values considered clinically significant after randomization should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor.
    - If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, and the Sponsor notified.
    - All protocol-required laboratory assessments must be conducted in accordance with the laboratory manual that will be developed by the central laboratory vendor and per the timelines outlined in the SoA (Section 1.3).
    - If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in subject management or are considered clinically significant by the Investigator (e.g., SAE or AE), then the results must be recorded in the eCRF.

Appendix 4 ECG Exclusion Criteria

A 12-lead ECG recording at Screening showing any of the following abnormalities:

- Sinus tachycardia ≥ 110 bpm (Sinus tachycardia ≥ 110 bpm should be confirmed by 2 additional readings at least 5 minutes apart.).
- Sinus bradycardia < 45 bpm (Sinus bradycardia < 45 bpm should be confirmed by 2 additional readings at least 5 minutes apart).
- Multifocal atrial tachycardia
- Junctional (heart rate > 100 bpm)
- Supraventricular tachycardia (> 100 bpm)
- Ventricular tachycardia
- Atrial fibrillation with rapid ventricular response (rate > 100 bpm).
- Atrial flutter
- Frequent VPCs (> 2 on a 10 sec ECG)
- Ventricular flutter
- Ventricular fibrillation
- Torsades de Pointes
- Wide QRS tachycardia (diagnosis unknown)
- Electrical alternans
- Pacemaker or ICD
- Idioventricular rhythm – heart rate < 100 bpm
- Mobitz type II second degree or third degree atrioventricular (AV) block.
- AV dissociation
- Bifascicular Block (RBBB plus LAHB or RBBB plus LPHB)
- Left bundle branch block
- Wolff Parkinson White Syndrome
- Brugada Syndrome pattern
- QTcF ≥ 480 msec when RBBB is present
- Subjects without complete right bundle branch block: QTc(F) ≥ 450 msec or an ECG that is unsuitable for QT measurements (e.g., poorly defined termination of the T wave).

Appendix 5 ECG Withdrawal Criteria

All ECGs conducted or overread after a subject has been randomized will be subject to ECG Withdrawal Criteria (including the pre-dose ECGs at Visit 2 and subsequent visits). A subject may be withdrawn from study medication if the 12-lead ECG recording during the study shows any of the following abnormalities:

The Investigator may withdraw subjects from study treatment for any other clinically significant finding (Section 7.0). Triplicate ECGs (over a brief period of time) should be performed on subjects experiencing any of the following or a clinically significant abnormality on their ECG per the Investigator's discretion, in order to confirm the abnormality in 2 of 3 measurements. The decision to withdraw a subject should also take into consideration the subject's medical history and prior/baseline ECGs.

- Sinus tachycardia ≥ 120 bpm (Sinus tachycardia ≥ 120 bpm should be confirmed by 2 additional readings at least 5 minutes apart.).
- Sinus bradycardia < 37 bpm (Sinus bradycardia < 37 bpm should be confirmed by 2 additional readings at least 5 minutes apart).
- Increase in heart rate ≥ 40 bpm relative to baseline.
- Multifocal atrial tachycardia
- Supraventricular tachycardia (> 100 bpm)
- Atrial fibrillation with rapid ventricular response (rate > 120 bpm).
- Atrial flutter with rapid ventricular response (rate > 120 bpm).
- Ventricular tachycardia (non-sustained, sustained, polymorphic or monomorphic)
- Ventricular flutter
- Ventricular fibrillation
- Torsades de Pointes
- Evidence of Mobitz type II second degree or third degree atrioventricular (AV) block.
- AV dissociation
- 2:1 AV block
- Bifascicular Block (RBBB plus LAHB or RBBB plus LPHB)
- An increase in QTcF > 60 msec from baseline ECG
- Uncorrected QT > 600 msec
- For subjects with QRS duration < 120 msec: QTc(F) ≥ 500 msec or an ECG that is unsuitable for QT measurements (e.g., poor defined termination of the T wave).
- For subjects with QRS duration ≥ 120 msec: QTc(F) ≥ 530 msec or an ECG that is unsuitable for QT measurements (e.g., poor defined termination of the T wave).
- Myocardial infarction (acute or recent) Note: Evidence of an old resolved Myocardial infarction is not exclusionary.

Appendix 6 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting

Definition of AE

- An AE is any untoward medical occurrence in a subject or subject, temporally associated with the use of blinded study medication, whether or not considered related to the blinded study medication.
- NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of blinded study medication.
- COPD exacerbations are an expected disease-related outcome. COPD exacerbations including daily expected COPD symptoms (e.g., dyspnea, chest tightness, wheezing, cough, sputum, that are not associated with the dosing event) will not be collected as an AE unless they meet the definition of an SAE.

Events Meeting the AE Definition

- Any abnormal laboratory test results (e.g., hematology, clinical chemistry) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (i.e., not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition other than COPD including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after blinded study medication administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either blinded study medication or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.
- “Lack of efficacy” or “failure of expected pharmacological action” per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms and/or clinical sequelae resulting from lack of efficacy will be reported as an AE or SAE if they fulfil the definition of an AE or SAE.

Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure should be assessed using the AE/SAE definitions.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:

a) Results in death.

b) Is life-threatening.

The term 'life-threatening' in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c) Requires inpatient hospitalization or prolongation of existing hospitalization.

In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d) Results in persistent disability/incapacity.

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e) Is a congenital anomaly/birth defect

f) Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording and Follow-up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in the eCRF. Each event must be recorded separately.
- It is **not** acceptable for the Investigator to send photocopies of the subject's medical records to Sponsor/Sponsor's designee in lieu of completion of the Verona Pharma /AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by groups such as the Sponsor/Sponsor's designee, Health Authority, or Ethics Committee. In this case, all subject identifiers, with the exception of the subject/patient number, will be redacted on the copies of the medical records before submission to records to the Sponsor/Sponsor's designee.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE; however, the signs and symptoms should be described in the narrative of the SAE form.

Assessment of Intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories:

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort, and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as 'serious' when it meets at least one of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Chronicity

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

Assessment of Causality

- The Investigator is obligated to assess the relationship between blinded study medication and each occurrence of each AE/SAE. The AE must be characterized as either 1) a reasonable possibility of causality or 2) no reasonable possibility of causality:
    - Reasonable possibility: There is a clear temporal relationship between the study intervention and the event onset; and the event is known to occur with the study intervention or there is a reasonable possibility that the study intervention caused the event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the event.
    - No reasonable possibility: There is no evidence suggesting that the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established (e.g. event is consistent with medical history).
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to blinded study medication administration will be considered and investigated.
- The Investigator will also consult the Investigator's Brochure (IB) in his/her assessment.

- For each AE/SAE, the Investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report to the Sponsor/Sponsor's designee. However, it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Sponsor/Sponsor's designee.
- The Investigator may change his/her opinion of causality considering follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Action and Outcome

- Action taken with blinded study medication (none, blinded study medication stopped, blinded study medication temporarily interrupted)
- Other actions (none, concomitant medication, study discontinuation, hospitalization, other)
- The outcome and date of outcome according to the following definitions:
  - Recovered or resolved (adverse event disappeared)
  - Recovering or resolving (subject 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 subject has been lost to follow-up)
- Seriousness (yes or no)

Follow-up of AEs and SAEs

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor/Sponsor's designee to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a subject dies during participation in the study or during a recognized follow-up period, the Investigator will provide the Sponsor/Sponsor's designee with a copy of any postmortem findings including histopathology, as applicable local regulatory requirements will allow.
- New or updated information will be recorded in the originally completed eCRF.
- The Investigator will submit any updated SAE data to the Sponsor/Sponsor's Designee within 24 hours of receipt of the information.

Reporting of SAEs

SAE Reporting to the Sponsor/Sponsor's designee via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to the Sponsor/Sponsor's designee will be the electronic data collection tool.
- If the electronic system is unavailable, then the study center will use the paper SAE data collection tool (see next section).
- The study center will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given study center, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a study center receives a report of a new SAE from a subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the study center can report this information on a paper SAE form (see next section) or to the Medical Monitor by telephone.
- Contacts for SAE reporting can be found in Appendix 2 on the Medical Monitor Contact Information page.

SAE Reporting to Sponsor/ Sponsor's Designee via Paper CRF if eCRF is not Available

- If the eCRF is not available, facsimile transmission of the SAE paper CRF may be used to transmit this information to the Medical Monitor.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection forms sent by overnight mail or courier service to the Medical Monitor.
- Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in Appendix 2 on the Medical Monitor Contact Information page.

Device Incidents

The Investigator, or responsible person according to local requirements (e.g., the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of device incidents.

A device incident is defined as an incident related to the failure of a medical device, deterioration in its effectiveness, or inadequacy in its labeling or directions that led to the death or serious deterioration in health of a patient, user, or other person, or could do so were it to recur.

Appendix 7 Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are not considered WOCBP

1. Premenopausal female with 1 of the following:

   a) Documented hysterectomy.

   b) Documented bilateral salpingectomy.

   c) Documented bilateral oophorectomy. Note: Documentation can come from the study center personnel's: review of the subject's medical records, medical examination, or medical history interview.

2. Postmenopausal female:

   a) Postmenopausal females are defined as amenorrhoeic for greater than 1 year with an appropriate clinical profile, e.g., age appropriate, > 45 years, in the absence of hormone replacement therapy.

Contraception Guidance

Female subjects

Female subjects of childbearing potential are eligible to participate if they are not breastfeeding and agree to use a highly effective method of contraception consistently and correctly as described in the table below, during the study starting at Visit 1 and for at least 30 days after the last dose of study treatment.

Table 12: Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of < 1% per year when used consistently and correctly.*
Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[b] • Oral • Intravaginal • Transdermal
Progestogen only hormonal contraception associated with inhibition of ovulation • Oral • Injectable

Table 12: Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Independent [a]
Implantable progestogen only hormonal contraception associated with inhibition of ovulation[b] • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) Bilateral tubal occlusion.
Vasectomized partner *A vasectomized partner is a highly effective birth control method provided that the partner is the sole male sexual partner of the WOCBP, and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.*
Sexual abstinence *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.*
NOTES: [a] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for subjects participating in clinical studies. [b] Hormonal contraception may be susceptible to interaction with the study treatment, which may reduce the efficacy of the contraceptive method. In this case, 2 highly effective methods of contraception should be utilized during the treatment period and for at least 30 days after the last dose of study treatment.

Pregnancy Testing:

- WOCBP should only be included after a negative highly sensitive pregnancy test at Screening (Visit 1).
- Additional pregnancy testing should be performed at times specified in the SoA (Section 1.3).

Pregnancy testing should be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected.

Collection of Pregnancy Information

Female subjects who become pregnant

- The Investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. Information will be recorded on

the appropriate form and submitted to the Sponsor within 24 hours of learning of a subject's pregnancy.

- The subject will be approached and asked to consent to be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the subject and the neonate, and the information will be forwarded to the Sponsor. Generally, follow-up will be no longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any post-study pregnancy related SAE considered reasonably related to the blinded study medication by the Investigator will be reported to the Sponsor as described in Section 8.7.5. While the Investigator is not obligated to actively seek this information in former subjects, he or she may learn of an SAE through spontaneous reporting.
- Any female subject who becomes pregnant while participating in the study will discontinue blinded study medication and be withdrawn from the study.

Appendix 8 Liver Safety

Phase III-IV liver chemistry stopping criteria and follow-up criteria have been designed to assure subject safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance). Phase III to IV liver chemistry stopping criteria 1 to 5 are defined below. Investigators may at their discretion refer subjects meeting liver safety criteria below to a specialist who may determine the appropriate or medically necessary additional or confirmatory laboratory tests or procedures as required by the protocol or otherwise deemed necessary (e.g., liver imaging).

1. ALT ≥ 3 × ULN and bilirubin ≥ 2 × ULN (35% direct bilirubin) (or ALT ≥ 3 × ULN and INR > 1.5 if international normalize ratio (INR) measured.

   Note: if serum bilirubin fractionation is not immediately available, withdraw study drug for that subject if ALT ≥ 3 × ULN and bilirubin ≥ 2 × ULN. Serum bilirubin fractionation should be performed if testing is available. If testing is unavailable, record presence of detectable urinary bilirubin on dipstick, indicating direct bilirubin elevations and suggesting liver injury.

2. ALT ≥ 8 × ULN.
3. ALT ≥ 5 × ULN but < 8 × ULN persists for ≥ 2 weeks.
4. ALT ≥ 3 × ULN if associated with symptoms (new or worsening) believed to be related to hepatitis (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, or jaundice) or hypersensitivity (such as fever, rash, or eosinophilia).
5. ALT ≥ 5 × ULN but < 8 × ULN and cannot be monitored weekly for ≥ 2 weeks.

When any of the liver stopping criteria 1 to 5 is met do the following:

- **Immediately** discontinue blinded study medication/investigational product for that subject.
- Report the event to PPD within 24 hours of learning its occurrence.
- Complete the liver event CRF and SAE data collection form if the event also meets the criteria for an SAE. All events of ALT ≥ 3 × ULN and bilirubin ≥ 2 × ULN (35% direct bilirubin) (or ALT ≥ 3 × ULN and INR > 1.5, if INR measured; INR measurement is not required and the threshold value stated will not apply to subjects receiving anticoagulants), termed ‘Hy’s Law’ must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).

Note: if serum bilirubin fractionation is not immediately available, discontinue blinded study medication/investigational product for that subject if ALT ≥ 3 × ULN and bilirubin ≥ 2 × ULN. Serum bilirubin fractionation should be performed if testing is available. If testing is unavailable, record presence of detectable urinary bilirubin on dipstick, indicating direct bilirubin elevations and suggesting liver injury.

- Complete the liver imaging and/or liver biopsy CRFs if these tests are performed.
- Perform liver event follow-up assessments and monitor the subject until liver chemistries resolve, stabilize, or return to baseline values as described below.
- Discontinue blinded study medication/investigational product after completion of the liver chemistry monitoring as described below.
- Do not restart investigational product.

In addition, for criterion 1:

- Make every reasonable attempt to have subjects return to clinic within 24 hours for repeat liver chemistries, liver event follow-up assessments (see below), and close monitoring.
- A specialist or hepatology consultation is recommended.
- Monitor subjects twice weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.

For criteria 2, 3, 4, and 5:

- Make every reasonable attempt to have subjects return to clinic within 24 to 72 hours for repeat liver chemistries and liver event follow-up assessments (see below).
- Monitor subjects weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize, or return to within baseline values; criterion 5 subjects should be monitored as frequently as possible.

Subjects with ALT ≥ 5 × ULN and < 8 × ULN which exhibit a decrease to ALT ≥ 3 × ULN, but < 5 × ULN and bilirubin < 2 × ULN without hepatitis symptoms or rash, and who can be monitored for 4 weeks:

- Notify the Medical Monitor within 24 hours of learning of the abnormality to discuss subject safety.
- Can continue investigational product.
- Must return weekly for repeat liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) until they resolve, stabilize, or return to within baseline.
- If at any time these subjects meet the liver chemistry stopping criteria, proceed as described above.
- If, after 4 weeks of monitoring, ALT < 3 × ULN and bilirubin < 2 × ULN, monitor subjects twice monthly until live chemistries normalize or return to within baseline values.

For criteria 1 to 5, make every attempt to carry out the liver event follow up assessments described below:

- Viral hepatitis serology including
  - Hepatitis A IgM antibody
  - Hepatitis B surface antigen and Hepatitis B Core antibody (IgM)
  - Hepatitis C RNA
  - Cytomegalovirus IgM antibody
  - Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing).
  - Hepatitis E IgM antibody
- Blood sample for PK analysis, obtained within 72 hours of the last dose. Record the date/time of the PK blood sample draw and the date/time of the last dose of blinded study medication/investigational product prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the SPM.
- Serum creatinine phosphokinase (CPK) and lactate dehydrogenase (LDH).
- Fractionate bilirubin if total bilirubin ≥ 2 × ULN.
- Obtain complete blood count with differential to assess eosinophilia.
- Record the appearance or worsening of clinical symptoms of hepatitis or hypersensitivity such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia as relevant on the AE report form.
- Record use of concomitant medications, acetaminophen, herbal remedies, or over the counter medications, or putative hepatotoxins, on the concomitant medications report form.
- Record alcohol use on the liver event alcohol intake case report form.

The following are required for subjects with ALT ≥ 3 × ULN and bilirubin ≥ 2 × ULN (35% direct) but are optional for the other abnormal liver chemistries:

- Anti-nuclear antibody, anti-smooth muscle antibody, and Type 1 anti-liver kidney microsomal antibodies and quantitative total immunoglobulin G (IgG or gamma globulins).

- Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week).
- Only in those with underlying chronic hepatitis B at study entry (identified by positive hepatitis B surface antigen): quantitative hepatitis B DNA and hepatitis delta antibody. NOTE: if hepatitis delta antibody assay cannot be performed, it can be replaced with a PCR of hepatitis D RNA virus where needed (Gal et al. 2005).

Appendix 9 Signature of Investigator

PROTOCOL TITLE: A Phase IIb, randomized, double-blind, placebo-controlled study to assess the efficacy, safety, and pharmacokinetics of glycopyrrolate inhalation solution over 1 week in subjects with COPD.

PROTOCOL NO: RPL554-CO-211

VERSION: 3.0

Version Date: 14 October 2024

This protocol is a confidential communication of Verona Pharma plc. I confirm that I have read this protocol, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices and the applicable laws and regulations. Acceptance of this document constitutes my agreement that no unpublished information contained herein will be published or disclosed without prior written approval from the Sponsor.

Instructions to the Investigator: Please SIGN and DATE this signature page. PRINT your name, title, and the name of the study center where the study will be conducted. Return the signed copy to the Sponsor.

I have read this protocol in its entirety and agree to conduct the study accordingly:

Signature of Investigator	Date

Printed Name _____

Investigator Title _____

Name/Address of Center _____

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