TITLE PAGE

Protocol Title: A randomised, placebo-controlled, double-blind (sponsor open), segmental LPS challenge study to investigate the pharmacodynamics of GSK2798745 in healthy participants

Protocol Number: 207464 / Amendment 02

Short Title: Effects of GSK2798745 on alveolar barrier disruption in a segmental LPS challenge model

Compound Number: GSK2798745

Sponsor Name and Legal Registered Address:

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SPONSOR SIGNATORY:

PPD

21 May 2018

Anya Ha PPD , PhD
Physician Project Lead
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Date

PPD

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Protocol Amendment 2 (2016N304618_02)	21-MAY-2018
Protocol Amendment 1 (2016N304618_01)	02-FEB-2018
Original Protocol (2016N304618_00)	24-OCT-2017

The original protocol (24-Oct-2017) was published internally only, it was not reviewed by the competent authority or the research ethics committee.

Protocol Amendment 1 (02-Feb-2018) was submitted to both the competent authority and the research ethics committee. The Summary of Changes table for Amendment 1 is in Appendix 7

Protocol Amendment 2 21-May-2018

Overall Rationale for the Amendment: The protocol was amended in response to comments from the competent authority.

(Note: 'Additions' are underlined and 'deletions' are striked off)

Section # and Name	Description of Change	Brief Rationale
Section 1. Synopsis and Section 4 Objectives and Endpoints	Endpoint: PK parameters of GSK2798745 in plasma (AUC [0-26~~24~~] and Cmax).	Error corrected from previous version
Section 6.2 Exclusion Criteria	Deleted: ~~Abnormal blood pressure as determined by the investigator.~~ Added: Values outside of the following ranges; 90-140 mmHg for systolic blood pressure, 50-90 mmHg for diastolic blood pressure and 50-90 beats/minute for heart rate. Added: An AV block greater than Grade 1	Requested by the competent authority

Section # and Name	Description of Change	Brief Rationale
Section 7.4 Blinding	All study staff involved in clinical assessments (which includes the investigator, sub-investigators, other site staff) and the participant will be blinded to the treatment allocated to individual participants and to post challenge ~~PD~~ total protein in BAL sample results.	Error corrected from previous version
Section 8.3 Study Stopping Criteria	Added: Participants who have been dosed at the time of the study halt will continue in the study, as planned. Further participants may be dosed only if, after review, the sponsor and investigator consider it safe to do so and only after ethics and regulatory approval of a substantial amendment.	Requested by the competent authority
Section 9.5 Adverse events	Added: Adverse event/serious adverse event reporting will meet the requirements set out in Section 12.2.4 of the ICH E3 Guidelines.	Requested by the competent authority
Section 9.5.3 Follow-up of AEs and SAEs	All AE/SAEs, will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up	Requested by the competent authority
Throughout	Updated the abbreviation table and minor formatting at few places.	Minor, therefore have not been summarized

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1. SYNOPSIS

Protocol Title: A randomised, placebo-controlled, double-blind (sponsor open), segmental LPS challenge study to investigate the pharmacodynamics of GSK2798745 in healthy participants

Short Title: Effects of GSK2798745 on alveolar barrier disruption in a segmental LPS challenge model

Rationale:

The influx of protein-rich fluid into the lung due to damage to the alveolar capillary barrier, with resultant adverse effects on respiratory function, is a fundamental underlying defect in Acute Respiratory Distress Syndrome (ARDS). In this Phase 1, proof-of-mechanism study, a segmental lipopolysaccharide (LPS) challenge will be used as a surrogate injury model to investigate the effects of Transient receptor potential vanilloid 4 (TRPV4) channel blockade on alveolar-septal barrier permeability in man. The LPS model has been widely used for the evaluation of anti-inflammatory agents, and will aid in the characterization of TRPV4 blockade on lung permeability and inflammation. It is hypothesised that TRPV4 inhibitors modulate the LPS response by reducing LPS induced calcium influx (either directly [e.g. via Toll-like receptor 4 (TLR4) mediated activation of endothelial cells] or indirectly [e.g. autocrine cytokine activation]). The assessment of TRPV4 mediated effects on LPS induced lung barrier damage in healthy volunteers is considered a viable strategy before conducting studies in an ARDS patient population.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To investigate the effect of GSK2798745 on alveolar-septal barrier permeability following LPS challenge in healthy volunteers.	• Baseline adjusted total protein concentration in bronchoalveolar lavage (BAL) samples at 24 h after segmental LPS challenge.
Secondary	
• To investigate the effect of GSK2798745 on neutrophil levels in LPS-challenged lungs.	• Baseline adjusted total and differential cell count of neutrophils in BAL samples at 24 h after segmental LPS challenge.

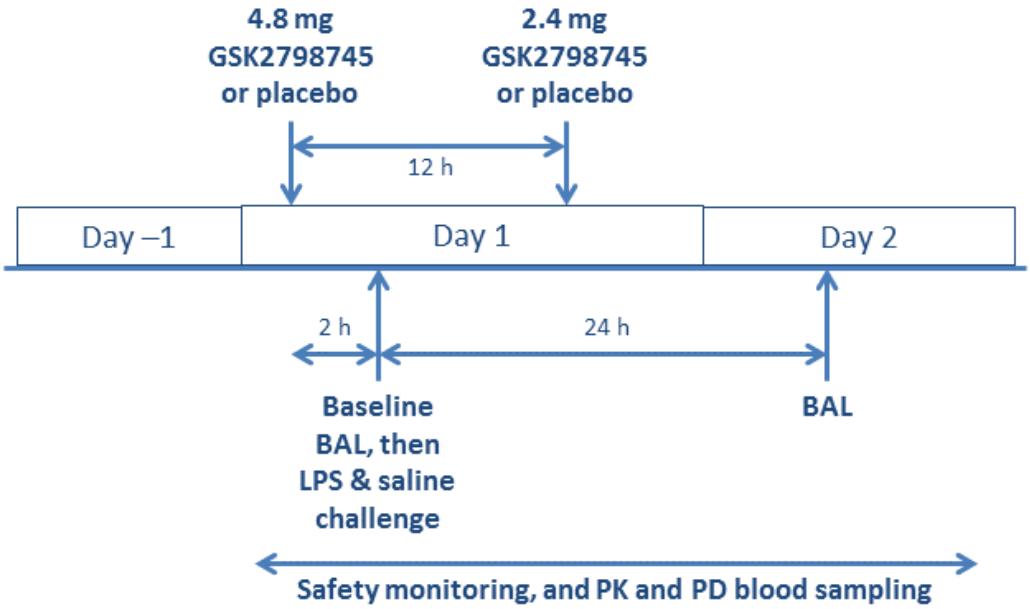
Objectives	Endpoints
• To assess the safety and tolerability of GSK2798745 in LPS-challenged, healthy volunteers.	• Adverse events (AEs), laboratory safety assessments, 12-lead electrocardiogram (ECG), physical examination, vital signs, spirometry (forced expiratory volume in 1 second [FEV1] and forced vital capacity [FVC]), faecal occult blood test (FOBT).
• To assess the pharmacokinetics (PK) of GSK2798745 in plasma in LPS-challenged, healthy volunteers	• PK parameters of GSK2798745 in plasma (AUC [0-26] and Cmax).

Overall Design:

This is a randomised, placebo-controlled, parallel group, double-blind (sponsor-open), segmental LPS challenge study of GSK2798745 in healthy participants.

Participants will be randomised 1:1 to take 2 single doses (3 tablets total): either 4.8 mg GSK2798745, then 2.4 mg GSK2798745 12 h later; or a dose of placebo, then another dose of placebo 12 h later. The first dose will be administered on Day 1 at 2 h before baseline BAL sampling from a segment in the left lower lobe. 4 ng/kg LPS will subsequently be instilled into the right middle segment and saline control into the lingula segment of the contralateral side. The second dose of study treatment will be administered 10 hours after LPS challenge followed by post-dose BAL sampling on Day 2. A schematic of the study is provided in Figure 1.

Figure 1 Study schematic



BAL: bronchoalveolar lavage; LPS: lipopolysaccharide; PD: pharmacodynamic; PK: pharmacokinetic.

Number of Participants:

Sufficient participants will be screened to ensure that a maximum of 60 evaluable participants (defined as all participants for whom results of the primary analysis can be determined) are enrolled onto the study: 30 for GSK2798745 and 30 for placebo. Interim analyses will be conducted, to assess the difference, if any, between treatments in the primary endpoint. Recruitment can continue whilst interim analyses are conducted. Depending on the results of the interim analyses, recruitment may be stopped.

Treatment Groups and Duration:

Each participant will take up to approximately 5 weeks to complete the study. Participants will be screened within 28 days before dosing and remain on site from the day before dosing (Day −1) until the day after dosing (Day 2). Participants will receive a follow up phone call 2 days after discharge, and return to the study site on Day 8 (± 1 day) for a follow-up visit.

2. SCHEDULE OF ACTIVITIES (SOA)

- The timing and number of planned study assessments, including safety, pharmacokinetic, pharmacodynamics and exploratory biomarker assessments may be altered during the course of the study based on newly available data (e.g., to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring.
- Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files, but will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the informed consent form (ICF).

2.1. Screening

	Screening (Days –28 to –2)
Outpatient visit	X
Informed consent	X
Inclusion and exclusion criteria	X
Demography	X
Full physical examination	X
Height and weight	X
Medical history	X
Past and current medical conditions	X
HIV, Hepatitis B and C screening	X
FSH and oestradiol[1]	X
Drug, alcohol and cotinine screen	X
C-SSRS	X
Laboratory assessments[2]	X
12-lead ECG[3]	X
Vital signs[4]	X
Spirometry[5]	X
FOBT[6]	X

C-SSRS: Columbia Suicide Severity Rating Scale; ECG: electrocardiogram; FOBT: Faecal Occult Blood Test; HIV: human immunodeficiency virus.

1. Postmenopausal females whose postmenopausal status is in doubt only.
2. Haematology, clinical chemistry (including liver chemistry), cardiac troponins, urinalysis and coagulation profile.
3. In triplicate.
4. Blood pressure and heart rate in triplicate. Single temperature measurement.
5. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). Triplicate measurement, the highest of which should be recorded in the case report form.
6. May be measured during the screening window or on Day –1. FOBT cards will be provided at screening and must be returned to the laboratory and analysed before dosing.

2.2. Treatment period

	Day –1[2]	Day 1[1]									Day 2[1]			Day 4
		Pre-dose	0 h	1 h	2 h	3 h	6 h	8 h	12 h	14 h	25.5 h	26 h	30 h	
Inpatient stay[2]		←==========→												
Telephone call[3]														X
Drug, alcohol and cotinine screen	X													
Laboratory assessments[4]	X												X	
12-lead ECG[5]	X	X											X	
Blood pressure and heart rate[5]	X	X		X			X		X	X			X	
Temperature[6]	X	X		X	X		X	X	X	X			X	
Spirometry[7]	X	X					X				X		X	
C-SSRS													X	
Randomisation		X												
Study treatment			X						X					
Pulse oximetry[8]					X							X		
Bronchoscopy, baseline BAL and challenge[9]					X									
Bronchoscopy and post-challenge BAL[10]												X		
Blood sample for exploratory biomarkers[11]					X[11]	X		X				X[11]		
Blood sample for PK		See footnote 12												
AE review		←==========→												
SAE review	←==========→													
Concomitant medication review	←==========→													

AE: adverse event; BAL: bronchoalveolar lavage; ECG: electrocardiogram; PK: pharmacokinetic; SAE: serious adverse event, C-SSRS: Columbia Suicide Severity Rating Scale.

1. Time points relative to the first dose on Day 1.
2. Admission on Day –1, at a time to allow all Day –1 procedures to be done; discharge on Day 2, at least 4 h after the bronchoscopy.
3. To check for any AEs.
4. Haematology, clinical chemistry (including liver chemistry), cardiac troponins, urinalysis and coagulation profile.
5. Triplicate on Day-1 and pre-dose. Single measurements at time points after dosing.
6. Immediately before BAL sampling/LPS challenge at the 2-h time point.
7. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). Triplicate measurements at each time point, the highest of which should be recorded in the case report form.
8. Pulse oximetry to be measured during bronchoscopy procedures. Only AEs to be recorded.
9. BAL samples for total protein, neutrophil counts, GSK2798745 and exploratory biomarkers. Baseline BAL sample from a segment in the left lower lobe, followed by segmental challenge of the lungs: LPS in right middle segment; saline control in the lingula segment of the contralateral side. Challenges to be done as close as possible to 2 h post-dose.
10. BAL samples for total protein, neutrophil counts, GSK2798745 and exploratory biomarkers. Baseline and post-dose measurements to be done by the same person, where possible.
11. Blood samples for exploratory biomarkers. The 2- and 26-h blood samples should be taken immediately before BAL sampling.
12. PK samples will be taken pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 6, 8, 12, 12.5, 13, 13.5, 14, 15, 24 and 26 h after the first dose. The 2- and 26-h blood sample should be taken immediately before BAL sampling

2.3. Follow-up/Early Withdrawal

	Follow-up/Early Withdrawal (Day 8 [± 1 day])
Outpatient visit	X
Full physical examination	X
Weight	X
Laboratory assessments[1]	X
12-lead ECG[2]	X
Vital signs[3]	X
Spirometry[4]	X
FOBT[5]	X
AE review	X
SAE review	X
Concomitant medication review	X

AE: adverse event; ECG: electrocardiogram; FOBT: Faecal Occult Blood Test; SAE: serious adverse event.

1. Haematology, clinical chemistry (including liver chemistry), cardiac troponins and urinalysis.
2. Single measurement.
3. Blood pressure, heart rate, temperature. Single measurement.
4. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). Triplicate measurement, the highest of which should be recorded in the case report form.
5. FOBT cards will be provided upon discharge from the unit and should be returned by the follow-up/early withdrawal visit.

3. INTRODUCTION

GSK2798745 is a potent and selective transient receptor potential vanilloid 4 (TRPV4) channel blocker being investigated for the treatment of acute respiratory distress syndrome (ARDS). TRPV4 blockade is expected to ameliorate the injury inflicted to the alveolar capillary barrier in ARDS patients and reduce the leakage of protein and fluid into the alveolar space. Studies investigating administration of GSK2798745 have been conducted in healthy volunteers and in patients with chronic heart failure.

GSK2798745 has been administered orally to healthy participants as single doses ranging from 0.25 to 12.5 mg. A dosage of 5 mg once daily has been administered for up to 14 days in healthy participants. Further, GSK2798745 at a dose of 2.4 mg has been evaluated as a single dose and subsequently as repeated doses for 7 days in participants with heart failure.

No clinically significant safety concerns were observed with single or repeat administration of GSK2798745 in either healthy volunteers or participants with heart failure.

3.1. Study Rationale

The influx of protein rich fluid into the lung due to damage to the alveolar capillary barrier, with resultant adverse effects on respiratory function is a fundamental underlying defect in ARDS. In this Phase 1 proof of mechanism study, a segmental lipopolysaccharide (LPS) challenge will be used as a surrogate injury model to investigate the effects of TRPV4 channel blockade on alveolar-septal barrier permeability in man. The LPS model has been widely used for the evaluation of anti-inflammatory agents [Zielen, 2015], and will aid in the characterization of TRPV4 blockade on lung permeability and inflammation.

Segmental LPS challenge, in which a small quantity of the endotoxin, LPS, is administered directly to one segment of the lung, is a model of focal and self-limiting lung inflammation. LPS induces pulmonary endothelial cell permeability [Fu, 2009] via calcium dependent cytoskeleton rearrangement [Bannerman, 1998; Gandhirajan, 2013]. LPS may also directly act on endothelial cells to cause permeability by degrading intercellular junctions [Bannerman, 1998]. The upregulation of a pro-inflammatory signalling cascade via NF-kB leading to endothelial activation and pro-inflammatory mediator release [Bosmann, 2012] may be regulated by the influx of calcium [Kandasamy, 2013], and may also induce further calcium influx and endothelial permeability [Lush, 2000; Tiruppathi, 2006].

Under normal conditions, concentrations of total protein in alveolar fluid are low. LPS induced endothelial damage leads to accumulation of protein rich fluid into the lung due to the migration of large molecules, such as albumin, from blood to the alveolar space. Preclinical and clinical studies have shown a positive correlation between amount of protein the bronchoalveolar lavage and extent of lung damage [Holter, 1986; Moazed, 2016; Yu, 2015]. In the lung, TRPV4, a Ca^{2+}-permeable non-selective cation channel, is widely expressed in cells involved in ARDS, namely microvascular endothelium,

alveolar epithelium, alveolar macrophages, and circulating neutrophils and monocytes, and is a known regulator of endothelial permeability [Narita, 2015; Suresh, 2015; Huh, 2012] and pulmonary oedema [Balakrishna, 2014; Hamanaka, 2007]. Therefore, TRPV4 may play an important role in LPS-induced alveolar permeability. The relevance of the TRPV4 channel to LPS induced injury has been confirmed in a mouse sepsis model. In this severe injury model, administration of a TRPV4 inhibitor 1 h prior to LPS injection (i.p.) increased survival by 70%. Although this study was not specifically designed to evaluate bronchoalveolar lavage (BAL) protein levels, total protein concentrations in BAL fluid of mice pre-treated with a TRPV4 inhibitor were reduced by approximately 20% after the LPS challenge in TRPV4 treated mice compared with controls [Dalsgaard, 2016].

It is hypothesised that TRPV4 inhibitors modulate the LPS response by reducing LPS induced calcium influx (either directly [e.g. via Toll-like receptor 4 (TLR4) mediated activation of endothelial cells] or indirectly [e.g. autocrine cytokine activation]).

Given the link to ARDS of endothelial damage and fluid leak, assessment of TRPV4 mediated effects on LPS induced lung barrier damage in healthy volunteers is important to establish whether beneficial effects may be observed in an ARDS patient population.

3.2. Background

ARDS is an acute inflammatory lung injury, associated with increased pulmonary vascular permeability, increased lung weight, and loss of aerated lung tissue [Bellani, 2016]. Treatment options remain limited to supportive care including mechanical ventilation and ARDS represents a significant unmet need accounting for over 10% of intensive care unit (ICU) admissions and a mortality rate of up to 40% [Bellani, 2016; Matthay, 2012]. The syndrome is characterised by acute hypoxemic respiratory failure and non-cardiogenic pulmonary oedema and may be precipitated by direct (e.g. pneumonia and aspiration) and indirect insults (e.g. sepsis and transfusion related acute lung injury ([TRALI]) to the lung. Dysregulated inflammation, inappropriate accumulation and activity of leukocytes and platelets, uncontrolled activation of coagulation pathways, and altered permeability of alveolar endothelial and epithelial barriers are central to the pathophysiology of ARDS [Matthay, 2012]. Damage to the alveolar-capillary membrane leads to increased vascular permeability, and the development of interstitial and alveolar protein rich oedema, leading to reduced gas exchange, ventilation perfusion mismatching and arterial hypoxaemia.

TRPV4 has been implicated as a key regulator of lung endothelial barrier integrity, and specifically, the integrity of the lung alveolar-capillary endothelium, which is most relevant to alveolar flooding associated with acute lung injury. TRPV4 activation by hydrostatic stretch in lung microvessels leads to increased endothelial $Ca2^{+}$ concentration and a diverse set of vascular responses, including an increase in endothelial permeability [Morty, 2014]. The importance of TRPV4 in maintaining pulmonary barrier function has been demonstrated in the settings of elevated pulmonary venous [Thorneloe, 2012] or airway pressure [Hamanaka, 2007], and following treatment with chemical and biological toxins such as Hydrochloric acid (HCl) and platelet activating factor [Balakrishna, 2014; Morty, 2014; Yin, 2016] . In these studies, TRPV4 blockade limited lung damage by

reducing plasma fluid leak into the alveolar space (thus increasing arterial oxygenation), and by modulating neutrophil and macrophage recruitment and activity and reducing overall mortality in response to LPS [Balakrishna, 2014; Morty, 2014; Yin, 2016; Dalsgaard, 2016]. This evidence suggests TRPV4 channel blockade may benefit patients with ARDS where alveolar capillary leak is a primary driver of injury, by reducing fluid leak, reducing ventilation perfusion mismatching, improving oxygenation and reducing the need for mechanical ventilation with potential reductions in mortality.

3.3. Benefit/Risk Assessment

3.3.1. Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of GSK2798745 may be found in the Investigator's Brochure.

All potential risks of GSK2798745 are based on pre-clinical data. No risks have been identified in the clinical studies of GSK2798745 conducted before the effective date of this protocol.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) [GSK2798745]		
Vascular lesions	Dogs (4-week study): at 30 mg/kg/day, 2 males had arterial lesions • One male: Heart – Coronary artery inflammation; Thymus – Arteriole inflammation with fibroplasia • One male: Epididymides – Artery degeneration/necrosis with inflammation Dogs (13-week study): At 10 mg/kg/day, 1 male and 1 female had arterial lesions • One male: Epididymides – Arteriole degeneration/necrosis with lymphocytic inflammation • One female: Bladder – Arteriole degeneration/necrosis with lymphocytic inflammation	Participant Monitoring: The arterial lesions noted in heart, thymus, epididymides, and urinary bladder cannot be monitored directly. There is currently no human translation biomarker or understanding of the underlying mechanism. Participant Exposure: Since these effects cannot be monitored directly in clinical studies, coverage of ≥30 fold will be maintained from the no-effect dose (3 mg/kg/day); exposure will not intentionally exceed the average daily area under concentration-time curve (AUC) of 0.513 hr*µg/mL and/or maximum observed plasma concentration (Cmax) of 0.050 µg/mL on an individual basis.
Myocardial toxicity	Dogs (4-week study): at 30 mg/kg/day, myofibre degeneration/necrosis and inflammation (2 animals)	Participant Selection: Participants with any history or current disease constituting a risk when taking the study treatment, including relevant history of acute coronary syndromes (including unstable angina), coronary angioplasty, or stenting, will be excluded. Participant Monitoring: Cardiac troponin levels will be monitored and ECGs will be done during the study. Participant Exposure: Exposure levels will be maintained below the threshold detailed in the Dose Justification Section (see Section 5.5).

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Mortality/moribund condition; poor viability	Dogs (4-week study): at 30 mg/kg/day, one male terminated early (Day 6) due to poor clinical condition. Another male had transient whole body shaking on Days 8 and 9. Dogs (13-week study): at 10 mg/kg/day one male was terminated early (Day 74) due to welfare reasons. Rats (micronucleus and comet study): mortality occurred following 1 to 3 doses at ≥600mg/kg/day	Participant Monitoring: Weight and adverse events reported by participants will be monitored.
Gastrointestinal (GI) and/or hepatic toxicity	GI toxicity: ≥3 mg/kg/day in dogs and at 30 and 300 mg/kg/day in rats, consisting of mucosal erosion/ulceration in the stomach and/or duodenum. Hepatic Toxicity: Biliary epithelial hypertrophy/hyperplasia and periductal mixed inflammatory cell infiltrate into the liver was observed at 300 mg/kg/day in rat (7-day study) and focal hepatocellular degeneration in 1 male dog at 30 mg/kg/day (4-week study)	Participant Selection: Participants with active ulcer disease or GI bleeding or those who are taking concomitant medications, including nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids, will be excluded. Assessment of faecal occult blood will be performed before and after dosing. Participant Monitoring: Participants will be monitored for GI intolerance (e.g by adverse events such as abdominal discomfort) and sequential clinical chemistry analysis, including liver enzymes. Follow-up faecal occult blood test (FOBT) will be conducted.
Testicular toxicity	Inconsistent finding in rats (4-week study): Spermatid retention at ≥60 mg/kg/day, however no effect observed in 13-week study. The observations in the 4-week study were not associated with degenerative changes in testes or epididymides. No spermatogenic abnormalities were observed in dogs.	Participant Exposure: A safety margin of ≥40 fold will be maintained from the no effect dose (60 mg/kg/day) in rats.
Skeletal muscle toxicity	Rat (4-week study): Myofiber necrosis: myofiber degeneration/regeneration; fibroplasia, at 300 mg/kg/day in the soleus muscle.	Participant Monitoring: Creatinine phosphokinase (CPK) levels will be taken before and after dosing.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Seizures and convulsions	Rats (micronucleus and comet study): convulsions observed at ≥600 mg/kg/day. Convulsions were not related to Cmax, nor occurred at any predictable time from dose administration. Dogs: No central nervous system (CNS) findings at 12 mg/kg/day in the dog 7-day EEG/CV study. In other compounds in the same series, convulsions have been observed.	Participant Selection: Participants with any history or current disease constituting a risk when taking the study treatment, including a history of seizure disorder or stroke within the last 5 years will be excluded from the study.
Low food consumption	Dogs (4-week study): 30 mg/kg/day reduced food consumption. Two males were terminated early (Day 10) due to extremely reduced food consumption. Rats (4-week study): 300 mg/kg/day had decreased food consumption.	Participant Monitoring: Weight will be monitored.
Effects on macrophages (Phospholipid accumulation)	Inconsistent effects observed in Rats (4-week study): ≥60 mg/kg/day in the lung (prominent alveolar macrophages); 300 mg/kg/day in the mesenteric lymph node (increased cellularity of sinus macrophages) and thymus (macrophage vacuolation; increased thymus weight). Consistent with phospholipid accumulation (phospholipidosis) based on ultrastructural appearance of mesenteric lymph nodes at 300 mg/kg/day. Findings were not associated with degenerative changes. In 13-week studies in rats, these effects were not observed.	Participant Exposure: A safety margin of ≥40 fold will be maintained from the no effect dose (60 mg/kg/day) in rats.
Theoretical Risk: Potential effects on vasoregulation .	TRPV4 mediates prostaglandin release from isolated human endothelial cells and in vivo in rats, supporting the potential for TRPV4 blockade to modulate blood pressure via prostaglandin release. No effect of GSK2798745 on blood	Participant Monitoring: Blood pressure will be monitored throughout the study.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	pressure was observed in preclinical or clinical studies.	
Theoretical Risk: Potential effect on hearing.	Genetic deletion of TRPV4 in mice has been shown to effect hearing. TRPV4 knockout (KO) mice at age 8 weeks exhibited normal hearing thresholds, but at age 24 weeks, had delayed-onset hearing loss; additionally, the cochlea was found to be vulnerable to acoustic injury with sound overexposure [Tabuchi, 2005]. Patients with Charcot-Marie-Tooth Disease Type 2C (CMT2C), an autosomal dominant axonal neuropathy related to TRPV4 gene mutations, demonstrate symptoms that include hearing loss caused by nerve damage in the inner ear (sensorineural hearing loss). These are predominantly gain of function TRPV4 abnormalities, in which the hearing loss is sporadic among family members; and relegated to some TRPV4 defects, but not in others. Although the exact mechanism is unclear, it has been suggested that the TRPV4 channel plays an important role in peripheral nerve function and that the alterations in TRPV4 in CMT2C may be due to increased channel activity leading to excessive calcium influx and a calcium overload. There is potential for benefit with GSK2798745, in that with cells (HEK293) expressing the CMT2C mutant channel, inhibitors of the TRPV4 channel were found to block the increased intracellular calcium concentrations and resultant cell death [Landouré, 2010]. In a study evaluating effects on heart failure with similar dosing exposure, a week-long duration and comprising an older participant demographic, audiometry testing was conducted. Although a variety of changes were observed, primarily	Participant Exposure: Dosing will be limited to a short duration of a single day.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	sporadic, low frequency and single frequency changes in those on treatment as well as placebo, none were assessed as a signal for concern by the reviewing expert audiologist.	
Theoretical risk: Potential suicidal ideation	GSK2798745 is considered to be a CNS-active drug. There has been some concern that some CNS-active drugs may be associated with an increased risk of suicidal thinking or behaviour when given to some patients with certain conditions. GSK considers it important to monitor for such events before or during clinical studies with CNS-active compounds. Participants being treated with GSK2798745 should be assessed at screening for suicidality.	Participant Selection: The risk of such an event would be very low in single-day dose trials in healthy volunteers; however, participants who, in the investigator/designee's judgement, poses a significant suicide risk, will be excluded from the study. A C-SSRS questionnaire will be completed before and after dosing.
Study procedures-related		
Risk of adverse events following delivery of LPS	Previously reported segmental LPS related adverse events include pleuritic pain, pyrexia, head ache, nausea and alveolitis [Hohlfeld, 2008; Holz, 2015]. It is known that LPS and pro-inflammatory mediators can cause gut permeability [Al-Sadi, 2014; Guo, 2013]. We expect the systemic exposure to LPS and systemic inflammatory response to LPS to be minimal following the segmental topical dose of 4ng/kg LPS. As such, we assess the risk to increased gut permeability, and potential increased bioavailability, with this regimen to be low.	Participant Monitoring: Participants will be monitored with safety assessments, including clinical laboratory tests, physical examination, ECGs, vital signs (including temperature), pulse oximetry and spirometry during the study. Experienced site staff will conduct the procedures. Participants will be carefully monitored and managed with standard procedures in the event of complications.
Risks associated with bronchoscopy and BAL sampling:	Procedure related complications include cough, transient fever, chills and myalgias, transient infiltrates, bronchospasm, transient fall of lung function, transient decrease in baseline PaO2 (partial pressure of oxygen in arterial blood).	

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Risk associated with blood draws	Fainting, mild pain, bruising, irritation or redness.	Experienced site staff will follow standard approaches for managing events related to blood draws.
Risks associated with spirometry	Shortness of breath, coughing, light-headedness or fainting, and/or chest tightness may be induced by spirometry testing.	Participants experiencing any of these symptoms will receive standard medical treatment by the study investigator.

3.3.2. Benefit Assessment

There will be no intended therapeutic clinical benefit to the participants taking part in the study as it is a healthy volunteer study. However, participants will undergo a medical evaluation during screening including physical exams, electrocardiograms (ECGs) and laboratory assessments which may provide important health information.

By taking part in this study, the participant will be contributing to the development of GSK2798745 for the treatment of ARDS, a syndrome with significant unmet need.

3.3.3. Overall Benefit:Risk Conclusion

No benefit to healthy participants is expected. The study activities have risks, however these risks are mitigated by: exclusion of participants with identified comorbidities; short (single day) duration of dosing with GSK2798745; exposure limited to previously approved exposure levels; and inclusion of safety margins, as well as safety monitoring by trained staff. The study will provide important information towards future development of TRPV4 blockers that may help patients with ARDS.

The design of the study is considered low risk to the participants and justified based on the safety information from the nonclinical studies and the previous clinical trials carried out on GSK2798745.

4. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
• To investigate the effect of GSK2798745 on alveolar-septal barrier permeability following LPS challenge in healthy volunteers.	• Baseline adjusted total protein concentration in bronchoalveolar lavage (BAL) samples at 24 h after segmental LPS challenge.
Secondary	
• To investigate the effect of GSK2798745 on neutrophil levels in LPS-challenged lungs.	• Baseline adjusted total and differential cell count of neutrophils in BAL samples at 24 h after segmental LPS challenge.
• To assess the safety and tolerability of GSK2798745 in LPS-challenged, healthy volunteers.	• Adverse events (AEs), laboratory safety assessments, 12-lead electrocardiogram (ECG), physical examination, vital signs, spirometry (forced expiratory volume in 1 second [FEV1] and forced vital capacity [FVC]), faecal occult blood test (FOBT).
• To assess the pharmacokinetics (PK) of GSK2798745 in plasma in LPS-challenged, healthy volunteers	• PK parameters of GSK2798745 in plasma (AUC [0-26] and Cmax).
Exploratory	
• To assess concentration of GSK2798745 in BAL.	• Levels of GSK2798745 in BAL samples at 2 and 26 h after dosing.
• To investigate the effect of GSK2798745 on exploratory markers of endothelial barrier permeability and/or injury, and inflammation, in LPS-challenged lungs.	• Baseline adjusted levels of exploratory biomarkers in blood and/or BAL.
• To possibly assess metabolite GSK3526876 (major metabolite of GSK2798745) in plasma and BAL samples.	• Plasma and BAL concentrations of metabolite GSK3526876.

Objectives	Endpoints
• To compare the PK of GSK2798745 with the effect of prophylactic dosing on alveolar-septal barrier permeability following LPS challenge in healthy volunteers.	• Comparison of PK parameters of GSK2798745 in plasma with baseline corrected total protein concentration in BAL.
• To investigate the effect of GSK2798745 on white blood cell levels (except neutrophils) in LPS-challenged lungs.	• Baseline adjusted total and differential cell count of white blood cells (except neutrophils) in BAL samples at 24 h after segmental LPS challenge.

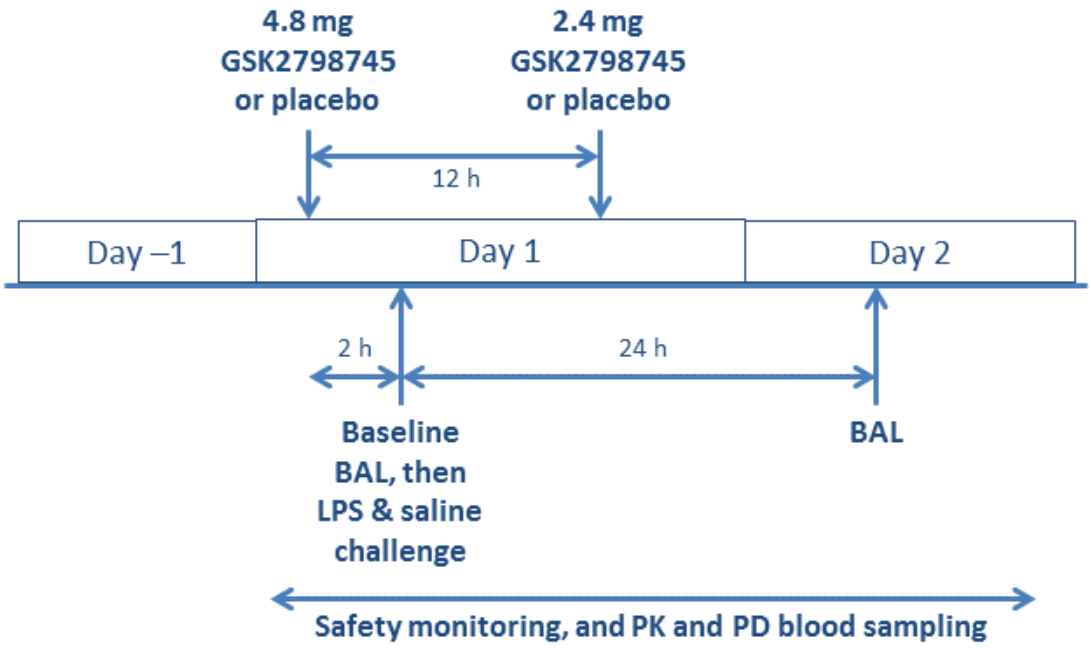
5. STUDY DESIGN

5.1. Overall Design

This is a randomised, placebo-controlled, parallel group, double-blind (sponsor-open), segmental LPS challenge study of GSK2798745 in healthy participants.

Participants will be randomised 1:1 to take 2 single doses (3 tablets total): either 4.8 mg GSK2798745, then 2.4 mg GSK2798745 12 h later; or a dose of placebo, then another dose of placebo 12 h later. Participants will undergo segmental LPS challenge to the lungs at 2 h after the first dose. BAL samples will be taken after dosing: immediately before and at 24 h after the LPS and saline challenge. Blood samples will be taken before and after dosing and the challenge. Safety will be monitored throughout. A schematic of the study is provided in Figure 2.

Figure 2 Study schematic



BAL: bronchoalveolar lavage; LPS: lipopolysaccharide; PD: pharmacodynamic; PK: pharmacokinetic.

Each participant will take up to approximately 5 weeks to complete the study. Participants will be screened within 28 days before dosing and remain on site from the day before dosing (Day −1) until the day after dosing (Day 2). The first dose will be administered on Day 1 at 2 h before baseline BAL sampling from a segment in the left lower lobe. 4 ng/kg LPS will subsequently be instilled into the right middle segment and saline control into the lingula segment of the contralateral side. The second dose of study treatment will be administered 10 hours after LPS challenge followed by post-dose BAL sampling on Day 2. Participants will receive a follow up phone call 2 days after discharge, and return to the study site on Day 8 (± 1 day) for a follow-up visit.

5.2. Number of Participants

Sufficient healthy participants will be screened to ensure that a maximum of 60 evaluable participants (defined as all participants for whom results of the primary analysis can be determined) are enrolled onto the study: 30 for GSK2798745 and 30 for placebo. Interim analyses will be conducted (as described in Section 10.3.4), to assess the difference, if any, between treatments in the primary endpoint. There are two interim analyses planned, depending on the results of the interim analyses, recruitment may be stopped. Participants who prematurely discontinue the study, and whose data are not evaluable (i.e. those for whom results of the primary analysis cannot be determined), may be replaced. Recruitment into the study can continue whilst interim analyses are taking place.

5.3. Participant and Study Completion

A participant is considered to have completed the study if he/she has completed all phases of the study including the follow-up visit.

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

5.4. Scientific Rationale for Study Design

- *Use of a placebo:* The randomised, placebo-controlled study design is a well-established methodology to evaluate potential treatment effects in experimental studies. The use of a placebo arm allows for a valid evaluation of changes in PD markers attributable to treatment versus those independent of treatment. Given that participants are healthy and treatment is not being withheld, the use of placebo is considered acceptable.
- *Parallel group:* The use of a parallel group design is considered to limit the burden of procedures and exposure of LPS to participants. A crossover design only minimally decreases the required number of evaluable participants and owing to the expected dropout rate in a crossover study, and the burden of procedures for each participant, a parallel group design was selected.
- *Blinding:* A double blind design is a standard methodology for randomised, controlled studies to avoid bias.
- *Selection of primary endpoint:* Changes in total protein concentrations to monitor influx of proteinaceous fluid into the lung has been used in several LPS challenge studies. Evidence points to the involvement of TRPV4 in changes to alveolar capillary barrier following LPS administration, with subsequent leak of proteinaceous fluid as measured by total protein. Given the influx of fluid with high concentrations of protein observed in ARDS patients, monitoring total protein changes is considered an acceptable surrogate to assess for target engagement of GSK2798745 and for potential effects in ARDS patients.
- *Timing of primary endpoint:* As previous studies show that total protein concentrations peak at 24 h after segmental LPS challenge [O'Grady, 2001; Holz, 2015], post-challenge BAL samples will be taken at 24 h after the challenge.

- *Timing of challenge:* Baseline BAL sampling prior to administration of study drug is not considered feasible due to the burden of bronchoscopy (i.e. two procedures required – one pre and one post dose to instil LPS). A baseline bronchoscopy is planned 2 h after administration of the first dose, with the aim of maximising exposure to GSK2798745 (in healthy volunteers, the median [range] Time to reach maximum plasma concentration (Tmax) of the tablet formulation of GSK2798745 to be used in this study is 1.5 h [1–3 h]). Drug on board at the time of baseline BAL fluid sampling is expected to have little to no effect on baseline BAL total protein concentrations as TRPV4 channels are quiescent until stimulated and blockade of these channels does not affect barrier permeability of 'healthy' endothelial cells [internal data]. The effects of LPS to induce endothelial permeability are believed to occur rapidly. Investigation of LPS induced permeability and markers of cell activation and inflammation over time consistently indicates that the initial events leading to increased barrier permeability and inflammation occur within first 2 h post intratracheal (i.t.) LPS exposure; where any earlier measurements are limited. LPS induces intracellular stress fibre formation within 5 min in both human dermal microvascular endothelial cells (HMVEC) endothelial and A549 epithelial cells [Ngamsri, 2010]. Increased levels of TNFα following i.t. LPS instillation are observed within 2 h in man [O'Grady, 2001] and other pro-inflammatory mediators within 4 h in a mouse model of acute lung injury (ALI) [Bosmann, 2012]. Permeability across an endothelial monolayer is observed after 0.5 h and peaks at 2 h post-LPS [Bannerman, 1998]. BAL total protein increases following LPS i.t. challenge in man as early as 2 h and reaches significance at 6 h post-LPS [Matthay , 2012] with significant increases in neutrophil counts also observed as early as 2 h. Looking to a systemic LPS model of sepsis with a higher LPS insult, earlier sampling is possible and high levels of inflammatory mediators are measured within 1 h, which reach plateau by 3 h. Therefore, despite our conservative treatment effect prediction (see Section 5.5), we expect that high plasma and/or alveolar fluid concentrations of GSK2798745 will be required at the time of and/or soon after the challenge, in order to inhibit the effects of LPS. Therefore, LPS will be administered shortly after time of Tmax (1.5-hours) to ensure sufficient drug on board.

5.5. Dose Justification

5.5.1. Dose Rationale

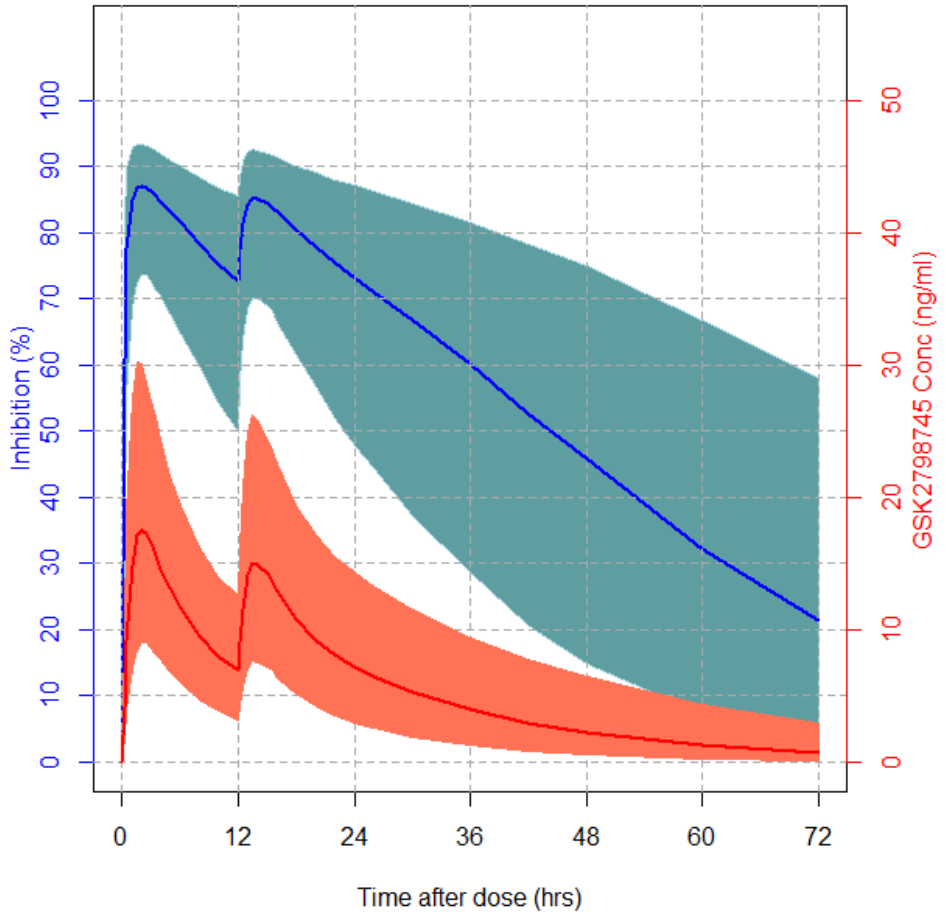
The first time in human study (TR4113787) for GSK2798745 evaluated doses from 0.25 mg to 12.5 mg as single doses, and 5 mg once daily repeat dosing for 14 days in healthy volunteers in cohorts 1–3. Cohorts 4 and 5 of the study were administered once daily 2.4 mg dose of capsules with food for 7 days to stable heart failure participants. Exposure from different tablet formulations administered with or without food was characterised in another clinical study in healthy volunteers (204725). Exposure from all available clinical data to date was analysed with a population pharmacokinetic (POP PK) approach accounting for participant weight, formulation, impact of food amongst other variables. Trial simulations were performed with this POP PK model with differing dosing regimen.

The estimated human IC50 of 2.1–3.2 ng/mL was derived from data from a rat study, which was conducted to assess the ability of different doses of GSK2798745 infusion to reduce the increased lung-to-bodyweight ratio induced by the TRPV4 agonist. The IC50 was also corrected for species differences using protein binding data and TRPV4 potency differences in *invitro* assays. To evaluate drug activity/efficacy at the intended dosing regimen, the percentage pulmonary oedema blockade was estimated using the population model and the corrected potency values derived from the rat pulmonary study.

Based on the simulations, the intended dosing scheme for this study is a 4.8 mg dose followed by a 2.4 mg dose at 12 h. Table 1 lists the predicted average percentage pulmonary oedema blockade over the 24-h period based on this potency range. The schematic in Figure 3 also depicts the range of GSK2798745 systemic exposure and the predicted percent inhibition by TRPV4 with the intended regimen. At 26 h post the first dose, when the primary endpoint sample will be taken, the predicted TRPV4 inhibition is 71.1% (44.4–86.3) [median (95% prediction interval)].

The intended dosing regimen also limits the daily ceiling human exposure at individual participant level to 30-fold of the exposure observed at the no observed adverse effect level (NOAEL) dose of 3 mg/kg from the 3-month dog safety study (D70496G). The current clinical doses are selected so that no participant intentionally exceeds the daily AUC of 513 ng*hr/mL and C_{max} of 50 ng.hr/mL. The likelihood of one or more participants of the 30 participants to be dosed with this regimen exceeding the threshold is listed in Table 1.

Figure 3 Predicted GSK2798745 exposure and corresponding effect on pulmonary oedema after doses of 4.8 mg then 2.4 mg, 12 h apart



Note: percentage inhibition based on the rat, agonist-driven, pulmonary oedema model.

Table 1 Predicted exposure, probability of exceeding threshold and predicted efficacy after doses of 4.8 mg then 2.4 mg, 12 h apart, administered to 30 participants

Percentage pulmonary oedema blockade over 24 h (median [95% PI])	Predicted exposure (median [95% PI])		% Probability that ≥1 of 30 participants will exceed the toxicokinetic limit[1]	
	AUC0–24 h (ng.h/mL)	Cmax (50 ng/mL)	AUC0–24 h (513 ng.h/mL)	Cmax (50 ng/mL)
79.1 [66.5–87.1]	272.4 [176.2–400.9]	23.1 [15.2–34.4]	5.6	1.6

PI: predicted interval.

1. The percentage of the 500 simulated studies (of 30 subjects each) in which ≥ 1 subject exceeds the toxicokinetic limit.

5.5.2. Treatment Effect Rationale

Data from a systemic LPS model [Dalsgaard, 2016] , and from other models of lung injury (chlorine gas exposed mice [Suresh, 2015], pulmonary venous pressure induced

injury of isolated mouse lungs [Narita, 2015], and a mouse model of intratracheal instillation of hydrochloric acid [Hamanaka, 2007]) suggest that TRPV4 blockade contributes 20–80% to a reduction in total protein in BAL fluid after the injury. Averaging the effect from those studies, the maximum effect possible in this segmental LPS model is estimated to be approximately 50%, assuming total TRPV4 channel blockade. Therefore, with estimated exposure expected to reduce total protein by approximately 71% in a pure agonist driven challenge, a median treatment effect of 35% might be expected in this LPS challenge. Based on pragmatic reasons and given some of the uncertainty around TRPV4 contribution to proteinaceous fluid influx as measured by total protein in a segmental LPS challenge, an estimated 30% treatment effect was nominally selected for sample size calculations.

6. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

6.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

AGE
1. Between 18 and 50 years of age inclusive, at the time of signing the informed consent.

TYPE OF PARTICIPANT AND DISEASE CHARACTERISTICS
2. Volunteers who are overtly healthy as determined by medical evaluation including medical history, physical examination, laboratory tests (including a normal coagulation profile), ECGs, vital signs and spirometry. *Note: In the event of out-of-range results of safety tests, the tests may be repeated once within the screening window. If a retest result is again outside the reference range and considered clinically significant by the investigator and GSK medical monitor, the participant will be considered a screen failure.*
3. Normal spirometry (FEV1 ≥80% of predicted, FEV1/FVC ratio ≥70%) at screening and before dosing.

WEIGHT
4. Body weight ≥50 kg and body mass index (BMI) within the range 19–29.9 kg/m^2 (inclusive).

SEX
5. Male or female. **a. Male participants:** A male participant must agree to use contraception, as described in Appendix 5, during the treatment period and for at least 7 days after the last dose of study treatment and refrain from donating sperm during this period. **b. Female participants:** A female is eligible to participate if she is not of childbearing potential, as defined in Appendix 5.

INFORMED CONSENT
6. Capable of giving signed informed consent which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

6.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTC INTERVAL)
1. Significant history of or current cardiovascular, respiratory (eg asthma, chronic obstructive pulmonary disorder (COPD), bronchiectasis, active Tuberculosis [TB]), hepatic, renal, gastrointestinal, endocrine, hematological, autoimmune or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; constituting a risk when taking the study treatment; or interfering with the interpretation of data.
2. Participant who, in the investigator/designee's judgement, poses a significant suicide risk. Evidence of serious suicide risk may include any history of suicidal behaviour and/or any evidence of suicidal ideation on any questionnaires e.g., Type 4 or 5 on the Columbia Suicide Severity Rating Scale (C-SSRS) in the last 5 years.
3. Active ulcer disease or gastrointestinal bleeding at the time of screening (positive faecal occult blood test [FOBT] at screening).
4. Values outside of the following ranges; 90-140 mmHg for systolic blood pressure, 50-90 mmHg for diastolic blood pressure and 50-90 beats/minute for heart rate.
5. An AV block greater than Grade 1.
6. ALT or bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
7. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
8. QTc >450 msec NOTES: the QTc is the QT interval corrected for heart rate according Fridericia's formula (QTcF).
9. At risk of Torsades de pointes (e.g. a personal history or a family history of sudden unexplained death, long QT, familial cardiac syndrome, or cardiomyopathy).
10. Chronic or acute infection within the 4 weeks before dosing, (e.g. upper and lower respiratory infection within the 4 weeks before dosing).
11. Major (as per investigator judgment) surgery within the last 12 weeks prior to randomisation or planned within 3 months of screening.

PRIOR/CONCOMITANT THERAPY
12. Use of prescription or non-prescription drugs (except paracetamol), including vitamins, herbal and dietary supplements (including St John's Wort) within 7 days or 5 half-lives (whichever is longer) before the first dose of study medication, unless, in the opinion of the investigator and GSK Medical Monitor, the medication will not interfere with the study procedures or compromise participant safety.
13. History of sensitivity to any of the study medications, or components thereof or a

PRIOR/CONCOMITANT THERAPY
history of drug or other allergy that, in the opinion of the investigator and/or GSK Medical Monitor, contraindicates their participation.

PRIOR/CONCURRENT CLINICAL STUDY EXPERIENCE
14. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within 3 months. 15. The participant has participated in a clinical trial and has received an investigational product within the following time period before the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer). 16. Exposure to more than four new chemical entities within 12 months before the first dosing day.

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
17. Presence of hepatitis B surface antigen (HBsAg) at screening 18. Positive hepatitis C antibody test result at screening. NOTE: Participants with positive Hepatitis C antibody due to prior resolved disease can be enrolled, only if a confirmatory negative Hepatitis C Ribonucleic acid (RNA) test is obtained. 19. Positive Hepatitis C RNA test result at screening or within 3 months prior to first dose of study treatment. NOTE: Test is optional and participants with negative Hepatitis C antibody test are not required to also undergo Hepatitis C RNA testing. 20. A positive pre-study drug/alcohol/cotinine screen. 21. A positive test for immunodeficiency virus (HIV) antibody. 22. Regular use of known drugs of abuse.

OTHER EXCLUSIONS
23. History of regular alcohol consumption within 6 months of the study defined as an average weekly intake of >21 units for males or >14 units for females. One unit is equivalent to 8 g of alcohol: a half-pint (~240 mL) of beer, 1 glass (125 mL) of wine or 1 (25 mL) measure of spirits. 24. Current smoker or a history of smoking within 6 months of screening, or a total pack year history of >5 pack years. [number of pack years = (number of cigarettes per day/20) x number of years smoked].

6.3. Lifestyle Restrictions

6.3.1. Meals and Dietary Restrictions

- Participants are not permitted to consume red wine, Seville oranges, grapefruit or grapefruit juice, and/or pomelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days before the start of study treatment until discharge from the clinical unit.
- Participants will be required to fast from midnight before the bronchoscopies on Day 1 and 2 until after the procedure and for at least 2 hours before dosing.

6.3.2. Caffeine, Alcohol, and Tobacco

- During the Treatment Period, participants will abstain from ingesting caffeine- or xanthine-containing products (eg, coffee, tea, cola drinks, and chocolate) for 24 hours before the start of dosing until after collection of the final pharmacokinetic (PK) and/or pharmacodynamic sample.
- During the Treatment Period, participants will abstain from alcohol for 24 hours before the start of dosing until after collection of the final PK and/or pharmacodynamic sample.
- Only non-smokers may be recruited into this study.

6.3.3. Activity

- Participants will abstain from strenuous exercise for 72 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities during studies (eg, watching television, reading).

6.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomised. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs).

Participants may be rescreened once. If rescreening is preformed, participants must be assigned a different unique participant identification number for the rescreening, and all screening procedures must be repeated. See the study reference manual (SRM) for more details.

7. TREATMENTS

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

7.1. Treatments Administered

Study Treatment Name:	**GSK2798745**	**Matching Placebo**	**Challenge Agent: GMP grade lipopolysaccharide from *Escherichia coli* (*E. Coli* Group 0113:H10:K Negative) for the segmental LPS challenge**
Dosage formulation:	White to slightly coloured, round biconvex tablet. Product: AP, Tab-A	White to slightly coloured, round biconvex tablet. Product code: CET, Tab-A..	LPS is available from stock lyophilized in a 1 microgram vial, formulated in 1% lactose and 0.1% PEG6000. Clear solution.
Unit dose strength(s)/Dosage level(s):	Unit dose strength 2.4 mg. Dosage Levels: 4.8 mg and 2.4 mg	Not applicable	4 ng/kg
Route of Administration	Oral	Oral	Direct application to the lung segment, via bronchoscopy
Dosing instructions:	Two tablets to be taken on the morning of Day 1, then one tablet to be taken 12 hours later.	Two tablets to be taken on the morning of Day 1, then one tablet to be taken 12 hours later.	One single instillation of 10 mL by bronchoscopy 2 hours after dosing with GSK2798745 or placebo on Day 1.

Study Treatment Name:	GSK2798745	Matching Placebo	Challenge Agent: GMP grade lipopolysaccharide from *Escherichia coli* (*E. Coli* Group 0113:H10:K Negative) for the segmental LPS challenge
Packaging and Labeling	GSK2798745 tablets will be provided in high-density polyethylene (HDPE) bottles with child-resistant closures that include induction-seal liners. Each bottle will be labelled as required per country requirement.	Placebo tablets will be provided in high-density polyethylene (HDPE) bottles with child-resistant closures that include induction-seal liners. Each bottle will be labelled as required per country requirement.	-
Manufacturer	GSK	GSK	List Laboratories, Inc.

Sterile saline (0.9%) for control challenge will be sourced by the clinical site.

7.1.1. Dose Modification

No dose modifications are permitted without submission of a substantial amendment to the protocol.

7.2. Challenge Agents

Bacterial endotoxin is a component of the cell wall of Gram-negative bacteria present in a variety of occupational and general environmental settings.

The dose of LPS used in this study is 4 ng/kg, which is 100 times lower than doses used in inhalation models of endotoxin-induced lung inflammation. This dose was chosen based on the data published by O'Grady et al [O'Grady, 2001]. In this study, lavage after 24-h in subjects challenged with 4 ng/kg of endotoxin revealed a localised inflammatory response that was neutrophil-predominant. The site performing this study has experience with this model. The LPS challenge agent to be used for the procedure is Good Manufacturing Practice (GMP)-grade product and will be sourced from List Laboratories, Inc. A certificate of analysis will be provided for each batch of the LPS challenge agent to ensure its quality and safety.

Reconstitution will be done under the responsibility and supervision of the investigator or qualified site staff who performs the bronchoscopy and administers the reconstituted LPS in individual dilution to the subject. The reconstitution will be done on the day of bronchoscopy immediately before the procedure (within 1 hour). If more than one subject per day will be investigated, the first process of reconstitution and administration of LPS has to be completed before a second process is started.

The reconstitution process is described as an example for the nominal dose of 10,000 EU of endotoxin per vial. Each vial of LPS contains a lyophilized solid containing 10,000 EU of endotoxin. Upon reconstitution with 5 mL of sterile saline 0.9%, the vial will contain 2,000 EU/mL = 2 EU/µL. Forty (40) µL/kg body weight will be withdrawn from the vial and transferred into a sterile endotoxin-free 30 mL-sample vial (Acila AG, Weiterstadt, Germany). Sterile saline 0.9% will be added to give a final volume of 20 mL. Ten (10) mL of this solution contains the application dose of 40 EU (4 ng)/kg, and will be filled into a 10 mL-syringe. This individual dilution will be administered to the participant by segmental pulmonary application during the bronchoscopy. The remaining 10 mL will be stored frozen (-20°C) as a retention sample.

The negative control will be performed with 10 mL of sterile saline 0.9% only. The reconstitution process will be adapted to the actual endotoxin content as determined in the Certificate of Analysis. The Investigator Site File will contain the current instructions for reconstitution in order to ensure the application dose of 40 EU/kg.

7.3. Method of Treatment Assignment

All participants will be centrally randomized using an Interactive Web Response System (IWRS). Before the study is initiated, the log in information and instructions for the IWRS will be provided to each site. Participants will be registered using the IWRS, and assigned a unique number (randomisation number). The randomisation number encodes the participant's treatment (GSK2798745 or placebo), according to the randomization schedule generated prior to the study by the Statistics Department at GSK. Each participant will be dispensed blinded study treatment, labelled with his/her unique randomisation number.

7.4. Blinding

This will be double blind study. All study staff involved in clinical assessments (which includes the investigator, sub-investigators, other site staff) and the participant will be blinded to the treatment allocated to individual participants and to post challenge total protein in BAL sample results. Selected sponsor study team members (and delegates if programming activities are outsourced) will be unblinded to perform the interim analysis. This may include the medical monitor, study statistician, study programmer (and delegates) and study pharmacokineticist. Access to unblinded data will be kept to the minimum set of individuals required to implement any interim analyses, but may include GSK management/review committees if alterations to the study conduct are required. Details of who were unblinded to what data and when will be included in the clinical study report. The IWRS will be programmed with blind-breaking instructions. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of

a participant's treatment assignment is warranted. Participant safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact GSK prior to unblinding a participant's treatment assignment unless this could delay emergency treatment of the participant. If a participant's treatment assignment is unblinded GSK must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and CRF, as applicable.

A participant whose treatment assignment is inadvertently unblinded (either to investigative staff or the participant themselves) will be permitted to remain in the study, although the accidental unblinding will be recorded as a protocol deviation and hence the participant will be subject to review as to their inclusion in analyses.

GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any participant with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the participant's treatment assignment, may be sent to investigators in accordance with local regulations and/or GSK policy.

7.5. Preparation/Handling/Storage/Accountability

1. The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.
2. Only participants enrolled in the study may receive study treatment and only authorised site staff may supply or administer study treatment. All study treatments 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 authorised site staff.
3. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).
4. Further guidance and information for the final disposition of unused study treatment are provided in the SRM.
5. Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff.
6. A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

7.6. Treatment Compliance

- When the individual dose for a participant is prepared from a bulk supply, the preparation of the dose will be confirmed by a second member of the study site staff.

- When participants are dosed at the site, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study treatment and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment. Study site personnel will examine each participant's mouth to ensure that the study treatment was ingested.
- GSK2798745 and the placebo will be orally administered to participants at the site.

7.7. Concomitant Therapy

7.7.1. Permitted Medications

Paracetamol, at doses of ≤2 grams/day, is permitted for use any time during the study. Rescue medication, such as salbutamol, is also permitted after bronchoscopy or spirometry procedure, if required, and other medication, such as midazolam, may be taken during bronchoscopies to aid the procedure. Other concomitant medication may be considered on a case-by-case basis by the investigator in consultation with the Medical Monitor if required.

7.7.2. Prohibited Medications

Except for the permitted medications noted above (Section 7.7.1), participants must abstain from taking prescription or non-prescription drugs (including vitamins and dietary or herbal supplements) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) before the start of study treatment until completion of the follow-up visit, unless, in the opinion of the investigator and sponsor, the medication will not interfere with the study.

7.8. Treatment after the End of the Study

Participants will not receive any additional treatment from GSK after completion of the study because only healthy volunteers are eligible for study participation.

8. DISCONTINUATION CRITERIA

8.1. Individual Stopping Criteria

8.1.1. Liver Chemistry Stopping Criteria

Increased monitoring criteria have been designed to assure participant safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance). These protocol guidelines are in alignment with FDA premarketing clinical liver safety guidance: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.

Increased monitoring will be performed for a participant if liver chemistry stopping criteria are met.

Liver Safety Required Actions and Follow up Assessments Section can be found in Appendix 6

8.1.2. QTc Stopping Criteria

The QTc should be based on single or averaged QTc values of triplicate electrocardiograms obtained over a brief (e.g., 5-10 minute) recording period.

A participant who meets either bulleted criterion below will be withdrawn from study:

- QTcF >500 msec
- Change from baseline of QTc >60 msec

8.1.3. Symptoms of Cardiac Ischaemia and Cardiac Troponin Stopping/Increased monitoring Criteria

8.1.3.1. Asymptomatic Participant

Cardiac troponin (cTn) will be measured before and at the end of the study. If any cTn assessment is >ULN or >2 times the participant's baseline value (Day −1), the participant should be contacted immediately to assess for symptoms of cardiac ischemia.

8.1.3.2. Symptomatic Participant

If a participant experiences symptoms of cardiac ischaemia (e.g. chest pain, increased shortness of breath, and diaphoresis), cardiology consultation should be obtained immediately. The participant should be evaluated by a cardiologist and undergo any clinically appropriate testing. The participant should be followed up until symptoms are resolved. If the event occurs after the first dose, but before the second, the participant should not receive the second dose.

8.1.4. Bronchoscopy Stopping Criteria

A participant will be withdrawn from the study if they experience symptomatic bradycardia or tachycardia requiring treatment as a consequence of bronchoscopy, BAL and LPS instillation. In addition, participants who have the following may also be withdrawn at the discretion of the investigator:

- Oxygen saturation <90% (on oxygen)
- Prolonged somnolence following administration of midazolam
- Significant hypertension/hypotension

8.2. Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance or administrative reasons.
- If the participant 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 participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.
- Refer to the SoA (Section 2.3) for data to be collected at the time of withdrawal.

8.3. Study Stopping Criteria

If there is a serious unexpected adverse event considered at least possibly related to the investigational product administration in one participant; or a severe non-serious AEs considered as, at least, possibly related to the investigational product administration in two participants, the study will be temporarily halted until full review of the events. In participants, significant changes from baseline measurements will be reviewed and participants will be followed until resolved. Participants who have been dosed at the time of the study halt will continue in the study, as planned. Further participants may be dosed only if, after review, the sponsor and investigator consider it safe to do so and only after ethics and regulatory approval of a substantial amendment.

8.4. Lost to Follow Up

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

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

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the Schedule of Activities (SoA).
- 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 participant should continue or discontinue study treatment.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA.
- The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 500 mL.
- Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

9.1. Bronchoscopy

Bronchoscopy will be performed in accordance with site standard operating procedures (SOPs), at 2 hours and 26 hours. These SOPs will reflect current standards of practice in hospital care and will include (but are not limited to) the following items:

- Oxygen supplementation will be given to all participants.
- Pulmonary function will be monitored before the bronchoscopy and after the bronchoscopy until FEV1 is within 20% of the pre-procedure value.
- All participants will be monitored in a recovery/holding room post bronchoscopy. Participants will be discharged only after approval is obtained from the supervising physician, and participants will be given a 24-hour contact number. Admission facilities will be available in the event a participant is not deemed fit for discharge or if overnight observation is deemed necessary by the study physician or site-specific policies.

Following bronchoscopies, participants will be monitored for at least 4 hours. However, should the investigator have any concern for participant safety, participants may be requested to remain resident for further observation.

9.2. Segmental lung challenge

Participants will undergo segmental challenge to the lungs, via bronchoscopy, at 2 hours after the first dose of investigational medicinal product (IMP): 10 mL LPS (4 ng/kg) will be instilled into the right middle segment and 10 mL saline control will be instilled into the lingula segment of the contralateral side.

Local SOPs will be followed and further details about the segmental LPS/saline challenge are provided in the SRM.

9.3. Pharmacodynamic Assessments

BAL samples will be taken, via bronchoscopy, to measure total protein and neutrophils counts. Baseline samples will be taken immediately before the LPS and saline challenges, from a segment in the left lower lobe, and post-challenge samples will be taken at 24 h after the LPS and saline challenges, from the challenged segments.

Details of BAL sample collection, processing, storage and shipping procedures are provided in the SRM.

9.4. Pharmacokinetics

Blood and BAL samples for assay of GSK2798745 will be collected at the time points indicated in the SoA (Section 2.2). Details of sample collection, processing, storage and shipping procedures are provided in the SRM.

PK analysis will be performed under the control of Platform Technologies and Science-IVIVT (PTS-IVIVT)/GlaxoSmithKline. Plasma and BAL concentrations of GSK2798745 will be determined using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Plasma and BAL samples may be analysed for the metabolite GSK3526876, GSK will store the remaining plasma and/or BAL for future possible metabolite investigations. Analysis of compound-related metabolites may be reported under a separate protocol.

9.5. Adverse Events

The definitions of an AE or SAE can be found in Appendix 4.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the participant to discontinue the study (see Section 8).

Adverse event/serious adverse event reporting will meet the requirements set out in Section 12.2.4 of the ICH E3 Guidelines.

9.5.1. Time Period and Frequency for Collecting AE and SAE Information

- All SAEs will be collected from the start of treatment until the follow-up visit. However, any SAEs assessed as related to study participation (e.g. study treatment, protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product, will be recorded from the time a participant consents to participate in the study.
- All AEs will be collected from the start of treatment until the follow-up visit.
- Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee within 24 hours, as indicated in Appendix 4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment 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 4.

9.5.2. Method of Detecting AEs and SAEs

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

9.5.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All AE/SAEs, will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 8.4). Further information on follow-up procedures is given in Appendix 4.

9.5.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment 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 treatment

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 a SAE or other specific safety information (eg: summary or listing of SAE) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

9.5.5. Pregnancy

- Details of all pregnancies in female partners of male participants will be collected after the start of study treatment and until the follow-up visit.
- If a pregnancy is reported, the investigator should inform GSK within 24 hours of learning of the pregnancy and should follow the procedures outlined in Section 12.5.

9.6. Treatment of Overdose

GSK does not recommend specific treatment for an overdose. The investigator (or physician in charge of the participant at the time) will use clinical judgment to treat any overdose.

In the event of an overdose, the investigator or treating physician should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the participant for AE/SAE and laboratory abnormalities until GSK2798745 can no longer be detected systemically (at least 5 days).
3. Obtain a plasma sample for PK analysis within 1 day from the date of the last dose of study treatment if requested by the Medical Monitor (determined on a case-by-case basis).
4. Document the quantity of the excess dose as well as the duration of the overdosing in the CRF.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the Medical Monitor based on the clinical evaluation of the participant.

9.7. Safety Assessments

Planned time points for all safety assessments are provided in the Schedule of Activities (SoA).

9.7.1. Physical Examinations

- A complete physical examination will include, at a minimum, measuring weight and assessments of the Skin, Cardiovascular, Respiratory, Gastrointestinal and Neurological systems. Height will be measured at screening only.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

9.7.2. Vital Signs

- Vital signs will be measured in a semi-supine position after 5 minutes' rest and will include temperature, systolic and diastolic blood pressure and heart rate
- At each time point before dosing, 3 readings of blood pressure and heart rate will be taken. The first reading should be rejected. The second and third readings should be averaged to give the measurement to be recorded in the CRF. At all other time points, single measurements will be taken.

9.7.3. Pulse Oximetry

- Pulse oximetry will be monitored during each bronchoscopy. Clinically significant results will be recorded as AEs.

9.7.4. Electrocardiograms

- Triplicate 12-lead ECGs will be obtained at each time point before dosing. At all other time-points a single 12-lead ECG will be obtained as outlined in the SoA (see Section 2) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 8.1.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.

- ECG to be measured in a semi-supine position after 5 minutes' rest.

9.7.5. Spirometry

Spirometry assessments will be performed from screening through the final visit as indicated in the SoA (Section 2). The following parameters will be assessed:

- Forced expiratory volume in one second (FEV_1)
- Forced vital capacity (FVC)

Measurements will be made in triplicate and the best recording (i.e. the highest FEV_1 and the highest FVC) from 3 technically acceptable manoeuvres will be recorded in the CRF. To fulfill the entry criteria, FEV_1 should be ≥80% of predicted and FEV1/FVC ratio ≥70%) at screening and pre-dose.

Details on performing the spirometry assessments are provided in the SRM.

9.7.6. Clinical Safety Laboratory Assessments

- Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- 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 CRF. The laboratory reports must be filed with the source documents.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study (until the follow-up visit) should be repeated until the values return to normal or baseline or are no longer considered significantly abnormal 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 aetiology should be identified and the sponsor notified.
- If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE or AE), then the results must be recorded in the CRF.

9.7.7. Faecal Occult Blood Test (FOBT)

Based on the preclinical finding of gastric erosions, FOBT will be performed before and after dosing (See Section 2). Details on FOBT are provided in the SRM.

9.7.8. Columbia Suicide Severity Rating Scale (C-SSRS)

Based on preclinical studies that have been conducted, GSK2798745 is considered to be a central nervous system (CNS)-active drug. There has been some concern that some CNS-active drugs may be associated with an increased risk of suicidal thinking or behaviour when given to some patients with certain conditions. Although GSK2798745 has not been shown to be associated with an increased risk of suicidal thinking or behaviour, GSK considers it important to monitor for such events.

The C-SSRS is a measure of suicidal ideation and behaviour, with demonstrated predictive validity and reliability. Sections of the C-SSRS include suicidal ideation, intensity of ideation, suicidal behaviour, and actual suicide attempt(s). The C-SSRS assesses lifetime and current suicidal thoughts and behaviours across these categories based on an increasing severity of a 1- to 5-rating scale. The semi-structured questionnaire is completed by a trained and experienced neurologist, psychiatrist, or

neuropsychologist, or another trained and experienced person approved by the Sponsor, who may be the Principal Investigator or a sub-investigator for the study. See SRM for details of the scale.

The C-SSRS will be performed at screening and after dosing before discharge (See Section 2).

9.8. Exploratory biomarkers

Blood and BAL samples for exploratory biomarker analysis of endothelial barrier permeability and/or injury and inflammation will be collected at the time points indicated in the SoA (Section 2).

Samples may also be used for research to develop methods or support identification of prognostic/diagnostic biomarkers associated with clinical outcomes in ARDS and related diseases.

Details of sample collection, processing, storage and shipping procedures are provided in the SRM.

Exploratory biomarker results may be entered into the clinical study database before or after database lock and unblinding, or may be reported separately.

9.9. Genetics

Genetics are not evaluated in this study.

10. STATISTICAL CONSIDERATIONS

The primary study objective is to investigate the effect of GSK2798745 relative to placebo on BAL total protein at 24 hours after segmental LPS challenge. In this study a Bayesian framework will be used (with non-informative priors) to estimate the posterior probability of any percentage reduction in mean 24 h post-LPS BAL protein level (GSK2798745 relative to placebo). It is anticipated that BAL total protein will be $\log_e$ transformed to improve normality before model fitting. Following back-transformation treatment comparisons will be expressed as percentage changes.

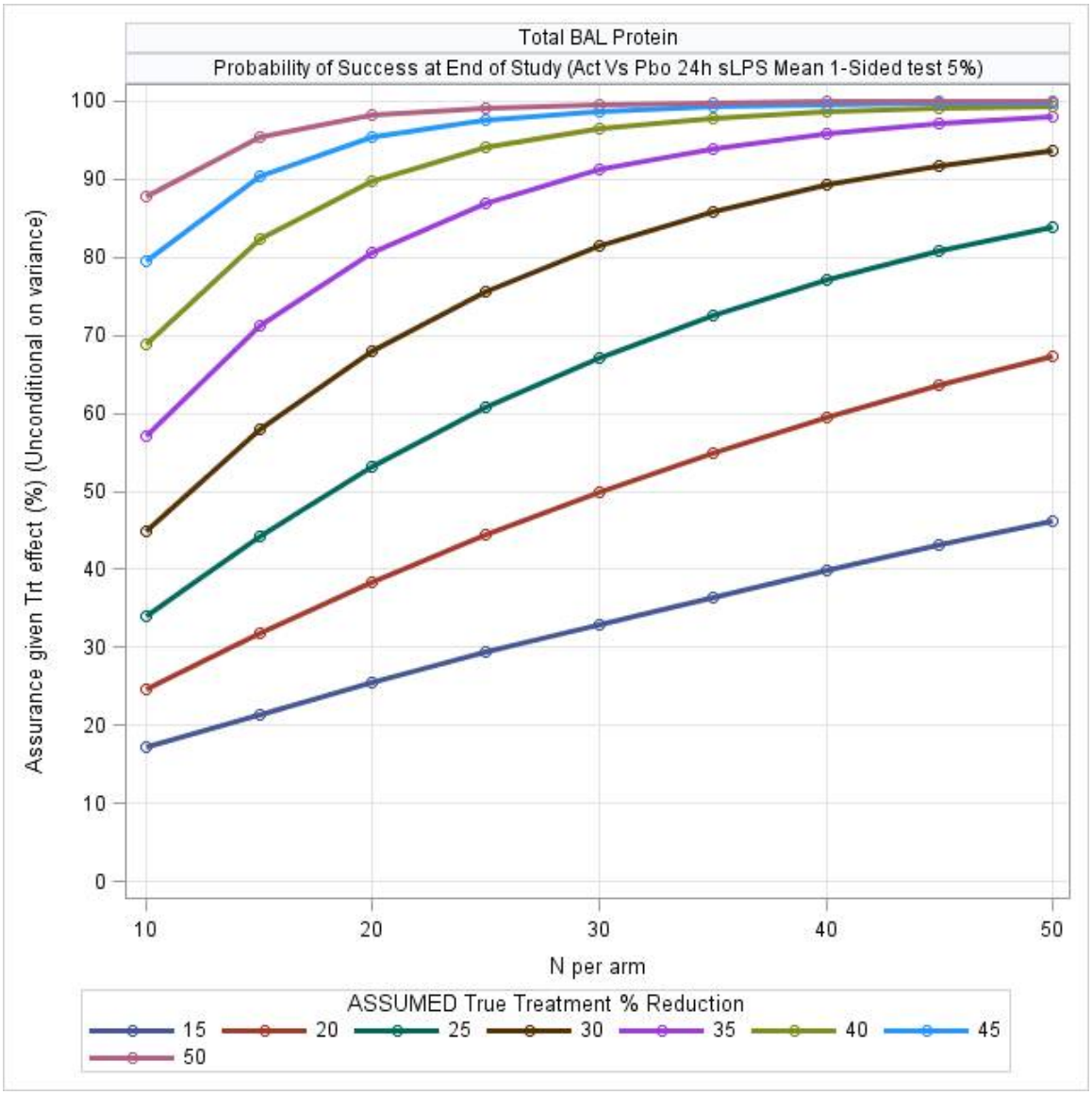
10.1. Sample Size Determination

The sample size of 30 participants per arm (1:1 allocation) is based on an upper limit of feasibility. Figure 4 illustrates the probability of study success for a range of sample sizes and assumed true treatment effects unconditional on variability.

Assuming a true treatment effect of a 30% reduction in mean BAL total protein in participants receiving GSK2798745 relative to placebo at 24 h after segmental LPS challenge to the lung, the probability of study success for the study is 82% (this excludes the possibility of success from a review of secondary data), unconditional on the variability of BAL total protein in segmental LPS challenged lungs. Outright end of study success is defined as at least a 95% posterior probability that the percentage reduction in the mean 24 h post-LPS BAL total protein level (GSK2798745 relative to placebo) exceeds zero.

Despite limited historical information for guidance on the true treatment effect for GSK2798745, 30% is believed to be a rational estimate. However, probability of study success is sensitive to the true treatment effect – for example, the probability of study success reduces to 50% if the true treatment effect is 20% (GSK2798745 relative to placebo) with a sample size of 30 per arm but increases to 96% if the true treatment effect is 40%.

Figure 4 Probability of Study Success (Unconditional on Variance) for a Range of Treatment Profiles and Sample Sizes



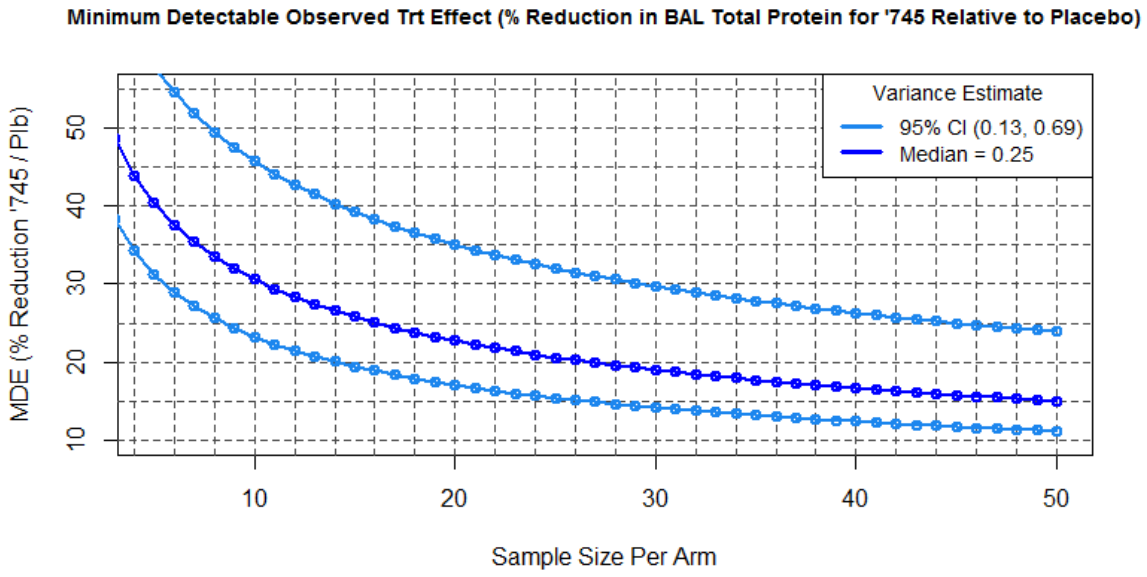
Estimates for the mean and variance of BAL total protein in segmental LPS challenged lungs were obtained from data in an observational study assessing the variability of the inflammatory response to segmental LPS challenge by the Fraunhofer Institute of Toxicology and Experimental Medicine, Germany [Holz, 2015] using a random effects model with total protein levels from 11 participants measured at baseline and 24 h over 2 study periods. The data were assumed to be representative of future segmental-LPS challenged lungs to be observed in this study.

The estimate of the mean BAL protein in 24 h post-LPS challenged lungs on the $\log_e$ scale is 5.47 (95% CI 5.23, 5.72). The variance estimate on the $\log_e$ scale is 0.25 (95% CI 0.13, 0.69). A posterior distribution was formed for the standard deviation and for each possible value of standard deviation from this distribution the power of the study was calculated and multiplied by its probability. The resulting values were all summed to

give probability of study success unconditional on variance, which is a more accurate representation of the probability of study success rather than assuming a single variance estimate with no uncertainty.

Figure 5 shows the minimum observed treatment effect in the study that would achieve success (expressed as a percentage reduction in BAL total protein for GSK2798745 relative to placebo) for a number of sample sizes. Assuming the variance in the study is the median obtained from the historical data, the minimum treatment effect to trigger success is 19% for a sample size of 30 participants per arm. As the sample size increases, the minimum observed treatment effect that would trigger success decreases due to the decreasing standard error (greater confidence in the observed treatment effect). This figure gives rationale for including the data review region since it is probable that moderate observed treatment effects will not achieve success with a sample size of 30 per arm.

Figure 5 Minimum Detectable Effect for Range of Sample Sizes



10.2. Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All participants who sign the ICF.
Evaluable	All participants for whom results of the primary analysis can be determined.
Safety	All randomized participants who take at least 1 dose of study treatment. Participants will be analysed according to the treatment they actually received.

Other populations, such as Per-Protocol population, may be defined in the RAP.

10.3. Statistical Analyses

10.3.1. Efficacy Analyses

Endpoint	Statistical Analysis Methods
Primary	The mean percentage reduction in BAL total protein (µg/mL) in segmental LPS challenged lungs, at 24 h post-challenge, for GSK2798745 relative to placebo, will be assessed using an analysis of covariance (ANCOVA) model (fitted in a Bayesian framework with non-informative priors for model parameters) testing that there is at least 95% posterior probability that the percentage reduction in 24 hour post-challenge mean BAL total protein (GSK2798745 relative to placebo) exceeds zero , adjusting for baseline BAL total protein. A sensitivity analysis will include 24 h BAL total protein in the saline segment (saline challenge will be administered to the contralateral lobe) as a covariate. Additional factors may be included as covariates if deemed appropriate.
Secondary	See Section 10.3.2 and Section 10.3.3
Exploratory	Will be described in the reporting and analysis plan.

10.3.2. Safety Analyses

All safety analyses will be performed on the Safety Population.

Endpoint	Statistical Analysis Methods
Secondary	For the safety data, no formal hypotheses will be tested. Safety data such as adverse events, electrocardiogram (ECG), physical examinations, vital signs, spirometry, FEV1 and FVC will be displayed in the form of listings, frequencies, summary statistics and graphs. Interpretation will be aided by clinical expertise. Full details, including example outputs, will be documented in the RAP.

10.3.3. Other Analyses

PK, pharmacodynamic, and biomarker exploratory analyses will be described in the reporting and analysis plan. The population PK analysis and pharmacodynamic analyses will be presented separately from the main clinical study report (CSR).

10.3.4. Interim Analyses

An interim analysis will occur once approximately 10 participants per arm have completed the treatment period to assess if the study should stop for futility or continue. Subsequently, if the decision at the first interim is to continue, an interim analysis will occur once approximately 20 participants per arm have completed the treatment period to assess whether the study should continue or stop for success or futility. If at this point the

study continues, the maximum 30 participants per arm will be recruited. The study then either meets the success criteria, undergoes analysis of secondary data (review) or is declared a failure. Enrolment into the study will continue whilst interim analyses are taking place.

This interim framework is being used to allow the possibility to reduce the number of participants enrolled into the study, either in the case of an overwhelming observed treatment effect or no/poor treatment effect. The interim stopping criteria for success is based on the probability of the study going on to meet the success criteria (predictive probability that the posterior probability is at least 0.95) whilst the stopping criteria for futility is based on the probability of the study going on to declare success or review (predictive probability that the posterior probability is at least 0.75, see Table 2).

Table 2 Interim Decision Criteria

Interim Analysis	**Success**	**Futility**
10 subjects per arm	N/A – cannot stop for success at the first interim.	Predictive Prob (PP $\geq$ 0.75) $\leq$ 0.075
20 subjects per arm	Predictive Prob (PP $\geq$ 0.95) $\geq$ 0.85	Predictive Prob (PP $\geq$ 0.75) $\leq$ 0.15

At each interim, the probability of the study going on to meet these criteria will be calculated. This probability value may be used to assist in determining whether there is sufficient rationale to continue/discontinue the study. If the probability of study success is sufficiently high, then the study may stop for success. Conversely, if the probability of study success or review is sufficiently low then the study may stop for futility. The probability thresholds for success and futility have been calibrated to balance the risk of declaring success despite a placebo-like drug (type 1 error) and declaring failure despite a positive true underlying treatment effect for GSK2798745 relative to placebo (type 2 error).

The predictive probability at each interim is calculated using predictive inference methods. The observed data at the interim is used to obtain estimates of the mean and standard error of the treatment effect for GSK2798745 relative to placebo. These estimates are then used to predict the treatment effect for participants in the remainder of the study using the predictive interim distribution. These two parts of data (observed and predicted) are combined to create a complete study. The end of study success criteria is then applied to this complete data set made up of observed and predicted participants. This process is performed 10,000 times to account for the uncertainty in the estimated treatment effect at the interim – i.e. the same observed data is combined with thousands of permutations for the remainder of the study, and each time the end of study success criteria is applied. The proportion of complete studies that meet the pre-defined success criteria gives the predicted probability of success if the study was to continue at the interim.

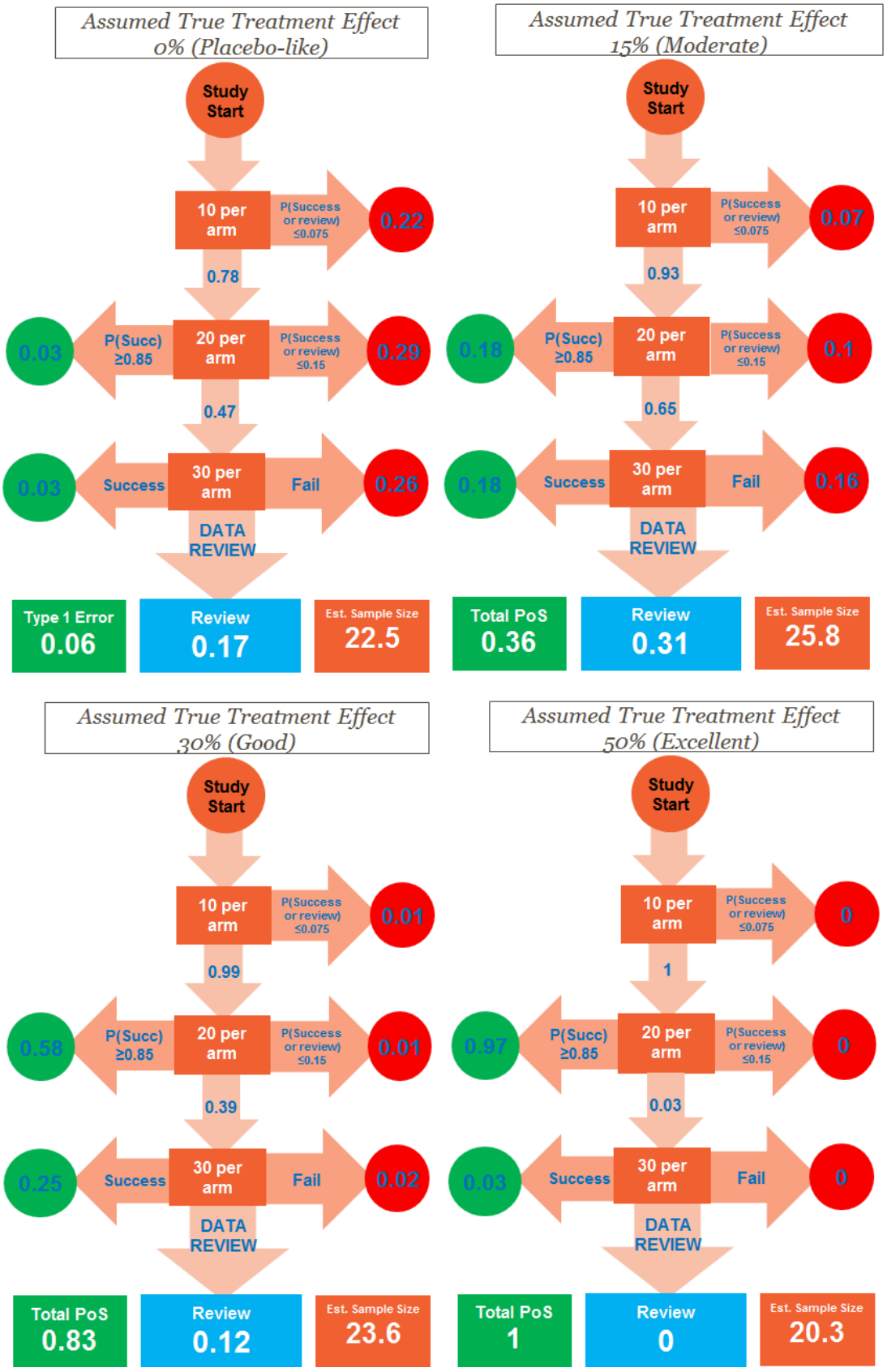
To inform the study design, a range of scenarios were reviewed to assess the operating characteristics when changing the total sample size, interim decision rules, interim analysis time points and assumed true treatment effects. Characteristics such as the overall probability of study success/failure, probability of success/failure at each interim, and the expected number of participants to be recruited were reviewed under a variety of design options.

Figure 6 show the operating characteristics of the chosen study design under true percentage reductions in total BAL protein for GSK2798745 relative to placebo of 0%, 15%, 30% and 50%, respectively, unconditional on variability. The schematic demonstrates all possible decision pathways the study may take. To obtain the operating characteristics of a given study design, 10,000 studies were simulated with each one taking a particular pathway and finishing in one of the pockets on the left or right of the diagram, or indeterminate (additional data review). The overall proportion of simulated studies reaching a particular conclusion gives the probability of a single study reaching that conclusion.

A stricter posterior probability futility threshold is implemented at the first interim to reduce the probability of incorrectly declaring futility. Furthermore, a minimum of 20 participants per arm must be recruited before declaring success. These rules are shown within the left (success) and right (futile) arrows. Under this design, assuming a true treatment effect of 30%, the overall probability of study success is approximately 83%, unconditional on variability. In addition, the probability of requiring a maximum of 20 participants per arm is approximately 61% (under the same assumption of true treatment effect of 30%). In the case of a placebo-like drug the probability of incorrectly declaring success is approximately 6% (akin to type 1 error rate). The blue boxes at the bottom of the charts show the probability that a study goes to data review.

The Reporting and Analysis Plan will describe the planned interim analyses in greater detail.

Figure 6 Interim Analysis Framework for Range of True Treatment Effects



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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase
AUC	Area under concentration-time curve
$AUC_{0–24}$	Area under the curve during 24 hours
$AUC_{0–26}$	Area under the curve during 26 hours
AV	Atrioventricular
BAL	Bronchoalveolar Lavage
BMI	Body mass index
BUN	Blood urea nitrogen
Ca^{2+}	Calcium
C_{max}	Maximum observed plasma concentration
CMT2C	Charcot-Marie-Tooth Disease Type 2C
CNS	Central Nervous System
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disorder
CPK	Creatinine phosphokinase
CRF	Case Report Form
CV	Cardiovascular
CSSRS	Columbia Suicide Severity Rating Scale
ECG	Electrocardiogram
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
EU	Endotoxin Unit
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in One Second
FVC	Forced vital capacity
FOBT	Faecal Occult Blood Test
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilance
GI	Gastrointestinal
GMP	Good Manufacturing Practice
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen
HCl	Hydrochloric acid
HDPE	High-density polyethylene
HIV	Human Immunodeficiency Virus
HMVEC	Human Dermal Microvascular Endothelial Cells

HPLC	High performance liquid chromatography
IB	Investigator's Brochure
IC_{50}	50% maximal inhibitory concentration
ICF	Informed consent form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IMP	Investigational medicinal product
IP	Investigational Product
i.t.	Intratracheal
IRB	Institutional Review Board
IWRS	Interactive Web Response System
Kg	Kilogram
KO	Knockout
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
MCH	Mean corpuscular haemogloin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligrams
mL	Milliliter
mmHg	Millimeter of mercury
MSDS	Material Safety Data Sheet
Msec	Milliseconds
mSV	MilliSievert
Ng	Nanogram
NOAEL	No observed adverse effect level
PK	Pharmacokinetic
POP PK	Population Pharmacokinetic
PTS-DMPK	Platform Technologies and Science-Drug Metabolism and Pharmacokinetics
QC	Quality control
QTcF	QT duration corrected for heart rate by Fridericia's formula
RAP	Reporting and Analysis Plan
RBC	Red blood cells
RNA	Ribonucleic acid
SAE	Serious adverse event(s)
SOA	Schedule of Activities
SRM	Study Reference Manual
SUSAR	Suspected Unexpected Serious Adverse Reactions
TB	Tuberculosis
TLR 4	Toll-like receptor 4
Tmax	Time to reach maximum plasma concentration

TRALI	Transfusion Related Acute Lung Injury
TRPV4	Transient receptor potential vanilloid 4
ULN	Upper Limit of Normal
WBC	White blood cells
WOCBP	Women of Child Bearing Potential

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
None

12.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed in Table 3 will be performed by the local laboratory.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 3 Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Haematology	Platelet Count RBC Count Haemoglobin Haematocrit	RBC Indices: MCV MCH %Reticulocytes	WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[1]	BUN CRP	Potassium	Aspartate Aminotransferase (AST)/ Serum Glutamic-Oxaloacetic Transaminase (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	Alanine Aminotransferase (ALT)/ Serum Glutamic-Pyruvic Transaminase (SGPT)	Total Protein
	Glucose (fasting not required)	Calcium	Alkaline phosphatase	CPK
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Tests	• Cardiac troponin (cTn) • Coagulation profile (Quick/INR, PTT and thrombocytes) • Faecal Occult Blood Test (FOBT)			
Other Screening Tests	• Follicle-stimulating hormone and estradiol (as needed in women of non-childbearing potential only) • Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and			

Laboratory Assessments	Parameters
	hepatitis C virus antibody) • Alcohol, cotinine and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines).

NOTES :

1. Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 8.1.1 and Appendix 6 All events of ALT ≥3 × upper limit of normal (ULN) and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).

12.3. Appendix 3: Study Governance Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
    - 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 ICH Good Clinical Practice (GCP) Guidelines
    - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Substantial amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- 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/EC
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

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.

Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative 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 participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.
- Participants who are rescreened are required to sign a new ICF.

Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant 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 participant.
- The participant 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.

Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Dissemination of Clinical Study Data

- Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.
- GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study participants, as appropriate.
- GSK will provide the investigator with the randomization codes for their site only after completion of the full statistical analysis.
- The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- 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 CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants 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 ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final Clinical Study Report (CSR)/ equivalent summary 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

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.
- Data reported on the CRF 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 the source document agreement (to be signed by the investigator (or delegate) at the site).

Study and Site Closure

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

The investigator may initiate study-site 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 site 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 participants by the investigator
- Discontinuation of further study treatment development

12.4. Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of a study treatment, whether or not considered related to the study treatment. • 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 a study treatment.

Events <u>Meeting</u> the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment 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 study treatment 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. •

Events <u>NOT</u> 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 participant'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 participant's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that

leads to the procedure is the AE.

- 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).

A 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 participant 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 participant 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 AE. 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, diarrhoea, 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 participant 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 AE and SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to GSK.
- 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.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficiently 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 an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- 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 study treatment administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, 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 GSK. 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** GSK**.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK 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 participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool
• The primary mechanism for reporting SAE to GSK will be the electronic data collection tool. • If the electronic system is unavailable for more than 24 hours, then the site will use the paper SAE data collection tool (see next section). • The site will enter the SAE data into the electronic system as soon as it becomes available. • The investigator or medically-qualified sub-investigator must show evidence within the eCRF (e.g., check review box, signature, etc.) of review and verification of the relationship of each SAE to IP/study participation (causality) within 72 hours of SAE entry into the eCRF. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the medical monitor (who is also the SAE coordinator) by telephone. • Contacts for SAE reporting can be found in SRM.

SAE Reporting to GSK via Paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the medical monitor (who is also the SAE coordinator)**.** • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. • 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 SRM.

12.5. Appendix 5: Contraceptive Guidance and Collection of Pregnancy Information

Definitions

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with ONE of the following:

- Documented hysterectomy
- Documented bilateral salpingectomy
- Documented bilateral oophorectomy

Note: Documentation can come from the site personnel's: review of participant's medical records, medical examination, or medical history interview.

3. Postmenopausal female

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
- Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance

Male participants

- Male participants with female partners of child-bearing potential are eligible to participate if they agree to ONE of the following during the protocol-defined time frame in Section 6.1:
- 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 an additional method of contraception with a failure rate of <1% per year as described in Table 4 when having penile-vaginal intercourse with a woman of childbearing potential

- Men 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 protocol-defined time frame.
- In addition, male participants must refrain from donating sperm from the time of first dose of study treatment until 2 weeks after last dose of study treatment.

Table 4 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 • oral • intravaginal • transdermal
Progestogen-only hormonal contraception associated with inhibition of ovulation • injectable
Highly Effective Methods That Are User Independent
• Implantable progestogen-only hormonal contraception associated with inhibition of ovulation • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • bilateral tubal occlusion
Vasectomized partner *(A vasectomized partner is a highly effective contraception 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 drug. 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 participant.)*

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 participants in clinical studies.

Collection of Pregnancy Information

Male participants with partners who become pregnant

- Investigator will attempt to collect pregnancy information on any male participant's female partner of a male study participant who becomes pregnant while participating in this study. This applies only to participants who receive study treatment.
- 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 GSK within 24 hours of learning of the partner's pregnancy.
- 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 GSK
- Generally, 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 foetal status (presence or absence of anomalies) or indication for procedure.

12.6. Appendix 6: Liver Safety: Required Actions and Follow-up Assessments

Phase I liver chemistry increased monitoring criteria and required follow up assessments

<table>
<tr><th colspan="2">Liver Chemistry Increased Monitoring Criteria</th></tr>
<tr><td>ALT-absolute</td><td>ALT≥3xULN
If ALT≥3xULN AND bilirubin[1,2] ≥ 2xULN (>35% direct bilirubin) or INR >1.5, Report as an SAE.
See additional Actions and Follow Up Assessments listed below</td></tr>
<tr><th colspan="2">Required Actions and Follow up Assessments</th></tr>
<tr><th>Actions</th><th>Follow Up Assessments</th></tr>
<tr><td>• Report the event to GSK within 24 hours
• Complete the liver event CRF, and complete an SAE data collection tool if the event also meets the criteria for an SAE[2]
• Perform liver event follow up assessments
• Monitor the participant until liver chemistries resolve, stabilise, or return to within baseline (see MONITORING below)
MONITORING:
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24 hrs
• Monitor participants twice weekly until liver chemistries resolve, stabilise or return to within baseline
• A specialist or haepatology consultation is recommended
If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24-</td><td>• Viral hepatitis serology[3]
• Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend
• Obtain blood sample for pharmacokinetic (PK) analysis, obtained within 48hrs of last dose[4]
• Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
• Fractionate bilirubin, if total bilirubin≥2xULN
• Obtain complete blood count with differential to assess eosinophilia
• Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE report form
• Record use of concomitant medications on the concomitant medications report form including acetaminophen, herbal remedies, other over the counter medications.
• Record alcohol use on the liver event alcohol intake case report form
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5:</td></tr>
</table>

Liver Chemistry Increased Monitoring Criteria	
72 hrs • Monitor participants weekly until liver chemistries resolve, stabilize or return to within baseline	• Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG) or gamma globulins. • Serum acetaminophen adduct high performance liquid chromatography (HPLC) assay (quantifies potential acetaminophen contribution to liver injury in participants with definite or likely acetaminophen use in the preceding week [James, 2009]. • Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease; complete Liver Imaging and/or Liver Biopsy CRF forms.

1. Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study treatment for that participant if ALT ≥ 3xULN and bilirubin ≥ 2xULN. Additionally, if serum bilirubin fractionation testing is unavailable, record presence of detectable urinary bilirubin on dipstick, indicating direct bilirubin elevations and suggesting liver injury.
2. All events of ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin) or ALT ≥ 3xULN and INR>1.5, if INR measured, which may indicate severe liver injury (possible 'Hy's Law'), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis); INR measurement is not required and the threshold value stated will not apply to participants receiving anticoagulants
3. Includes: 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

12.7. Appendix 7: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC).

Protocol Amendment 1 02-FEB-2018

Overall Rationale for the Amendment: The primary reason for amending the protocol is to update the number of time-points for exploratory biomarkers. The number of blood samples for exploratory biomarkers has been reduced.

Section # and Name	Description of Change	Brief Rationale
Section 2.2 Treatment Period of Schedule of Activities	Removal of time-points (Pre-dose, 6h, 12h, 14h) for exploratory biomarker blood sample.	No longer required.
Section 3.3.1 Risk Assessment	Vascular lesions: The following correction was made under Summary of Data/Rationale for Risk: Dogs (~~12~~**13**-week study): At 10 mg/kg/day, 1 male and 1 female had arterial lesions.	Error corrected from previous version.
Section 4 Exploratory Objectives and Endpoints	The following was added: Objective: To **possibly** assess metabolite GSK3526876 (major metabolite of GSK2798745) in plasma **and BAL samples.** Endpoint: Plasma **and BAL** concentrations of metabolite GSK3526876.	Updated to also include BAL. Amended to allow flexibility, depending on the availability of an assay.
Section 5.4 Scientific Rationale for study design	The following correction was made: Drug on board at the time of baseline BAL fluid sampling is expected to have little to no effect on baseline BAL total protein concentrations as TRPV4 channels are quiescent until stimulated and blockade of these channels does **not** affect barrier permeability of 'healthy' endothelial cells [internal data].	Error corrected from previous version.
5.5.2 Treatment Effect Rational	Data from **a** ~~the~~ systemic LPS model ~~described in Section 5.4[Shyamsundar, 2009]~~ [Dalsgaard, 2016].	Error corrected from previous version.

Section # and Name	Description of Change	Brief Rationale
Section 7.2 Challenge Agents	Removal of wording: The LPS challenge agent to be used for the procedure is Good Manufacturing Practice (GMP)-grade product and will be sourced ~~**by GSK**~~ from List Laboratories, Inc.	Wording not required.
Section 7.7.1 Permitted Medications	The following was added: Rescue medication, such as salbutamol, is also permitted after bronchoscopy or spirometry procedure, if required**, and other medication, such as midazolam, may be taken during bronchoscopies to aid the procedure.**	Text added on use of midazolam during bronchoscopy as a rescue medication.
9.4 Pharmacokinetics	The following changes were made: Plasma **and BAL** samples ~~will also~~ **may be** analysed for the metabolite GSK3526876,	Amended to allow flexibility, depending on the availability of an assay.

TITLE PAGE

Protocol Title: A randomised, placebo-controlled, double-blind (sponsor open), segmental LPS challenge study to investigate the pharmacodynamics of GSK2798745 in healthy participants

Protocol Number: 207464 / Amendment 01

Short Title: Effects of GSK2798745 on alveolar barrier disruption in a segmental LPS challenge model

Compound Number: GSK2798745

Sponsor Name and Legal Registered Address:

GlaxoSmithKline Research & Development Limited
980 Great West Road
Brentford
Middlesex, TW8 9GS
UK

Medical Monitor Name and Contact Information can be found in the Study Reference Manual

Regulatory Agency Identifying Number(s): 2017-002388-16

Approval Date: 02-FEB-2018

207464

SPONSOR SIGNATORY:

PPD

2 Feb, 2018

Anya Harry, MD, PhD
Physician Project Lead
Director, Clinical Development, Respiratory

Date

CONFIDENTIAL

207464

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Protocol Amendment 1 (2016N304618_01)	02-FEB-2018
Original Protocol (2016N304618_00)	24-OCT-2017

The original protocol (24-Oct-2017) was published internally only, it was not reviewed by the competent authority or the research ethics committee.

Protocol Amendment 1 02-FEB-2018

Overall Rationale for the Amendment: The primary reason for amending the protocol is to update the number of time-points for exploratory biomarkers. The number of blood samples for exploratory biomarkers has been reduced.

Section # and Name	Description of Change	Brief Rationale
Section 2.2 Treatment Period of Schedule of Activities	Removal of time-points (Pre-dose, 6h, 12h, 14h) for exploratory biomarker blood sample.	No longer required.
Section 3.3.1 Risk Assessment	Vascular lesions: The following correction was made under Summary of Data/Rationale for Risk: Dogs (~~12~~**13**-week study): At 10 mg/kg/day, 1 male and 1 female had arterial lesions.	Error corrected from previous version.
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Section 5.4 Scientific Rationale for study design	The following correction was made: Drug on board at the time of baseline BAL fluid sampling is expected to have little to no effect on baseline BAL total protein concentrations as TRPV4 channels are quiescent until stimulated and blockade of these channels does **not** affect barrier permeability of 'healthy' endothelial cells [internal data].	Error corrected from previous version.
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Section 7.2 Challenge Agents	Removal of wording: The LPS challenge agent to be used for the procedure is Good	Wording not required.

Section # and Name	Description of Change	Brief Rationale
	Manufacturing Practice (GMP)-grade product and will be sourced ~~**by GSK**~~ from List Laboratories, Inc.	
Section 7.7.1 Permitted Medications	The following was added: Rescue medication, such as salbutamol, is also permitted after bronchoscopy or spirometry procedure, if required**, and other medication, such as midazolam, may be taken during bronchoscopies to aid the procedure.**	Text added on use of midazolam during bronchoscopy as a rescue medication.
9.4 Pharmacokinetics	The following changes were made: Plasma **and BAL** samples ~~will also~~ **may be** analysed for the metabolite GSK3526876,	Amended to allow flexibility, depending on the availability of an assay.

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PAGE

1. SYNOPSIS

Protocol Title: A randomised, placebo-controlled, double-blind (sponsor open), segmental LPS challenge study to investigate the pharmacodynamics of GSK2798745 in healthy participants

Short Title: Effects of GSK2798745 on alveolar barrier disruption in a segmental LPS challenge model

Rationale:

The influx of protein-rich fluid into the lung due to damage to the alveolar capillary barrier, with resultant adverse effects on respiratory function, is a fundamental underlying defect in Acute Respiratory Distress Syndrome (ARDS). In this Phase 1, proof-of-mechanism study, a segmental lipopolysaccharide (LPS) challenge will be used as a surrogate injury model to investigate the effects of Transient receptor potential vanilloid 4 (TRPV4) channel blockade on alveolar-septal barrier permeability in man. The LPS model has been widely used for the evaluation of anti-inflammatory agents, and will aid in the characterization of TRPV4 blockade on lung permeability and inflammation. It is hypothesised that TRPV4 inhibitors modulate the LPS response by reducing LPS induced calcium influx (either directly [e.g. via Toll-like receptor 4 (TLR4) mediated activation of endothelial cells] or indirectly [e.g. autocrine cytokine activation]). The assessment of TRPV4 mediated effects on LPS induced lung barrier damage in healthy volunteers is considered a viable strategy before conducting studies in an ARDS patient population.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To investigate the effect of GSK2798745 on alveolar-septal barrier permeability following LPS challenge in healthy volunteers.	• Baseline adjusted total protein concentration in bronchoalveolar lavage (BAL) samples at 24 h after segmental LPS challenge.
Secondary	
• To investigate the effect of GSK2798745 on neutrophil levels in LPS-challenged lungs.	• Baseline adjusted total and differential cell count of neutrophils in BAL samples at 24 h after segmental LPS challenge.

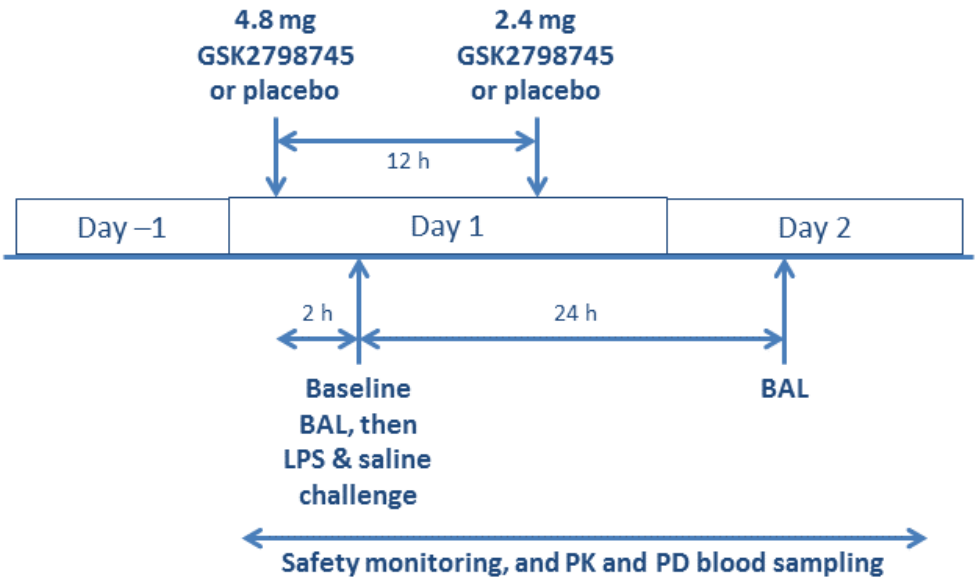
Objectives	Endpoints
• To assess the safety and tolerability of GSK2798745 in LPS-challenged, healthy volunteers.	• Adverse events (AEs), laboratory safety assessments, 12-lead electrocardiogram (ECG), physical examination, vital signs, spirometry (forced expiratory volume in 1 second [FEV1] and forced vital capacity [FVC]), faecal occult blood test (FOBT).
• To assess the pharmacokinetics (PK) of GSK2798745 in plasma in LPS-challenged, healthy volunteers	• PK parameters of GSK2798745 in plasma (AUC (0-24) and Cmax).

Overall Design:

This is a randomised, placebo-controlled, parallel group, double-blind (sponsor-open), segmental LPS challenge study of GSK2798745 in healthy participants.

Participants will be randomised 1:1 to take 2 single doses (3 tablets total): either 4.8 mg GSK2798745, then 2.4 mg GSK2798745 12 h later; or a dose of placebo, then another dose of placebo 12 h later. The first dose will be administered on Day 1 at 2 h before baseline BAL sampling from a segment in the left lower lobe. 4 ng/kg LPS will subsequently be instilled into the right middle segment and saline control into the lingula segment of the contralateral side. The second dose of study treatment will be administered 10 hours after LPS challenge followed by post-dose BAL sampling on Day 2. A schematic of the study is provided in Figure 1.

Figure 1 Study schematic



BAL: bronchoalveolar lavage; LPS: lipopolysaccharide; PD: pharmacodynamic; PK: pharmacokinetic.

Number of Participants:

Sufficient participants will be screened to ensure that a maximum of 60 evaluable participants (defined as all participants for whom results of the primary analysis can be determined) are enrolled onto the study: 30 for GSK2798745 and 30 for placebo. Interim analyses will be conducted, to assess the difference, if any, between treatments in the primary endpoint. Recruitment can continue whilst interim analyses are conducted. Depending on the results of the interim analyses, recruitment may be stopped.

Treatment Groups and Duration:

Each participant will take up to approximately 5 weeks to complete the study. Participants will be screened within 28 days before dosing and remain on site from the day before dosing (Day −1) until the day after dosing (Day 2). Participants will receive a follow up phone call 2 days after discharge, and return to the study site on Day 8 (± 1 day) for a follow-up visit.

2. SCHEDULE OF ACTIVITIES (SOA)

- The timing and number of planned study assessments, including safety, pharmacokinetic, pharmacodynamics and exploratory biomarker assessments may be altered during the course of the study based on newly available data (e.g., to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring.
- Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files, but will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the informed consent form (ICF).

2.1. Screening

	Screening (Days –28 to –2)
Outpatient visit	X
Informed consent	X
Inclusion and exclusion criteria	X
Demography	X
Full physical examination	X
Height and weight	X
Medical history	X
Past and current medical conditions	X
HIV, Hepatitis B and C screening	X
FSH and oestradiol[1]	X
Drug, alcohol and cotinine screen	X
C-SSRS	X
Laboratory assessments[2]	X
12-lead ECG[3]	X
Vital signs[4]	X
Spirometry[5]	X
FOBT[6]	X

C-SSRS: Columbia Suicide Severity Rating Scale; ECG: electrocardiogram; FOBT: Faecal Occult Blood Test; HIV: human immunodeficiency virus.

1. Postmenopausal females whose postmenopausal status is in doubt only.
2. Haematology, clinical chemistry (including liver chemistry), cardiac troponins, urinalysis and coagulation profile.
3. In triplicate.
4. Blood pressure and heart rate in triplicate. Single temperature measurement.
5. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). Triplicate measurement, the highest of which should be recorded in the case report form.
6. May be measured during the screening window or on Day –1. FOBT cards will be provided at screening and must be returned to the laboratory and analysed before dosing.

2.2. Treatment period

		Day 1[1]									Day 2[1]			
	Day –1[2]	Pre-dose	0 h	1 h	2 h	3 h	6 h	8 h	12 h	14 h	25.5 h	26 h	30 h	Day 4
Inpatient stay[2]		←==========	==========	==========	==========	==========	==========	==========	==========	==========	==========	==========	==========→	
Telephone call[3]														X
Drug, alcohol and cotinine screen	X													
Laboratory assessments[4]	X												X	
12-lead ECG[5]	X	X											X	
Blood pressure and heart rate[5]	X	X		X			X		X	X			X	
Temperature[6]	X	X		X	X		X	X	X	X			X	
Spirometry[7]	X	X					X				X		X	
C-SSRS													X	
Randomisation		X												
Study treatment			X						X					
Pulse oximetry[8]					X							X		
Bronchoscopy, baseline BAL and challenge[9]					X									
Bronchoscopy and post-challenge BAL[10]												X		
Blood sample for exploratory biomarkers[11]					X[11]	X		X				X[11]		
Blood sample for PK		See footnote 12												
AE review		←==========	==========	==========	==========	==========	==========	==========	==========	==========	==========	==========	==========→	
SAE review	←==========	==========	==========	==========	==========	==========	==========	==========	==========	==========	==========	==========	==========→	
Concomitant medication review	←==========	==========	==========	==========	==========	==========	==========	==========	==========	==========	==========	==========	==========→	

AE: adverse event; BAL: bronchoalveolar lavage; ECG: electrocardiogram; PK: pharmacokinetic; SAE: serious adverse event, C-SSRS: Columbia Suicide Severity Rating Scale.

1. Time points relative to the first dose on Day 1.
2. Admission on Day –1, at a time to allow all Day –1 procedures to be done; discharge on Day 2, at least 4 h after the bronchoscopy.

3. To check for any AEs.
4. Haematology, clinical chemistry (including liver chemistry), cardiac troponins, urinalysis and coagulation profile.
5. Triplicate on Day-1 and pre-dose. Single measurements at time points after dosing.
6. Immediately before BAL sampling/LPS challenge at the 2-h time point.
7. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). Triplicate measurements at each time point, the highest of which should be recorded in the case report form.
8. Pulse oximetry to be measured during bronchoscopy procedures. Only AEs to be recorded.
9. BAL samples for total protein, neutrophil counts, GSK2798745 and exploratory biomarkers. Baseline BAL sample from a segment in the left lower lobe, followed by segmental challenge of the lungs: LPS in right middle segment; saline control in the lingula segment of the contralateral side. Challenges to be done as close as possible to 2 h post-dose.
10. BAL samples for total protein, neutrophil counts, GSK2798745 and exploratory biomarkers. Baseline and post-dose measurements to be done by the same person, where possible.
11. Blood samples for exploratory biomarkers. The 2- and 26-h blood samples should be taken immediately before BAL sampling.
12. PK samples will be taken pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 6, 8, 12, 12.5, 13, 13.5, 14, 15, 24 and 26 h after the first dose. The 2- and 26-h blood sample should be taken immediately before BAL sampling

2.3. Follow-up/Early Withdrawal

	Follow-up/Early Withdrawal (Day 8 [± 1 day])
Outpatient visit	X
Full physical examination	X
Weight	X
Laboratory assessments[1]	X
12-lead ECG[2]	X
Vital signs[3]	X
Spirometry[4]	X
FOBT[5]	X
AE review	X
SAE review	X
Concomitant medication review	X

AE: adverse event; ECG: electrocardiogram; FOBT: Faecal Occult Blood Test; SAE: serious adverse event.

1. Haematology, clinical chemistry (including liver chemistry), cardiac troponins and urinalysis.
2. Single measurement.
3. Blood pressure, heart rate, temperature. Single measurement.
4. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). Triplicate measurement, the highest of which should be recorded in the case report form.
5. FOBT cards will be provided upon discharge from the unit and should be returned by the follow-up/early withdrawal visit.

3. INTRODUCTION

GSK2798745 is a potent and selective transient receptor potential vanilloid 4 (TRPV4) channel blocker being investigated for the treatment of acute respiratory distress syndrome (ARDS). TRPV4 blockade is expected to ameliorate the injury inflicted to the alveolar capillary barrier in ARDS patients and reduce the leakage of protein and fluid into the alveolar space. Studies investigating administration of GSK2798745 have been conducted in healthy volunteers and in patients with chronic heart failure.

GSK2798745 has been administered orally to healthy participants as single doses ranging from 0.25 to 12.5 mg. A dosage of 5 mg once daily has been administered for up to 14 days in healthy participants. Further, GSK2798745 at a dose of 2.4 mg has been evaluated as a single dose and subsequently as repeated doses for 7 days in participants with heart failure.

No clinically significant safety concerns were observed with single or repeat administration of GSK2798745 in either healthy volunteers or participants with heart failure.

3.1. Study Rationale

The influx of protein rich fluid into the lung due to damage to the alveolar capillary barrier, with resultant adverse effects on respiratory function is a fundamental underlying defect in ARDS. In this Phase 1 proof of mechanism study, a segmental lipopolysaccharide (LPS) challenge will be used as a surrogate injury model to investigate the effects of TRPV4 channel blockade on alveolar-septal barrier permeability in man. The LPS model has been widely used for the evaluation of anti-inflammatory agents [Zielen, 2015], and will aid in the characterization of TRPV4 blockade on lung permeability and inflammation.

Segmental LPS challenge, in which a small quantity of the endotoxin, LPS, is administered directly to one segment of the lung, is a model of focal and self-limiting lung inflammation. LPS induces pulmonary endothelial cell permeability [Fu, 2009] via calcium dependent cytoskeleton rearrangement [Bannerman, 1998; Gandhirajan, 2013]. LPS may also directly act on endothelial cells to cause permeability by degrading intercellular junctions [Bannerman, 1998]. The upregulation of a pro-inflammatory signalling cascade via NF-kB leading to endothelial activation and pro-inflammatory mediator release [Bosmann, 2012] may be regulated by the influx of calcium [Kandasamy, 2013], and may also induce further calcium influx and endothelial permeability [Lush, 2000; Tiruppathi, 2006].

Under normal conditions, concentrations of total protein in alveolar fluid are low. LPS induced endothelial damage leads to accumulation of protein rich fluid into the lung due to the migration of large molecules, such as albumin, from blood to the alveolar space. Preclinical and clinical studies have shown a positive correlation between amount of protein the bronchoalveolar lavage and extent of lung damage [Holter, 1986; Moazed, 2016; Yu, 2015]. In the lung, TRPV4, a Ca^{2+}-permeable non-selective cation channel, is widely expressed in cells involved in ARDS, namely microvascular endothelium,

alveolar epithelium, alveolar macrophages, and circulating neutrophils and monocytes, and is a known regulator of endothelial permeability [Narita, 2015; Suresh, 2015; Huh, 2012] and pulmonary oedema [Balakrishna, 2014; Hamanaka, 2007]. Therefore, TRPV4 may play an important role in LPS-induced alveolar permeability. The relevance of the TRPV4 channel to LPS induced injury has been confirmed in a mouse sepsis model. In this severe injury model, administration of a TRPV4 inhibitor 1 h prior to LPS injection (i.p.) increased survival by 70%. Although this study was not specifically designed to evaluate bronchoalveolar lavage (BAL) protein levels, total protein concentrations in BAL fluid of mice pre-treated with a TRPV4 inhibitor were reduced by approximately 20% after the LPS challenge in TRPV4 treated mice compared with controls [Dalsgaard, 2016].

It is hypothesised that TRPV4 inhibitors modulate the LPS response by reducing LPS induced calcium influx (either directly [e.g. via Toll-like receptor 4 (TLR4) mediated activation of endothelial cells] or indirectly [e.g. autocrine cytokine activation]).

Given the link to ARDS of endothelial damage and fluid leak, assessment of TRPV4 mediated effects on LPS induced lung barrier damage in healthy volunteers is important to establish whether beneficial effects may be observed in an ARDS patient population.

3.2. Background

ARDS is an acute inflammatory lung injury, associated with increased pulmonary vascular permeability, increased lung weight, and loss of aerated lung tissue [Bellani, 2016]. Treatment options remain limited to supportive care including mechanical ventilation and ARDS represents a significant unmet need accounting for over 10% of intensive care unit (ICU) admissions and a mortality rate of up to 40% [Bellani, 2016; Matthay, 2012]. The syndrome is characterised by acute hypoxemic respiratory failure and non-cardiogenic pulmonary oedema and may be precipitated by direct (e.g. pneumonia and aspiration) and indirect insults (e.g. sepsis and transfusion related acute lung injury ([TRALI]) to the lung. Dysregulated inflammation, inappropriate accumulation and activity of leukocytes and platelets, uncontrolled activation of coagulation pathways, and altered permeability of alveolar endothelial and epithelial barriers are central to the pathophysiology of ARDS [Matthay, 2012]. Damage to the alveolar-capillary membrane leads to increased vascular permeability, and the development of interstitial and alveolar protein rich oedema, leading to reduced gas exchange, ventilation perfusion mismatching and arterial hypoxaemia.

TRPV4 has been implicated as a key regulator of lung endothelial barrier integrity, and specifically, the integrity of the lung alveolar-capillary endothelium, which is most relevant to alveolar flooding associated with acute lung injury. TRPV4 activation by hydrostatic stretch in lung microvessels leads to increased endothelial $Ca2^{+}$ concentration and a diverse set of vascular responses, including an increase in endothelial permeability [Morty, 2014]. The importance of TRPV4 in maintaining pulmonary barrier function has been demonstrated in the settings of elevated pulmonary venous [Thorneloe, 2012] or airway pressure [Hamanaka, 2007], and following treatment with chemical and biological toxins such as Hydrochloric acid (HCl) and platelet activating factor [Balakrishna, 2014; Morty, 2014; Yin, 2016] . In these studies, TRPV4 blockade limited lung damage by

reducing plasma fluid leak into the alveolar space (thus increasing arterial oxygenation), and by modulating neutrophil and macrophage recruitment and activity and reducing overall mortality in response to LPS [Balakrishna, 2014; Morty, 2014; Yin, 2016; Dalsgaard, 2016]. This evidence suggests TRPV4 channel blockade may benefit patients with ARDS where alveolar capillary leak is a primary driver of injury, by reducing fluid leak, reducing ventilation perfusion mismatching, improving oxygenation and reducing the need for mechanical ventilation with potential reductions in mortality.

3.3. Benefit/Risk Assessment

3.3.1. Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of GSK2798745 may be found in the Investigator's Brochure.

All potential risks of GSK2798745 are based on pre-clinical data. No risks have been identified in the clinical studies of GSK2798745 conducted before the effective date of this protocol.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) [GSK2798745]		
Vascular lesions	Dogs (4-week study): at 30 mg/kg/day, 2 males had arterial lesions • One male: Heart – Coronary artery inflammation; Thymus – Arteriole inflammation with fibroplasia • One male: Epididymides – Artery degeneration/necrosis with inflammation Dogs (13-week study): At 10 mg/kg/day, 1 male and 1 female had arterial lesions • One male: Epididymides – Arteriole degeneration/necrosis with lymphocytic inflammation • One female: Bladder – Arteriole degeneration/necrosis with lymphocytic inflammation	Participant Monitoring: The arterial lesions noted in heart, thymus, epididymides, and urinary bladder cannot be monitored directly. There is currently no human translation biomarker or understanding of the underlying mechanism. Participant Exposure: Since these effects cannot be monitored directly in clinical studies, coverage of ≥30 fold will be maintained from the no-effect dose (3 mg/kg/day); exposure will not intentionally exceed the average daily area under concentration-time curve (AUC) of 0.513 hr*µg/mL and/or maximum observed plasma concentration (Cmax) of 0.050 µg/mL on an individual basis.
Myocardial toxicity	Dogs (4-week study): at 30 mg/kg/day, myofibre degeneration/necrosis and inflammation (2 animals)	Participant Selection: Participants with any history or current disease constituting a risk when taking the study treatment, including relevant history of acute coronary syndromes (including unstable angina), coronary angioplasty, or stenting, will be excluded. Participant Monitoring: Cardiac troponin levels will be monitored and ECGs will be done during the study. Participant Exposure: Exposure levels will be maintained below the threshold detailed in the Dose Justification Section (see Section 5.5).

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Mortality/moribund condition; poor viability	Dogs (4-week study): at 30 mg/kg/day, one male terminated early (Day 6) due to poor clinical condition. Another male had transient whole body shaking on Days 8 and 9. Dogs (13-week study): at 10 mg/kg/day one male was terminated early (Day 74) due to welfare reasons. Rats (micronucleus and comet study): mortality occurred following 1 to 3 doses at ≥600mg/kg/day	Participant Monitoring: Weight and adverse events reported by participants will be monitored.
Gastrointestinal (GI) and/or hepatic toxicity	GI toxicity: ≥3 mg/kg/day in dogs and at 30 and 300 mg/kg/day in rats, consisting of mucosal erosion/ulceration in the stomach and/or duodenum. Hepatic Toxicity: Biliary epithelial hypertrophy/hyperplasia and periductal mixed inflammatory cell infiltrate into the liver was observed at 300 mg/kg/day in rat (7-day study) and focal hepatocellular degeneration in 1 male dog at 30 mg/kg/day (4-week study)	Participant Selection: Participants with active ulcer disease or GI bleeding or those who are taking concomitant medications, including nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids, will be excluded. Assessment of faecal occult blood will be performed before and after dosing. Participant Monitoring: Participants will be monitored for GI intolerance (e.g by adverse events such as abdominal discomfort) and sequential clinical chemistry analysis, including liver enzymes. Follow-up faecal occult blood test (FOBT) will be conducted.
Testicular toxicity	Inconsistent finding in rats (4-week study): Spermatid retention at ≥60 mg/kg/day, however no effect observed in 13-week study. The observations in the 4-week study were not associated with degenerative changes in testes or epididymides. No spermatogenic abnormalities were observed in dogs.	Participant Exposure: A safety margin of ≥40 fold will be maintained from the no effect dose (60 mg/kg/day) in rats.
Skeletal muscle toxicity	Rat (4-week study): Myofiber necrosis: myofiber degeneration/regeneration; fibroplasia, at 300 mg/kg/day in the soleus muscle.	Participant Monitoring: Creatinine phosphokinase (CPK) levels will be taken before and after dosing.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Seizures and convulsions	Rats (micronucleus and comet study): convulsions observed at ≥600 mg/kg/day. Convulsions were not related to Cmax, nor occurred at any predictable time from dose administration. Dogs: No central nervous system (CNS) findings at 12 mg/kg/day in the dog 7-day EEG/CV study. In other compounds in the same series, convulsions have been observed.	Participant Selection: Participants with any history or current disease constituting a risk when taking the study treatment, including a history of seizure disorder or stroke within the last 5 years will be excluded from the study.
Low food consumption	Dogs (4-week study): 30 mg/kg/day reduced food consumption. Two males were terminated early (Day 10) due to extremely reduced food consumption. Rats (4-week study): 300 mg/kg/day had decreased food consumption.	Participant Monitoring: Weight will be monitored.
Effects on macrophages (Phospholipid accumulation)	Inconsistent effects observed in Rats (4-week study): ≥60 mg/kg/day in the lung (prominent alveolar macrophages); 300 mg/kg/day in the mesenteric lymph node (increased cellularity of sinus macrophages) and thymus (macrophage vacuolation; increased thymus weight). Consistent with phospholipid accumulation (phospholipidosis) based on ultrastructural appearance of mesenteric lymph nodes at 300 mg/kg/day. Findings were not associated with degenerative changes. In 13-week studies in rats, these effects were not observed.	Participant Exposure: A safety margin of ≥40 fold will be maintained from the no effect dose (60 mg/kg/day) in rats.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Theoretical Risk: Potential effects on vasoregulation .	TRPV4 mediates prostaglandin release from isolated human endothelial cells and in vivo in rats, supporting the potential for TRPV4 blockade to modulate blood pressure via prostaglandin release. No effect of GSK2798745 on blood pressure was observed in preclinical or clinical studies.	Participant Monitoring: Blood pressure will be monitored throughout the study.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Theoretical Risk: Potential effect on hearing.	Genetic deletion of TRPV4 in mice has been shown to effect hearing. TRPV4 knockout (KO) mice at age 8 weeks exhibited normal hearing thresholds, but at age 24 weeks, had delayed-onset hearing loss; additionally, the cochlea was found to be vulnerable to acoustic injury with sound overexposure [Tabuchi, 2005]. Patients with Charcot-Marie-Tooth Disease Type 2C (CMT2C), an autosomal dominant axonal neuropathy related to TRPV4 gene mutations, demonstrate symptoms that include hearing loss caused by nerve damage in the inner ear (sensorineural hearing loss). These are predominantly gain of function TRPV4 abnormalities, in which the hearing loss is sporadic among family members; and relegated to some TRPV4 defects, but not in others. Although the exact mechanism is unclear, it has been suggested that the TRPV4 channel plays an important role in peripheral nerve function and that the alterations in TRPV4 in CMT2C may be due to increased channel activity leading to excessive calcium influx and a calcium overload. There is potential for benefit with GSK2798745, in that with cells (HEK293) expressing the CMT2C mutant channel, inhibitors of the TRPV4 channel were found to block the increased intracellular calcium concentrations and resultant cell death [Landouré, 2010]. In a study evaluating effects on heart failure with similar dosing exposure, a week-long duration and comprising an older participant demographic, audiometry testing was conducted. Although a variety of changes were observed, primarily sporadic, low frequency and single frequency changes in those on treatment as well as placebo, none were assessed as a signal for concern by the reviewing expert audiologist.	Participant Exposure: Dosing will be limited to a short duration of a single day.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Theoretical risk: Potential suicidal ideation	GSK2798745 is considered to be a CNS-active drug. There has been some concern that some CNS-active drugs may be associated with an increased risk of suicidal thinking or behaviour when given to some patients with certain conditions. GSK considers it important to monitor for such events before or during clinical studies with CNS-active compounds. Participants being treated with GSK2798745 should be assessed at screening for suicidality.	Participant Selection: The risk of such an event would be very low in single-day dose trials in healthy volunteers; however, participants who, in the investigator/designee's judgement, poses a significant suicide risk, will be excluded from the study. A C-SSRS questionnaire will be completed before and after dosing.
Study procedures-related		
Risk of adverse events following delivery of LPS	Previously reported segmental LPS related adverse events include pleuritic pain, pyrexia, head ache, nausea and alveolitis [Hohlfeld, 2008; Holz, 2015]. It is known that LPS and pro-inflammatory mediators can cause gut permeability [Al-Sadi, 2014; Guo, 2013]. We expect the systemic exposure to LPS and systemic inflammatory response to LPS to be minimal following the segmental topical dose of 4ng/kg LPS. As such, we assess the risk to increased gut permeability, and potential increased bioavailability, with this regimen to be low.	Participant Monitoring: Participants will be monitored with safety assessments, including clinical laboratory tests, physical examination, ECGs, vital signs (including temperature), pulse oximetry and spirometry during the study. Experienced site staff will conduct the procedures. Participants will be carefully monitored and managed with standard procedures in the event of complications.
Risks associated with bronchoscopy and BAL sampling:	Procedure related complications include cough, transient fever, chills and myalgias, transient infiltrates, bronchospasm, transient fall of lung function, transient decrease in baseline PaO2 (partial pressure of oxygen in arterial blood).	

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Risk associated with blood draws	Fainting, mild pain, bruising, irritation or redness.	Experienced site staff will follow standard approaches for managing events related to blood draws.
Risks associated with spirometry	Shortness of breath, coughing, light-headedness or fainting, and/or chest tightness may be induced by spirometry testing.	Participants experiencing any of these symptoms will receive standard medical treatment by the study investigator.

3.3.2. Benefit Assessment

There will be no intended therapeutic clinical benefit to the participants taking part in the study as it is a healthy volunteer study. However, participants will undergo a medical evaluation during screening including physical exams, electrocardiograms (ECGs) and laboratory assessments which may provide important health information.

By taking part in this study, the participant will be contributing to the development of GSK2798745 for the treatment of ARDS, a syndrome with significant unmet need.

3.3.3. Overall Benefit:Risk Conclusion

No benefit to healthy participants is expected. The study activities have risks, however these risks are mitigated by: exclusion of participants with identified comorbidities; short (single day) duration of dosing with GSK2798745; exposure limited to previously approved exposure levels; and inclusion of safety margins, as well as safety monitoring by trained staff. The study will provide important information towards future development of TRPV4 blockers that may help patients with ARDS.

The design of the study is considered low risk to the participants and justified based on the safety information from the nonclinical studies and the previous clinical trials carried out on GSK2798745.

4. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
• To investigate the effect of GSK2798745 on alveolar-septal barrier permeability following LPS challenge in healthy volunteers.	• Baseline adjusted total protein concentration in bronchoalveolar lavage (BAL) samples at 24 h after segmental LPS challenge.
Secondary	
• To investigate the effect of GSK2798745 on neutrophil levels in LPS-challenged lungs.	• Baseline adjusted total and differential cell count of neutrophils in BAL samples at 24 h after segmental LPS challenge.
• To assess the safety and tolerability of GSK2798745 in LPS-challenged, healthy volunteers.	• Adverse events (AEs), laboratory safety assessments, 12-lead electrocardiogram (ECG), physical examination, vital signs, spirometry (forced expiratory volume in 1 second [FEV1] and forced vital capacity [FVC]), faecal occult blood test (FOBT).

Objectives	Endpoints
• To assess the pharmacokinetics (PK) of GSK2798745 in plasma in LPS-challenged, healthy volunteers	• PK parameters of GSK2798745 in plasma (AUC (0-24) and Cmax).
Exploratory	
• To assess concentration of GSK2798745 in BAL.	• Levels of GSK2798745 in BAL samples at 2 and 26 h after dosing.
• To investigate the effect of GSK2798745 on exploratory markers of endothelial barrier permeability and/or injury, and inflammation, in LPS-challenged lungs.	• Baseline adjusted levels of exploratory biomarkers in blood and/or BAL.
• To possibly assess metabolite GSK3526876 (major metabolite of GSK2798745) in plasma and BAL samples.	• Plasma and BAL concentrations of metabolite GSK3526876.
• To compare the PK of GSK2798745 with the effect of prophylactic dosing on alveolar-septal barrier permeability following LPS challenge in healthy volunteers.	• Comparison of PK parameters of GSK2798745 in plasma with baseline corrected total protein concentration in BAL.
• To investigate the effect of GSK2798745 on white blood cell levels (except neutrophils) in LPS-challenged lungs.	• Baseline adjusted total and differential cell count of white blood cells (except neutrophils) in BAL samples at 24 h after segmental LPS challenge.

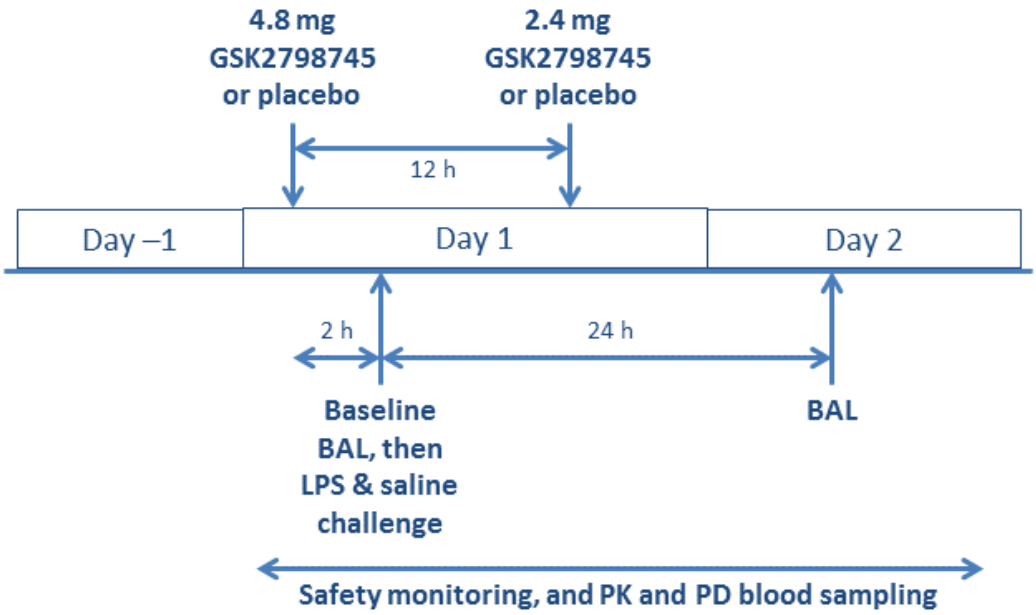
5. STUDY DESIGN

5.1. Overall Design

This is a randomised, placebo-controlled, parallel group, double-blind (sponsor-open), segmental LPS challenge study of GSK2798745 in healthy participants.

Participants will be randomised 1:1 to take 2 single doses (3 tablets total): either 4.8 mg GSK2798745, then 2.4 mg GSK2798745 12 h later; or a dose of placebo, then another dose of placebo 12 h later. Participants will undergo segmental LPS challenge to the lungs at 2 h after the first dose. BAL samples will be taken after dosing: immediately before and at 24 h after the LPS and saline challenge. Blood samples will be taken before and after dosing and the challenge. Safety will be monitored throughout. A schematic of the study is provided in Figure 2.

Figure 2 Study schematic



BAL: bronchoalveolar lavage; LPS: lipopolysaccharide; PD: pharmacodynamic; PK: pharmacokinetic.

Each participant will take up to approximately 5 weeks to complete the study. Participants will be screened within 28 days before dosing and remain on site from the day before dosing (Day −1) until the day after dosing (Day 2). The first dose will be administered on Day 1 at 2 h before baseline BAL sampling from a segment in the left lower lobe. 4 ng/kg LPS will subsequently be instilled into the right middle segment and saline control into the lingula segment of the contralateral side. The second dose of study treatment will be administered 10 hours after LPS challenge followed by post-dose BAL sampling on Day 2. Participants will receive a follow up phone call 2 days after discharge, and return to the study site on Day 8 (± 1 day) for a follow-up visit.

5.2. Number of Participants

Sufficient healthy participants will be screened to ensure that a maximum of 60 evaluable participants (defined as all participants for whom results of the primary analysis can be determined) are enrolled onto the study: 30 for GSK2798745 and 30 for placebo. Interim analyses will be conducted (as described in Section 10.3.4), to assess the difference, if any, between treatments in the primary endpoint. There are two interim analyses planned, depending on the results of the interim analyses, recruitment may be stopped. Participants who prematurely discontinue the study, and whose data are not evaluable (i.e. those for whom results of the primary analysis cannot be determined), may be replaced. Recruitment into the study can continue whilst interim analyses are taking place.

5.3. Participant and Study Completion

A participant is considered to have completed the study if he/she has completed all phases of the study including the follow-up visit.

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

5.4. Scientific Rationale for Study Design

- *Use of a placebo:* The randomised, placebo-controlled study design is a well-established methodology to evaluate potential treatment effects in experimental studies. The use of a placebo arm allows for a valid evaluation of changes in PD markers attributable to treatment versus those independent of treatment. Given that participants are healthy and treatment is not being withheld, the use of placebo is considered acceptable.
- *Parallel group:* The use of a parallel group design is considered to limit the burden of procedures and exposure of LPS to participants. A crossover design only minimally decreases the required number of evaluable participants and owing to the expected dropout rate in a crossover study, and the burden of procedures for each participant, a parallel group design was selected.
- *Blinding:* A double blind design is a standard methodology for randomised, controlled studies to avoid bias.
- *Selection of primary endpoint:* Changes in total protein concentrations to monitor influx of proteinaceous fluid into the lung has been used in several LPS challenge studies. Evidence points to the involvement of TRPV4 in changes to alveolar capillary barrier following LPS administration, with subsequent leak of proteinaceous fluid as measured by total protein. Given the influx of fluid with high concentrations of protein observed in ARDS patients, monitoring total protein changes is considered an acceptable surrogate to assess for target engagement of GSK2798745 and for potential effects in ARDS patients.
- *Timing of primary endpoint:* As previous studies show that total protein concentrations peak at 24 h after segmental LPS challenge [O'Grady, 2001; Holz, 2015], post-challenge BAL samples will be taken at 24 h after the challenge.
- *Timing of challenge:* Baseline BAL sampling prior to administration of study drug is not considered feasible due to the burden of bronchoscopy (i.e. two procedures required – one pre and one post dose to instil LPS). A baseline bronchoscopy is planned 2 h after administration of the first dose, with the aim of maximising exposure to GSK2798745 (in healthy volunteers, the median [range] Time to reach maximum plasma concentration (Tmax) of the tablet formulation of GSK2798745 to be used in this study is 1.5 h [1–3 h]). Drug on board at the time of baseline BAL fluid sampling is expected to have little to no effect on baseline BAL total protein concentrations as TRPV4 channels are quiescent until stimulated and blockade of these channels does not affect barrier permeability of 'healthy' endothelial cells [internal data]. The effects of LPS to induce endothelial permeability are believed to occur rapidly. Investigation of LPS induced permeability and markers of cell activation and inflammation over time consistently indicates that the initial events leading to increased barrier permeability and inflammation occur within first 2 h post

intratracheal (i.t.) LPS exposure; where any earlier measurements are limited. LPS induces intracellular stress fibre formation within 5 min in both human dermal microvascular endothelial cells (HMVEC) endothelial and A549 epithelial cells [Ngamsri, 2010]. Increased levels of TNFα following i.t. LPS instillation are observed within 2 h in man [O'Grady, 2001] and other pro-inflammatory mediators within 4 h in a mouse model of acute lung injury (ALI) [Bosmann, 2012]. Permeability across an endothelial monolayer is observed after 0.5 h and peaks at 2 h post-LPS [Bannerman, 1998]. BAL total protein increases following LPS i.t. challenge in man as early as 2 h and reaches significance at 6 h post-LPS [Matthay , 2012] with significant increases in neutrophil counts also observed as early as 2 h. Looking to a systemic LPS model of sepsis with a higher LPS insult, earlier sampling is possible and high levels of inflammatory mediators are measured within 1 h, which reach plateau by 3 h. Therefore, despite our conservative treatment effect prediction (see Section 5.5), we expect that high plasma and/or alveolar fluid concentrations of GSK2798745 will be required at the time of and/or soon after the challenge, in order to inhibit the effects of LPS. Therefore, LPS will be administered shortly after time of Tmax (1.5-hours) to ensure sufficient drug on board.

5.5. Dose Justification

5.5.1. Dose Rationale

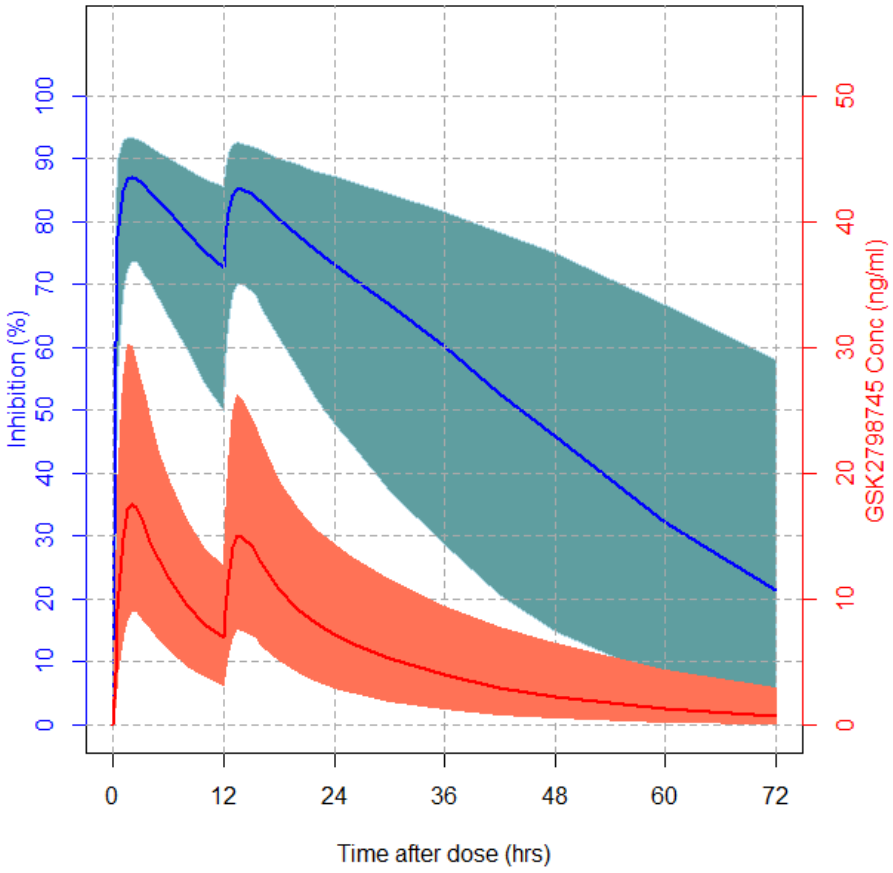
The first time in human study (TR4113787) for GSK2798745 evaluated doses from 0.25 mg to 12.5 mg as single doses, and 5 mg once daily repeat dosing for 14 days in healthy volunteers in cohorts 1–3. Cohorts 4 and 5 of the study were administered once daily 2.4 mg dose of capsules with food for 7 days to stable heart failure participants. Exposure from different tablet formulations administered with or without food was characterised in another clinical study in healthy volunteers (204725). Exposure from all available clinical data to date was analysed with a population pharmacokinetic (POP PK) approach accounting for participant weight, formulation, impact of food amongst other variables. Trial simulations were performed with this POP PK model with differing dosing regimen.

The estimated human IC50 of 2.1–3.2 ng/mL was derived from data from a rat study, which was conducted to assess the ability of different doses of GSK2798745 infusion to reduce the increased lung-to-bodyweight ratio induced by the TRPV4 agonist. The IC50 was also corrected for species differences using protein binding data and TRPV4 potency differences in *invitro* assays. To evaluate drug activity/efficacy at the intended dosing regimen, the percentage pulmonary oedema blockade was estimated using the population model and the corrected potency values derived from the rat pulmonary study.

Based on the simulations, the intended dosing scheme for this study is a 4.8 mg dose followed by a 2.4 mg dose at 12 h. Table 1 lists the predicted average percentage pulmonary oedema blockade over the 24-h period based on this potency range. The schematic in Figure 3 also depicts the range of GSK2798745 systemic exposure and the predicted percent inhibition by TRPV4 with the intended regimen. At 26 h post the first dose, when the primary endpoint sample will be taken, the predicted TRPV4 inhibition is 71.1% (44.4–86.3) [median (95% prediction interval)].

The intended dosing regimen also limits the daily ceiling human exposure at individual participant level to 30-fold of the exposure observed at the no observed adverse effect level (NOAEL) dose of 3 mg/kg from the 3-month dog safety study (D70496G). The current clinical doses are selected so that no participant intentionally exceeds the daily AUC of 513 ng*hr/mL and C_{max} of 50 ng.hr/mL. The likelihood of one or more participants of the 30 participants to be dosed with this regimen exceeding the threshold is listed in Table 1.

Figure 3 Predicted GSK2798745 exposure and corresponding effect on pulmonary oedema after doses of 4.8 mg then 2.4 mg, 12 h apart



Note: percentage inhibition based on the rat, agonist-driven, pulmonary oedema model.

Table 1 Predicted exposure, probability of exceeding threshold and predicted efficacy after doses of 4.8 mg then 2.4 mg, 12 h apart, administered to 30 participants

Percentage pulmonary oedema blockade over 24 h (median [95% PI])	Predicted exposure (median [95% PI])		% Probability that ≥1 of 30 participants will exceed the toxicokinetic limit[1]	
	AUC0–24 h (ng.h/mL)	Cmax (50 ng/mL)	AUC0–24 h (513 ng.h/mL)	Cmax (50 ng/mL)
79.1 [66.5–87.1]	272.4 [176.2–400.9]	23.1 [15.2–34.4]	5.6	1.6

PI: predicted interval.

1. The percentage of the 500 simulated studies (of 30 subjects each) in which ≥ 1 subject exceeds the toxicokinetic limit.

5.5.2. Treatment Effect Rationale

Data from a systemic LPS model [Dalsgaard, 2016] , and from other models of lung injury (chlorine gas exposed mice [Suresh, 2015], pulmonary venous pressure induced injury of isolated mouse lungs [Narita, 2015], and a mouse model of intratracheal instillation of hydrochloric acid [Hamanaka, 2007]) suggest that TRPV4 blockade contributes 20–80% to a reduction in total protein in BAL fluid after the injury. Averaging the effect from those studies, the maximum effect possible in this segmental LPS model is estimated to be approximately 50%, assuming total TRPV4 channel blockade. Therefore, with estimated exposure expected to reduce total protein by approximately 71% in a pure agonist driven challenge, a median treatment effect of 35% might be expected in this LPS challenge. Based on pragmatic reasons and given some of the uncertainty around TRPV4 contribution to proteinaceous fluid influx as measured by total protein in a segmental LPS challenge, an estimated 30% treatment effect was nominally selected for sample size calculations.

6. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

6.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

AGE
1. Between 18 and 50 years of age inclusive, at the time of signing the informed consent.

TYPE OF PARTICIPANT AND DISEASE CHARACTERISTICS
2. Volunteers who are overtly healthy as determined by medical evaluation including medical history, physical examination, laboratory tests (including a normal coagulation profile), ECGs, vital signs and spirometry.

Note: In the event of out-of-range results of safety tests, the tests may be repeated once within the screening window. If a retest result is again outside the reference range and considered clinically significant by the investigator and GSK medical monitor, the participant will be considered a screen failure.

3. Normal spirometry (FEV1 ≥80% of predicted, FEV1/FVC ratio ≥70%) at screening and before dosing.

WEIGHT
4. Body weight ≥50 kg and body mass index (BMI) within the range 19–29.9 kg/m^2 (inclusive).

SEX
5. Male or female. **a. Male participants:** A male participant must agree to use contraception, as described in Appendix 5, during the treatment period and for at least 7 days after the last dose of study treatment and refrain from donating sperm during this period. **b. Female participants:** A female is eligible to participate if she is not of childbearing potential, as defined in Appendix 5.

INFORMED CONSENT
6. Capable of giving signed informed consent which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

6.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTC INTERVAL)
1. Significant history of or current cardiovascular, respiratory (eg asthma, chronic obstructive pulmonary disorder (COPD), bronchiectasis, active Tuberculosis [TB]), hepatic, renal, gastrointestinal, endocrine, hematological, autoimmune or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; constituting a risk when taking the study treatment; or interfering with the interpretation of data.
2. Participant who, in the investigator/designee's judgement, poses a significant suicide risk. Evidence of serious suicide risk may include any history of suicidal behaviour and/or any evidence of suicidal ideation on any questionnaires e.g., Type 4 or 5 on the Columbia Suicide Severity Rating Scale (C-SSRS) in the last 5 years.

3. Active ulcer disease or gastrointestinal bleeding at the time of screening (positive faecal occult blood test [FOBT] at screening).
4. Abnormal blood pressure as determined by the investigator.
5. ALT or bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
6. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
7. QTc >450 msec

   NOTES: the QTc is the QT interval corrected for heart rate according Fridericia's formula (QTcF).
8. At risk of Torsades de pointes (e.g. a personal history or a family history of sudden unexplained death, long QT, familial cardiac syndrome, or cardiomyopathy).
9. Chronic or acute infection within the 4 weeks before dosing, (e.g. upper and lower respiratory infection within the 4 weeks before dosing).
10. Major (as per investigator judgment) surgery within the last 12 weeks prior to randomisation or planned within 3 months of screening.

PRIOR/CONCOMITANT THERAPY

11. Use of prescription or non-prescription drugs (except paracetamol), including vitamins, herbal and dietary supplements (including St John's Wort) within 7 days or 5 half-lives (whichever is longer) before the first dose of study medication, unless, in the opinion of the investigator and GSK Medical Monitor, the medication will not interfere with the study procedures or compromise participant safety.
12. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator and/or GSK Medical Monitor, contraindicates their participation.

PRIOR/CONCURRENT CLINICAL STUDY EXPERIENCE

13. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within 3 months.
14. The participant has participated in a clinical trial and has received an investigational product within the following time period before the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
15. Exposure to more than four new chemical entities within 12 months before the first dosing day.

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA

16. Presence of hepatitis B surface antigen (HBsAg) at screening

17. Positive hepatitis C antibody test result at screening.

    NOTE: Participants with positive Hepatitis C antibody due to prior resolved disease can be enrolled, only if a confirmatory negative Hepatitis C Ribonucleic acid (RNA) test is obtained.

18. Positive Hepatitis C RNA test result at screening or within 3 months prior to first dose of study treatment.

    NOTE: Test is optional and participants with negative Hepatitis C antibody test are not required to also undergo Hepatitis C RNA testing.

19. A positive pre-study drug/alcohol/cotinine screen.
20. A positive test for immunodeficiency virus (HIV) antibody.
21. Regular use of known drugs of abuse.

OTHER EXCLUSIONS
22. History of regular alcohol consumption within 6 months of the study defined as an average weekly intake of >21 units for males or >14 units for females. One unit is equivalent to 8 g of alcohol: a half-pint (~240 mL) of beer, 1 glass (125 mL) of wine or 1 (25 mL) measure of spirits.
23. Current smoker or a history of smoking within 6 months of screening, or a total pack year history of >5 pack years. [number of pack years = (number of cigarettes per day/20) x number of years smoked].

6.3. Lifestyle Restrictions

6.3.1. Meals and Dietary Restrictions

- Participants are not permitted to consume red wine, Seville oranges, grapefruit or grapefruit juice, and/or pomelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days before the start of study treatment until discharge from the clinical unit.
- Participants will be required to fast from midnight before the bronchoscopies on Day 1 and 2 until after the procedure and for at least 2 hours before dosing.

6.3.2. Caffeine, Alcohol, and Tobacco

- During the Treatment Period, participants will abstain from ingesting caffeine- or xanthine-containing products (eg, coffee, tea, cola drinks, and chocolate) for 24 hours before the start of dosing until after collection of the final pharmacokinetic (PK) and/or pharmacodynamic sample.
- During the Treatment Period, participants will abstain from alcohol for 24 hours before the start of dosing until after collection of the final PK and/or pharmacodynamic sample.
- Only non-smokers may be recruited into this study.

6.3.3. Activity

- Participants will abstain from strenuous exercise for 72 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities during studies (eg, watching television, reading).

6.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomised. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs).

Participants may be rescreened once. If rescreening is preformed, participants must be assigned a different unique participant identification number for the rescreening, and all screening procedures must be repeated. See the study reference manual (SRM) for more details.

7. TREATMENTS

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

7.1. Treatments Administered

Study Treatment Name:	**GSK2798745**	**Matching Placebo**	**Challenge Agent: GMP grade lipopolysaccharide from *Escherichia coli* (*E. Coli* Group 0113:H10:K Negative) for the segmental LPS challenge**
Dosage formulation:	White to slightly coloured, round biconvex tablet. Product: AP, Tab-A	White to slightly coloured, round biconvex tablet. Product code: CET, Tab-A..	LPS is available from stock lyophilized in a 1 microgram vial, formulated in 1% lactose and 0.1% PEG6000. Clear solution.
Unit dose strength(s)/Dosage level(s):	Unit dose strength 2.4 mg. Dosage Levels: 4.8 mg and 2.4 mg	Not applicable	4 ng/kg
Route of Administration	Oral	Oral	Direct application to the lung segment, via bronchoscopy
Dosing instructions:	Two tablets to be taken on the morning of Day 1, then one tablet to be taken 12 hours later.	Two tablets to be taken on the morning of Day 1, then one tablet to be taken 12 hours later.	One single instillation of 10 mL by bronchoscopy 2 hours after dosing with GSK2798745 or placebo on Day 1.
Packaging and Labeling	GSK2798745 tablets will be provided in high-density polyethylene (HDPE) bottles with child-resistant closures that include induction-seal liners. Each bottle will be labelled as required per country requirement.	Placebo tablets will be provided in high-density polyethylene (HDPE) bottles with child-resistant closures that include induction-seal liners. Each bottle will be labelled as required per country requirement.	-
Manufacturer	GSK	GSK	List Laboratories, Inc.

Sterile saline (0.9%) for control challenge will be sourced by the clinical site.

7.1.1. Dose Modification

No dose modifications are permitted without submission of a substantial amendment to the protocol.

7.2. Challenge Agents

Bacterial endotoxin is a component of the cell wall of Gram-negative bacteria present in a variety of occupational and general environmental settings.

The dose of LPS used in this study is 4 ng/kg, which is 100 times lower than doses used in inhalation models of endotoxin-induced lung inflammation. This dose was chosen based on the data published by O'Grady et al [O'Grady, 2001]. In this study, lavage after 24-h in subjects challenged with 4 ng/kg of endotoxin revealed a localised inflammatory response that was neutrophil-predominant. The site performing this study has experience with this model. The LPS challenge agent to be used for the procedure is Good Manufacturing Practice (GMP)-grade product and will be sourced from List Laboratories, Inc. A certificate of analysis will be provided for each batch of the LPS challenge agent to ensure its quality and safety.

Reconstitution will be done under the responsibility and supervision of the investigator or qualified site staff who performs the bronchoscopy and administers the reconstituted LPS in individual dilution to the subject. The reconstitution will be done on the day of bronchoscopy immediately before the procedure (within 1 hour). If more than one subject per day will be investigated, the first process of reconstitution and administration of LPS has to be completed before a second process is started.

The reconstitution process is described as an example for the nominal dose of 10,000 EU of endotoxin per vial. Each vial of LPS contains a lyophilized solid containing 10,000 EU of endotoxin. Upon reconstitution with 5 mL of sterile saline 0.9%, the vial will contain 2,000 EU/mL = 2 EU/µL. Forty (40) µL/kg body weight will be withdrawn from the vial and transferred into a sterile endotoxin-free 30 mL-sample vial (Acila AG, Weiterstadt, Germany). Sterile saline 0.9% will be added to give a final volume of 20 mL. Ten (10) mL of this solution contains the application dose of 40 EU (4 ng)/kg, and will be filled into a 10 mL-syringe. This individual dilution will be administered to the participant by segmental pulmonary application during the bronchoscopy. The remaining 10 mL will be stored frozen (-20°C) as a retention sample.

The negative control will be performed with 10 mL of sterile saline 0.9% only. The reconstitution process will be adapted to the actual endotoxin content as determined in the Certificate of Analysis. The Investigator Site File will contain the current instructions for reconstitution in order to ensure the application dose of 40 EU/kg.

7.3. Method of Treatment Assignment

All participants will be centrally randomized using an Interactive Web Response System (IWRS). Before the study is initiated, the log in information and instructions for the

IWRS will be provided to each site. Participants will be registered using the IWRS, and assigned a unique number (randomisation number). The randomisation number encodes the participant's treatment (GSK2798745 or placebo), according to the randomization schedule generated prior to the study by the Statistics Department at GSK. Each participant will be dispensed blinded study treatment, labelled with his/her unique randomisation number.

7.4. Blinding

This will be double blind study. All study staff involved in clinical assessments (which includes the investigator, sub-investigators, other site staff) and the participant will be blinded to the treatment allocated to individual participants and to post challenge PD results. Selected sponsor study team members (and delegates if programming activities are outsourced) will be unblinded to perform the interim analysis. This may include the medical monitor, study statistician, study programmer (and delegates) and study pharmacokineticist. Access to unblinded data will be kept to the minimum set of individuals required to implement any interim analyses, but may include GSK management/review committees if alterations to the study conduct are required. Details of who were unblinded to what data and when will be included in the clinical study report. The IWRS will be programmed with blind-breaking instructions. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a participant's treatment assignment is warranted. Participant safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact GSK prior to unblinding a participant's treatment assignment unless this could delay emergency treatment of the participant. If a participant's treatment assignment is unblinded GSK must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and CRF, as applicable.

A participant whose treatment assignment is inadvertently unblinded (either to investigative staff or the participant themselves) will be permitted to remain in the study, although the accidental unblinding will be recorded as a protocol deviation and hence the participant will be subject to review as to their inclusion in analyses.

GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any participant with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the participant's treatment assignment, may be sent to investigators in accordance with local regulations and/or GSK policy.

7.5. Preparation/Handling/Storage/Accountability

1. The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.
2. Only participants enrolled in the study may receive study treatment and only authorised site staff may supply or administer study treatment. All study treatments 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 authorised site staff.

3. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).
4. Further guidance and information for the final disposition of unused study treatment are provided in the SRM.
5. Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff.
6. A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

7.6. Treatment Compliance

- When the individual dose for a participant is prepared from a bulk supply, the preparation of the dose will be confirmed by a second member of the study site staff.
- When participants are dosed at the site, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study treatment and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment. Study site personnel will examine each participant's mouth to ensure that the study treatment was ingested.
- GSK2798745 and the placebo will be orally administered to participants at the site.

7.7. Concomitant Therapy

7.7.1. Permitted Medications

Paracetamol, at doses of ≤2 grams/day, is permitted for use any time during the study. Rescue medication, such as salbutamol, is also permitted after bronchoscopy or spirometry procedure, if required, and other medication, such as midazolam, may be taken during bronchoscopies to aid the procedure. Other concomitant medication may be considered on a case-by-case basis by the investigator in consultation with the Medical Monitor if required.

7.7.2. Prohibited Medications

Except for the permitted medications noted above (Section 7.7.1), participants must abstain from taking prescription or non-prescription drugs (including vitamins and dietary or herbal supplements) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) before the start of study treatment until completion of the follow-up visit, unless, in the opinion of the investigator and sponsor, the medication will not interfere with the study.

7.8. Treatment after the End of the Study

Participants will not receive any additional treatment from GSK after completion of the study because only healthy volunteers are eligible for study participation.

8. DISCONTINUATION CRITERIA

8.1. Individual Stopping Criteria

8.1.1. Liver Chemistry Stopping Criteria

Increased monitoring criteria have been designed to assure participant safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance). These protocol guidelines are in alignment with FDA premarketing clinical liver safety guidance: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.

Increased monitoring will be performed for a participant if liver chemistry stopping criteria are met.

Liver Safety Required Actions and Follow up Assessments Section can be found in Appendix 6

8.1.2. QTc Stopping Criteria

The QTc should be based on single or averaged QTc values of triplicate electrocardiograms obtained over a brief (e.g., 5-10 minute) recording period.

A participant who meets either bulleted criterion below will be withdrawn from study:

- QTcF >500 msec
- Change from baseline of QTc >60 msec

8.1.3. Symptoms of Cardiac Ischaemia and Cardiac Troponin Stopping/Increased monitoring Criteria

8.1.3.1. Asymptomatic Participant

Cardiac troponin (cTn) will be measured before and at the end of the study. If any cTn assessment is >ULN or >2 times the participant's baseline value (Day −1), the participant should be contacted immediately to assess for symptoms of cardiac ischemia.

8.1.3.2. Symptomatic Participant

If a participant experiences symptoms of cardiac ischaemia (e.g. chest pain, increased shortness of breath, and diaphoresis), cardiology consultation should be obtained immediately. The participant should be evaluated by a cardiologist and undergo any clinically appropriate testing. The participant should be followed up until symptoms are

resolved. If the event occurs after the first dose, but before the second, the participant should not receive the second dose.

8.1.4. Bronchoscopy Stopping Criteria

A participant will be withdrawn from the study if they experience symptomatic bradycardia or tachycardia requiring treatment as a consequence of bronchoscopy, BAL and LPS instillation. In addition, participants who have the following may also be withdrawn at the discretion of the investigator:

- Oxygen saturation <90% (on oxygen)
- Prolonged somnolence following administration of midazolam
- Significant hypertension/hypotension

8.2. Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance or administrative reasons.
- If the participant 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 participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.
- Refer to the SoA (Section 2.3) for data to be collected at the time of withdrawal.

8.3. Study Stopping Criteria

If there is a serious unexpected adverse event considered at least possibly related to the investigational product administration in one participant; or a severe non-serious AEs considered as, at least, possibly related to the investigational product administration in two participants, the study will be temporarily halted until full review of the events. In participants, significant changes from baseline measurements will be reviewed and participants will be followed until resolved.

8.4. Lost to Follow Up

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

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

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the Schedule of Activities (SoA).
- 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 participant should continue or discontinue study treatment.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA.
- The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 500 mL.
- Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

9.1. Bronchoscopy

Bronchoscopy will be performed in accordance with site standard operating procedures (SOPs), at 2 hours and 26 hours. These SOPs will reflect current standards of practice in hospital care and will include (but are not limited to) the following items:

- Oxygen supplementation will be given to all participants.
- Pulmonary function will be monitored before the bronchoscopy and after the bronchoscopy until FEV1 is within 20% of the pre-procedure value.
- All participants will be monitored in a recovery/holding room post bronchoscopy. Participants will be discharged only after approval is obtained from the

supervising physician, and participants will be given a 24-hour contact number. Admission facilities will be available in the event a participant is not deemed fit for discharge or if overnight observation is deemed necessary by the study physician or site-specific policies.

Following bronchoscopies, participants will be monitored for at least 4 hours. However, should the investigator have any concern for participant safety, participants may be requested to remain resident for further observation.

9.2. Segmental lung challenge

Participants will undergo segmental challenge to the lungs, via bronchoscopy, at 2 hours after the first dose of investigational medicinal product (IMP): 10 mL LPS (4 ng/kg) will be instilled into the right middle segment and 10 mL saline control will be instilled into the lingula segment of the contralateral side.

Local SOPs will be followed and further details about the segmental LPS/saline challenge are provided in the SRM.

9.3. Pharmacodynamic Assessments

BAL samples will be taken, via bronchoscopy, to measure total protein and neutrophils counts. Baseline samples will be taken immediately before the LPS and saline challenges, from a segment in the left lower lobe, and post-challenge samples will be taken at 24 h after the LPS and saline challenges, from the challenged segments.

Details of BAL sample collection, processing, storage and shipping procedures are provided in the SRM.

9.4. Pharmacokinetics

Blood and BAL samples for assay of GSK2798745 will be collected at the time points indicated in the SoA (Section 2.2). Details of sample collection, processing, storage and shipping procedures are provided in the SRM.

PK analysis will be performed under the control of Platform Technologies and Science-IVIVT (PTS-IVIVT)/GlaxoSmithKline. Plasma and BAL concentrations of GSK2798745 will be determined using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Plasma and BAL samples may be analysed for the metabolite GSK3526876, GSK will store the remaining plasma and/or BAL for future possible metabolite investigations. Analysis of compound-related metabolites may be reported under a separate protocol.

9.5. Adverse Events

The definitions of an AE or SAE can be found in Appendix 4.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the participant to discontinue the study (see Section 8).

9.5.1. Time Period and Frequency for Collecting AE and SAE Information

- All SAEs will be collected from the start of treatment until the follow-up visit. However, any SAEs assessed as related to study participation (e.g. study treatment, protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product, will be recorded from the time a participant consents to participate in the study.
- All AEs will be collected from the start of treatment until the follow-up visit.
- Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee within 24 hours, as indicated in Appendix 4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment 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 4.

9.5.2. Method of Detecting AEs and SAEs

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

9.5.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs, will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 8.4). Further information on follow-up procedures is given in Appendix 4.

9.5.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment 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 treatment 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 a SAE or other specific safety information (eg: summary or listing of SAE) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

9.5.5. Pregnancy

- Details of all pregnancies in female partners of male participants will be collected after the start of study treatment and until the follow-up visit.
- If a pregnancy is reported, the investigator should inform GSK within 24 hours of learning of the pregnancy and should follow the procedures outlined in Section 12.5.

9.6. Treatment of Overdose

GSK does not recommend specific treatment for an overdose. The investigator (or physician in charge of the participant at the time) will use clinical judgment to treat any overdose.

In the event of an overdose, the investigator or treating physician should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the participant for AE/SAE and laboratory abnormalities until GSK2798745 can no longer be detected systemically (at least 5 days).
3. Obtain a plasma sample for PK analysis within 1 day from the date of the last dose of study treatment if requested by the Medical Monitor (determined on a case-by-case basis).
4. Document the quantity of the excess dose as well as the duration of the overdosing in the CRF.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the Medical Monitor based on the clinical evaluation of the participant.

9.7. Safety Assessments

Planned time points for all safety assessments are provided in the Schedule of Activities (SoA).

9.7.1. Physical Examinations

- A complete physical examination will include, at a minimum, measuring weight and assessments of the Skin, Cardiovascular, Respiratory, Gastrointestinal and Neurological systems. Height will be measured at screening only.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

9.7.2. Vital Signs

- Vital signs will be measured in a semi-supine position after 5 minutes' rest and will include temperature, systolic and diastolic blood pressure and heart rate
- At each time point before dosing, 3 readings of blood pressure and heart rate will be taken. The first reading should be rejected. The second and third readings should be averaged to give the measurement to be recorded in the CRF. At all other time points, single measurements will be taken.

9.7.3. Pulse Oximetry

- Pulse oximetry will be monitored during each bronchoscopy. Clinically significant results will be recorded as AEs.

9.7.4. Electrocardiograms

- Triplicate 12-lead ECGs will be obtained at each time point before dosing. At all other time-points a single 12-lead ECG will be obtained as outlined in the SoA (see Section 2) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 8.1.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- ECG to be measured in a semi-supine position after 5 minutes' rest.

9.7.5. Spirometry

Spirometry assessments will be performed from screening through the final visit as indicated in the SoA (Section 2). The following parameters will be assessed:

- Forced expiratory volume in one second (FEV_1)
- Forced vital capacity (FVC)

Measurements will be made in triplicate and the best recording (i.e. the highest FEV_1 and the highest FVC) from 3 technically acceptable manoeuvres will be recorded in the CRF. To fulfill the entry criteria, FEV_1 should be ≥80% of predicted and FEV1/FVC ratio ≥70%) at screening and pre-dose.

Details on performing the spirometry assessments are provided in the SRM.

9.7.6. Clinical Safety Laboratory Assessments

- Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- 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 CRF. The laboratory reports must be filed with the source documents.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study (until the follow-up visit) should be repeated until the values return to normal or baseline or are no longer considered significantly abnormal 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 aetiology should be identified and the sponsor notified.
- If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE or AE), then the results must be recorded in the CRF.

9.7.7. Faecal Occult Blood Test (FOBT)

Based on the preclinical finding of gastric erosions, FOBT will be performed before and after dosing (See Section 2). Details on FOBT are provided in the SRM.

9.7.8. Columbia Suicide Severity Rating Scale (C-SSRS)

Based on preclinical studies that have been conducted, GSK2798745 is considered to be a central nervous system (CNS)-active drug. There has been some concern that some CNS-active drugs may be associated with an increased risk of suicidal thinking or behaviour when given to some patients with certain conditions. Although GSK2798745 has not been shown to be associated with an increased risk of suicidal thinking or behaviour, GSK considers it important to monitor for such events.

The C-SSRS is a measure of suicidal ideation and behaviour, with demonstrated predictive validity and reliability. Sections of the C-SSRS include suicidal ideation, intensity of ideation, suicidal behaviour, and actual suicide attempt(s). The C-SSRS assesses lifetime and current suicidal thoughts and behaviours across these categories based on an increasing severity of a 1- to 5-rating scale. The semi-structured questionnaire is completed by a trained and experienced neurologist, psychiatrist, or

neuropsychologist, or another trained and experienced person approved by the Sponsor, who may be the Principal Investigator or a sub-investigator for the study. See SRM for details of the scale.

The C-SSRS will be performed at screening and after dosing before discharge (See Section 2).

9.8. Exploratory biomarkers

Blood and BAL samples for exploratory biomarker analysis of endothelial barrier permeability and/or injury and inflammation will be collected at the time points indicated in the SoA (Section 2).

Samples may also be used for research to develop methods or support identification of prognostic/diagnostic biomarkers associated with clinical outcomes in ARDS and related diseases.

Details of sample collection, processing, storage and shipping procedures are provided in the SRM.

Exploratory biomarker results may be entered into the clinical study database before or after database lock and unblinding, or may be reported separately.

9.9. Genetics

Genetics are not evaluated in this study.

10. STATISTICAL CONSIDERATIONS

The primary study objective is to investigate the effect of GSK2798745 relative to placebo on BAL total protein at 24 hours after segmental LPS challenge. In this study a Bayesian framework will be used (with non-informative priors) to estimate the posterior probability of any percentage reduction in mean 24 h post-LPS BAL protein level (GSK2798745 relative to placebo). It is anticipated that BAL total protein will be $\log_e$ transformed to improve normality before model fitting. Following back-transformation treatment comparisons will be expressed as percentage changes.

10.1. Sample Size Determination

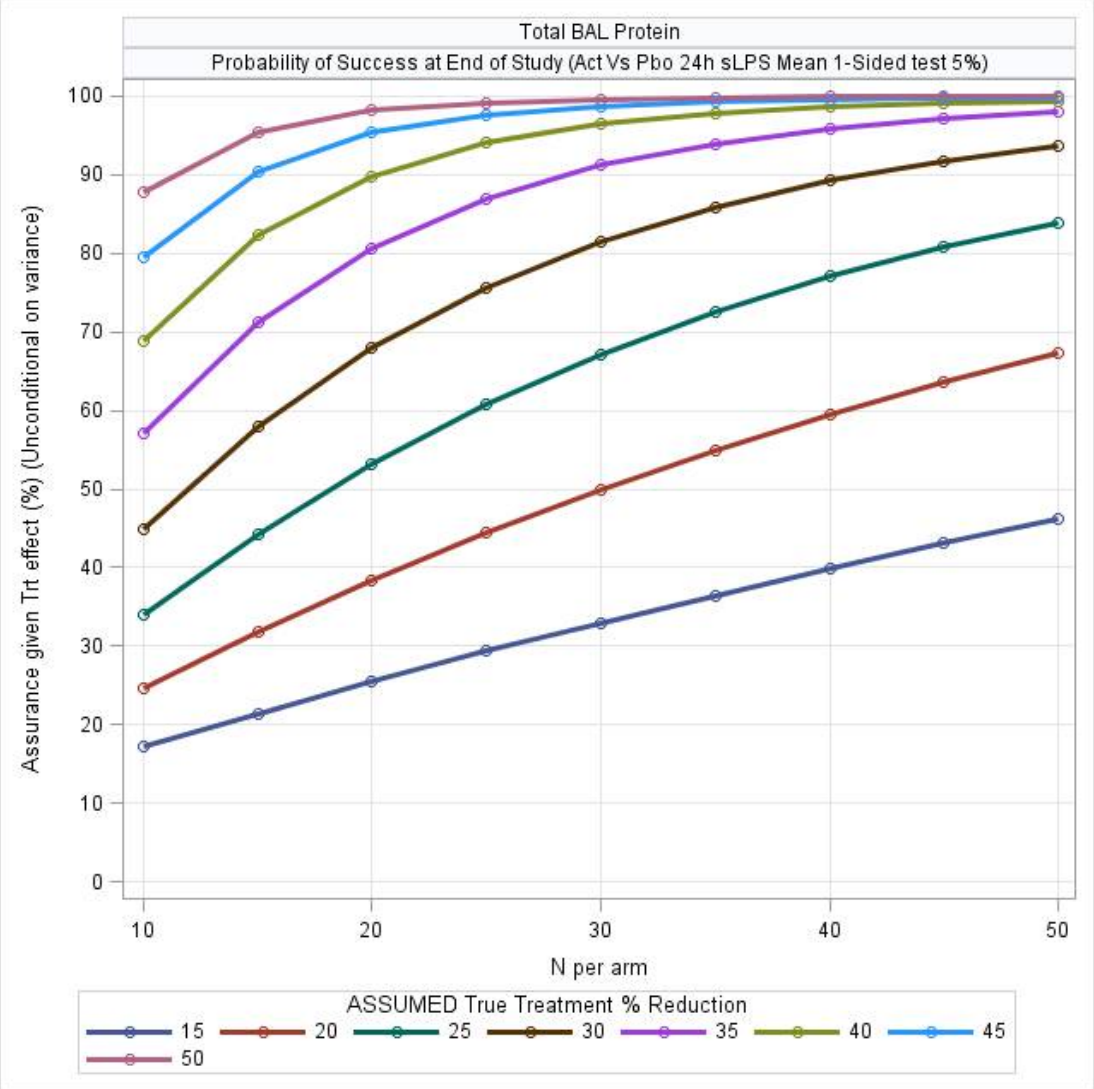
The sample size of 30 participants per arm (1:1 allocation) is based on an upper limit of feasibility. Figure 4 illustrates the probability of study success for a range of sample sizes and assumed true treatment effects unconditional on variability.

Assuming a true treatment effect of a 30% reduction in mean BAL total protein in participants receiving GSK2798745 relative to placebo at 24 h after segmental LPS challenge to the lung, the probability of study success for the study is 82% (this excludes the possibility of success from a review of secondary data), unconditional on the variability of BAL total protein in segmental LPS challenged lungs. Outright end of study success is defined as at least a 95% posterior probability that the percentage reduction in

the mean 24 h post-LPS BAL total protein level (GSK2798745 relative to placebo) exceeds zero.

Despite limited historical information for guidance on the true treatment effect for GSK2798745, 30% is believed to be a rational estimate. However, probability of study success is sensitive to the true treatment effect – for example, the probability of study success reduces to 50% if the true treatment effect is 20% (GSK2798745 relative to placebo) with a sample size of 30 per arm but increases to 96% if the true treatment effect is 40%.

Figure 4 Probability of Study Success (Unconditional on Variance) for a Range of Treatment Profiles and Sample Sizes



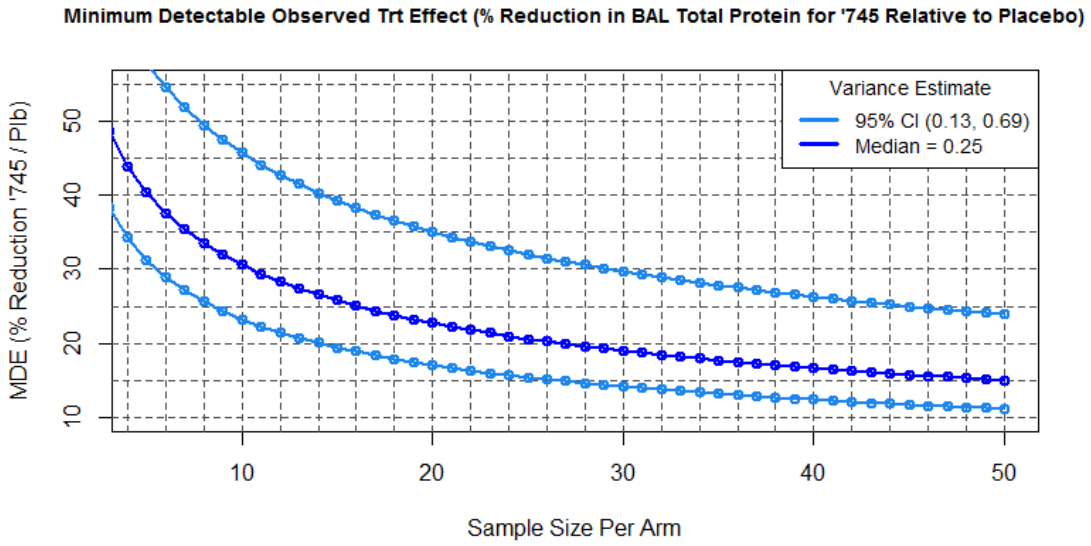
Estimates for the mean and variance of BAL total protein in segmental LPS challenged lungs were obtained from data in an observational study assessing the variability of the inflammatory response to segmental LPS challenge by the Fraunhofer Institute of

Toxicology and Experimental Medicine, Germany [Holz, 2015] using a random effects model with total protein levels from 11 participants measured at baseline and 24 h over 2 study periods. The data were assumed to be representative of future segmental-LPS challenged lungs to be observed in this study.

The estimate of the mean BAL protein in 24 h post-LPS challenged lungs on the $\log_e$ scale is 5.47 (95% CI 5.23, 5.72). The variance estimate on the $\log_e$ scale is 0.25 (95% CI 0.13, 0.69). A posterior distribution was formed for the standard deviation and for each possible value of standard deviation from this distribution the power of the study was calculated and multiplied by its probability. The resulting values were all summed to give probability of study success unconditional on variance, which is a more accurate representation of the probability of study success rather than assuming a single variance estimate with no uncertainty.

Figure 5 shows the minimum observed treatment effect in the study that would achieve success (expressed as a percentage reduction in BAL total protein for GSK2798745 relative to placebo) for a number of sample sizes. Assuming the variance in the study is the median obtained from the historical data, the minimum treatment effect to trigger success is 19% for a sample size of 30 participants per arm. As the sample size increases, the minimum observed treatment effect that would trigger success decreases due to the decreasing standard error (greater confidence in the observed treatment effect). This figure gives rationale for including the data review region since it is probable that moderate observed treatment effects will not achieve success with a sample size of 30 per arm.

Figure 5 Minimum Detectable Effect for Range of Sample Sizes



10.2. Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All participants who sign the ICF.
Evaluable	All participants for whom results of the primary analysis can be determined.
Safety	All randomized participants who take at least 1 dose of study treatment. Participants will be analysed according to the treatment they actually received.

Other populations, such as Per-Protocol population, may be defined in the RAP.

10.3. Statistical Analyses

10.3.1. Efficacy Analyses

Endpoint	Statistical Analysis Methods
Primary	The mean percentage reduction in BAL total protein (µg/mL) in segmental LPS challenged lungs, at 24 h post-challenge, for GSK2798745 relative to placebo, will be assessed using an analysis of covariance (ANCOVA) model (fitted in a Bayesian framework with non-informative priors for model parameters) testing that there is at least 95% posterior probability that the percentage reduction in 24 hour post-challenge mean BAL total protein (GSK2798745 relative to placebo) exceeds zero , adjusting for baseline BAL total protein. A sensitivity analysis will include 24 h BAL total protein in the saline segment (saline challenge will be administered to the contralateral lobe) as a covariate. Additional factors may be included as covariates if deemed appropriate.
Secondary	See Section 10.3.2 and Section 10.3.3
Exploratory	Will be described in the reporting and analysis plan.

10.3.2. Safety Analyses

All safety analyses will be performed on the Safety Population.

Endpoint	Statistical Analysis Methods
Secondary	For the safety data, no formal hypotheses will be tested. Safety data such as adverse events, electrocardiogram (ECG), physical examinations, vital signs, spirometry, FEV1 and FVC will be displayed in the form of listings, frequencies, summary statistics and graphs. Interpretation will be aided by clinical expertise. Full details, including example outputs, will be documented in the RAP.

10.3.3. Other Analyses

PK, pharmacodynamic, and biomarker exploratory analyses will be described in the reporting and analysis plan. The population PK analysis and pharmacodynamic analyses will be presented separately from the main clinical study report (CSR).

10.3.4. Interim Analyses

An interim analysis will occur once approximately 10 participants per arm have completed the treatment period to assess if the study should stop for futility or continue. Subsequently, if the decision at the first interim is to continue, an interim analysis will occur once approximately 20 participants per arm have completed the treatment period to assess whether the study should continue or stop for success or futility. If at this point the study continues, the maximum 30 participants per arm will be recruited. The study then either meets the success criteria, undergoes analysis of secondary data (review) or is declared a failure. Enrolment into the study will continue whilst interim analyses are taking place.

This interim framework is being used to allow the possibility to reduce the number of participants enrolled into the study, either in the case of an overwhelming observed treatment effect or no/poor treatment effect. The interim stopping criteria for success is based on the probability of the study going on to meet the success criteria (predictive probability that the posterior probability is at least 0.95) whilst the stopping criteria for futility is based on the probability of the study going on to declare success or review (predictive probability that the posterior probability is at least 0.75, see Table 2).

Table 2 Interim Decision Criteria

Interim Analysis	Success	Futility
10 subjects per arm	N/A – cannot stop for success at the first interim.	Predictive Prob (PP $\geq$ 0.75) $\leq$ 0.075
20 subjects per arm	Predictive Prob (PP $\geq$ 0.95) $\geq$ 0.85	Predictive Prob (PP $\geq$ 0.75) $\leq$ 0.15

At each interim, the probability of the study going on to meet these criteria will be calculated. This probability value may be used to assist in determining whether there is sufficient rationale to continue/discontinue the study. If the probability of study success is sufficiently high, then the study may stop for success. Conversely, if the probability of study success or review is sufficiently low then the study may stop for futility. The probability thresholds for success and futility have been calibrated to balance the risk of declaring success despite a placebo-like drug (type 1 error) and declaring failure despite a positive true underlying treatment effect for GSK2798745 relative to placebo (type 2 error).

The predictive probability at each interim is calculated using predictive inference methods. The observed data at the interim is used to obtain estimates of the mean and standard error of the treatment effect for GSK2798745 relative to placebo. These estimates are then used to predict the treatment effect for participants in the remainder of the study using the predictive interim distribution. These two parts of data (observed and predicted) are combined to create a complete study. The end of study success criteria is then applied to this complete data set made up of observed and predicted participants. This process is performed 10,000 times to account for the uncertainty in the estimated treatment effect at the interim – i.e. the same observed data is combined with thousands of permutations for the remainder of the study, and each time the end of study success criteria is applied. The proportion of complete studies that meet the pre-defined success criteria gives the predicted probability of success if the study was to continue at the interim.

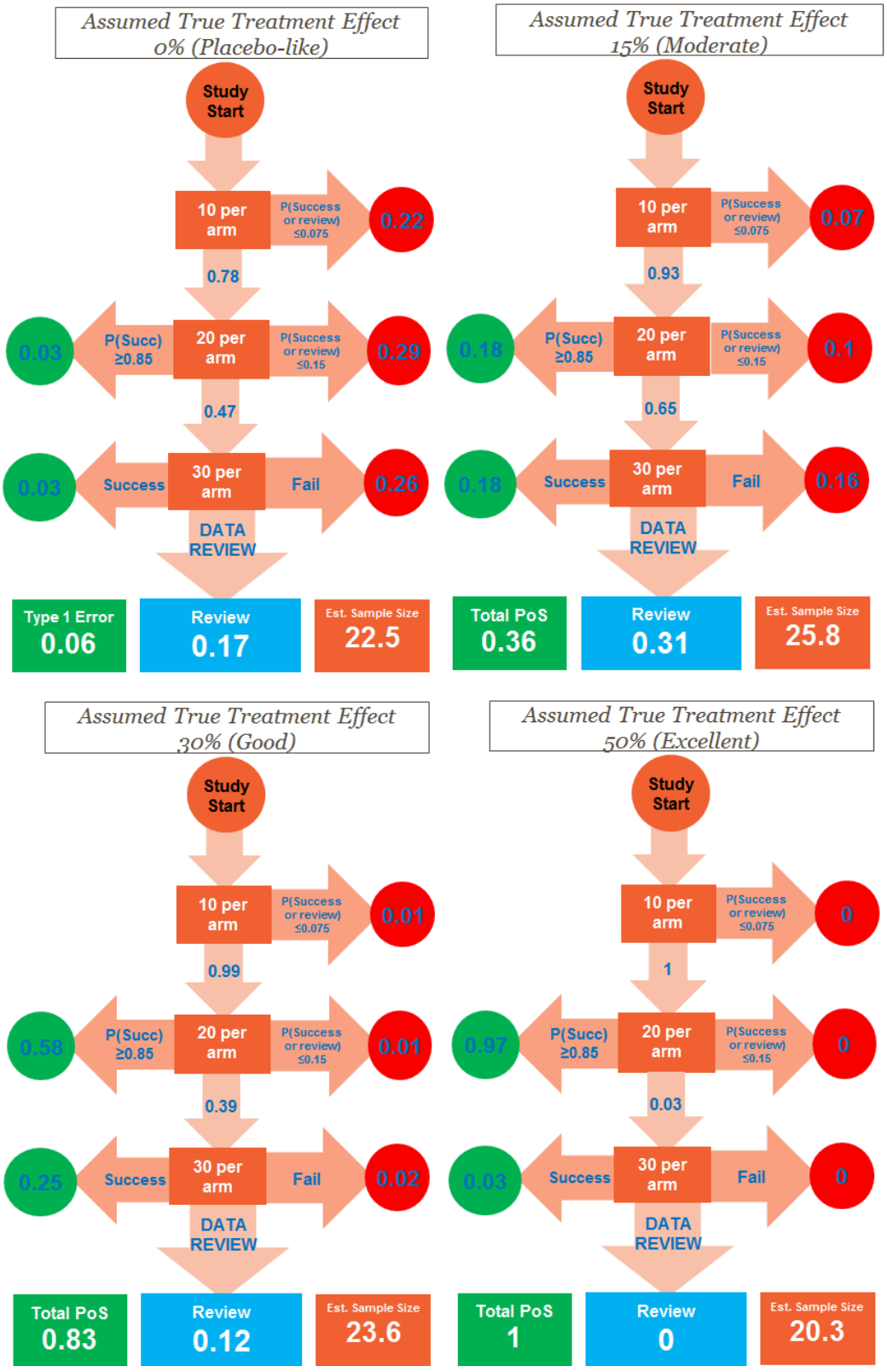
To inform the study design, a range of scenarios were reviewed to assess the operating characteristics when changing the total sample size, interim decision rules, interim analysis time points and assumed true treatment effects. Characteristics such as the overall probability of study success/failure, probability of success/failure at each interim, and the expected number of participants to be recruited were reviewed under a variety of design options.

Figure 6 show the operating characteristics of the chosen study design under true percentage reductions in total BAL protein for GSK2798745 relative to placebo of 0%, 15%, 30% and 50%, respectively, unconditional on variability. The schematic demonstrates all possible decision pathways the study may take. To obtain the operating characteristics of a given study design, 10,000 studies were simulated with each one taking a particular pathway and finishing in one of the pockets on the left or right of the diagram, or indeterminate (additional data review). The overall proportion of simulated studies reaching a particular conclusion gives the probability of a single study reaching that conclusion.

A stricter posterior probability futility threshold is implemented at the first interim to reduce the probability of incorrectly declaring futility. Furthermore, a minimum of 20 participants per arm must be recruited before declaring success. These rules are shown within the left (success) and right (futile) arrows. Under this design, assuming a true treatment effect of 30%, the overall probability of study success is approximately 83%, unconditional on variability. In addition, the probability of requiring a maximum of 20 participants per arm is approximately 61% (under the same assumption of true treatment effect of 30%). In the case of a placebo-like drug the probability of incorrectly declaring success is approximately 6% (akin to type 1 error rate). The blue boxes at the bottom of the charts show the probability that a study goes to data review.

The Reporting and Analysis Plan will describe the planned interim analyses in greater detail.

Figure 6 Interim Analysis Framework for Range of True Treatment Effects



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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase
AUC	Area under concentration-time curve
$AUC_{0–24}$	Area under the curve during 24 hours
BAL	Bronchoalveolar Lavage
BMI	Body mass index
BUN	Blood urea nitrogen
Ca^{2+}	Calcium
C_{max}	Maximum observed plasma concentration
CMT2C	Charcot-Marie-Tooth Disease Type 2C
CNS	Central Nervous System
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disorder
CPK	Creatinine phosphokinase
CRF	Case Report Form
CV	Cardiovascular
CSSRS	Columbia Suicide Severity Rating Scale
ECG	Electrocardiogram
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
EU	Endotoxin Unit
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in One Second
FVC	Forced vital capacity
FOBT	Faecal Occult Blood Test
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilance
GI	Gastrointestinal
GMP	Good Manufacturing Practice
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen
HCl	Hydrochloric acid
HDPE	High-density polyethylene
HIV	Human Immunodeficiency Virus
HMVEC	Human Dermal Microvascular Endothelial Cells
HPLC	High performance liquid chromatography
IB	Investigator's Brochure

IC_{50}	50% maximal inhibitory concentration
ICF	Informed consent form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IMP	Investigational medicinal product
IP	Investigational Product
i.t.	Intratracheal
IRB	Institutional Review Board
IWRS	Interactive Web Response System
Kg	Kilogram
KO	Knockout
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
MCH	Mean corpuscular haemogloin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligrams
mL	Milliliter
MSDS	Material Safety Data Sheet
Msec	Milliseconds
mSV	MilliSievert
Ng	Nanogram
NOAEL	No observed adverse effect level
PK	Pharmacokinetic
POP PK	Population Pharmacokinetic
PTS-DMPK	Platform Technologies and Science-Drug Metabolism and Pharmacokinetics
QC	Quality control
QTcF	QT duration corrected for heart rate by Fridericia's formula
RAP	Reporting and Analysis Plan
RBC	Red blood cells
RNA	Ribonucleic acid
SAE	Serious adverse event(s)
SOA	Schedule of Activities
SRM	Study Reference Manual
SUSAR	Suspected Unexpected Serious Adverse Reactions
TB	Tuberculosis
TLR 4	Toll-like receptor 4
Tmax	Time to reach maximum plasma concentration
TRALI	Transfusion Related Acute Lung Injury
TRPV4	Transient receptor potential vanilloid 4
ULN	Upper Limit of Normal

WBC	White blood cells
WOCBP	Women of Child Bearing Potential

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
None

12.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed in Table 3 will be performed by the local laboratory.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 3 Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Haematology	Platelet Count RBC Count Haemoglobin Haematocrit	RBC Indices: MCV MCH %Reticulocytes	WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[1]	BUN CRP	Potassium	Aspartate Aminotransferase (AST)/ Serum Glutamic-Oxaloacetic Transaminase (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	Alanine Aminotransferase (ALT)/ Serum Glutamic-Pyruvic Transaminase (SGPT)	Total Protein
	Glucose (fasting not required)	Calcium	Alkaline phosphatase	CPK
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Tests	• Cardiac troponin (cTn) • Coagulation profile (Quick/INR, PTT and thrombocytes) • Faecal Occult Blood Test (FOBT)			
Other Screening Tests	• Follicle-stimulating hormone and estradiol (as needed in women of non-childbearing potential only) • Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and			

Laboratory Assessments	Parameters
	hepatitis C virus antibody) • Alcohol, cotinine and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines).

NOTES :

1. Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 8.1.1 and Appendix 6 All events of ALT ≥3 × upper limit of normal (ULN) and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).

12.3. Appendix 3: Study Governance Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
  - 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 ICH Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Substantial amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- 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/EC
  - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

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.

Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative 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 participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.
- Participants who are rescreened are required to sign a new ICF.

Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant 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 participant.
- The participant 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.

Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Dissemination of Clinical Study Data

- Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.
- GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study participants, as appropriate.
- GSK will provide the investigator with the randomization codes for their site only after completion of the full statistical analysis.
- The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- 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 CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants 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 ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final Clinical Study Report (CSR)/ equivalent summary 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

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.
- Data reported on the CRF 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 the source document agreement (to be signed by the investigator (or delegate) at the site).

Study and Site Closure

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

The investigator may initiate study-site 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 site 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 participants by the investigator
- Discontinuation of further study treatment development

12.4. Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of a study treatment, whether or not considered related to the study treatment. • 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 a study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment 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 study treatment 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. •

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 participant'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 participant's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that

leads to the procedure is the AE.

- 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).

A 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 participant 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 participant 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 AE. 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, diarrhoea, 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 participant 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 AE and SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to GSK.
- 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.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficiently 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 an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- 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 study treatment administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, 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 GSK. 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** GSK**.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK 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 participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool
• The primary mechanism for reporting SAE to GSK will be the electronic data collection tool. • If the electronic system is unavailable for more than 24 hours, then the site will use the paper SAE data collection tool (see next section). • The site will enter the SAE data into the electronic system as soon as it becomes available. • The investigator or medically-qualified sub-investigator must show evidence within the eCRF (e.g., check review box, signature, etc.) of review and verification of the relationship of each SAE to IP/study participation (causality) within 72 hours of SAE entry into the eCRF. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the medical monitor (who is also the SAE coordinator) by telephone. • Contacts for SAE reporting can be found in SRM.

SAE Reporting to GSK via Paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the medical monitor (who is also the SAE coordinator)**.** • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. • 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 SRM.

12.5. Appendix 5: Contraceptive Guidance and Collection of Pregnancy Information

Definitions

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with ONE of the following:

- Documented hysterectomy
- Documented bilateral salpingectomy
- Documented bilateral oophorectomy

Note: Documentation can come from the site personnel's: review of participant's medical records, medical examination, or medical history interview.

3. Postmenopausal female

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
- Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance

Male participants

- Male participants with female partners of child-bearing potential are eligible to participate if they agree to ONE of the following during the protocol-defined time frame in Section 6.1:
- 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 an additional method of contraception with a failure rate of <1% per year as described in Table 4 when having penile-vaginal intercourse with a woman of childbearing potential

- Men 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 protocol-defined time frame.
- In addition, male participants must refrain from donating sperm from the time of first dose of study treatment until 2 weeks after last dose of study treatment.

Table 4 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 • oral • intravaginal • transdermal
Progestogen-only hormonal contraception associated with inhibition of ovulation • injectable
Highly Effective Methods That Are User Independent
• Implantable progestogen-only hormonal contraception associated with inhibition of ovulation • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • bilateral tubal occlusion
Vasectomized partner *(A vasectomized partner is a highly effective contraception 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 drug. 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 participant.)*

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 participants in clinical studies.

Collection of Pregnancy Information

Male participants with partners who become pregnant

- Investigator will attempt to collect pregnancy information on any male participant's female partner of a male study participant who becomes pregnant while participating in this study. This applies only to participants who receive study treatment.
- 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 GSK within 24 hours of learning of the partner's pregnancy.
- 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 GSK
- Generally, 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 foetal status (presence or absence of anomalies) or indication for procedure.

12.6. Appendix 6: Liver Safety: Required Actions and Follow-up Assessments

Phase I liver chemistry increased monitoring criteria and required follow up assessments

Liver Chemistry Increased Monitoring Criteria	
ALT-absolute	ALT≥3xULN If ALT≥3xULN **AND bilirubin**[1,2] ≥ 2xULN (>35% direct bilirubin) or **INR** >1.5, Report as an SAE. See additional Actions and Follow Up Assessments listed below

Required Actions and Follow up Assessments	
Actions	**Follow Up Assessments**
• Report the event to GSK **within 24 hours** • Complete the liver event CRF, and complete an SAE data collection tool if the event also meets the criteria for an SAE[2] • Perform liver event follow up assessments • Monitor the participant until liver chemistries resolve, stabilise, or return to within baseline (see **MONITORING** below) **MONITORING:** **If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24 hrs** • Monitor participants twice weekly until liver chemistries resolve, stabilise or return to within baseline • A specialist or haepatology consultation is recommended **If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24-**	• Viral hepatitis serology[3] • Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend • Obtain blood sample for pharmacokinetic (PK) analysis, obtained within 48hrs of last dose[4] • Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). • Fractionate bilirubin, if total bilirubin≥2xULN • Obtain complete blood count with differential to assess eosinophilia • Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE report form • Record use of concomitant medications on the concomitant medications report form including acetaminophen, herbal remedies, other over the counter medications. • Record alcohol use on the liver event alcohol intake case report form **If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5:**

Liver Chemistry Increased Monitoring Criteria	
72 hrs • Monitor participants weekly until liver chemistries resolve, stabilize or return to within baseline	• Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG) or gamma globulins. • Serum acetaminophen adduct high performance liquid chromatography (HPLC) assay (quantifies potential acetaminophen contribution to liver injury in participants with definite or likely acetaminophen use in the preceding week [James, 2009]. • Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease; complete Liver Imaging and/or Liver Biopsy CRF forms.

1. Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study treatment for that participant if ALT ≥ 3xULN and bilirubin ≥ 2xULN. Additionally, if serum bilirubin fractionation testing is unavailable, record presence of detectable urinary bilirubin on dipstick, indicating direct bilirubin elevations and suggesting liver injury.
2. All events of ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin) or ALT ≥ 3xULN and INR>1.5, if INR measured, which may indicate severe liver injury (possible 'Hy's Law'), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis); INR measurement is not required and the threshold value stated will not apply to participants receiving anticoagulants
3. Includes: 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

12.7. Appendix 7: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC).

TITLE PAGE

Protocol Title: A randomised, placebo-controlled, double-blind (sponsor open), segmental LPS challenge study to investigate the pharmacodynamics of GSK2798745 in healthy participants

Protocol Number: 207464

Short Title: Effects of GSK2798745 on alveolar barrier disruption in a segmental LPS challenge model

Compound Number: GSK2798745

Sponsor Name and Legal Registered Address:

GlaxoSmithKline Research & Development Limited
980 Great West Road
Brentford
Middlesex, TW8 9GS
UK

Medical Monitor Name and Contact Information can be found in the Study Reference Manual

Regulatory Agency Identifying Number(s): 2017-002388-16

Approval Date: 24-OCT-2017

207464

SPONSOR SIGNATORY:

PPD

24 October 2017

Anya Harry,
Physician Project Lead
Director, Clinical Development, Respiratory

Date

PPD

CONFIDENTIAL

207464

TABLE OF CONTENTS

PAGE

1. SYNOPSIS

Protocol Title: A randomised, placebo-controlled, double-blind (sponsor open), segmental LPS challenge study to investigate the pharmacodynamics of GSK2798745 in healthy participants

Short Title: Effects of GSK2798745 on alveolar barrier disruption in a segmental LPS challenge model

Rationale:

The influx of protein-rich fluid into the lung due to damage to the alveolar capillary barrier, with resultant adverse effects on respiratory function, is a fundamental underlying defect in Acute Respiratory Distress Syndrome (ARDS). In this Phase 1, proof-of-mechanism study, a segmental lipopolysaccharide (LPS) challenge will be used as a surrogate injury model to investigate the effects of Transient receptor potential vanilloid 4 (TRPV4) channel blockade on alveolar-septal barrier permeability in man. The LPS model has been widely used for the evaluation of anti-inflammatory agents, and will aid in the characterization of TRPV4 blockade on lung permeability and inflammation. It is hypothesised that TRPV4 inhibitors modulate the LPS response by reducing LPS induced calcium influx (either directly [e.g. via Toll-like receptor 4 (TLR4) mediated activation of endothelial cells] or indirectly [e.g. autocrine cytokine activation]). The assessment of TRPV4 mediated effects on LPS induced lung barrier damage in healthy volunteers is considered a viable strategy before conducting studies in an ARDS patient population.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
• To investigate the effect of GSK2798745 on alveolar-septal barrier permeability following LPS challenge in healthy volunteers.	• Baseline adjusted total protein concentration in bronchoalveolar lavage (BAL) samples at 24 h after segmental LPS challenge.
Secondary	
• To investigate the effect of GSK2798745 on neutrophil levels in LPS-challenged lungs.	• Baseline adjusted total and differential cell count of neutrophils in BAL samples at 24 h after segmental LPS challenge.

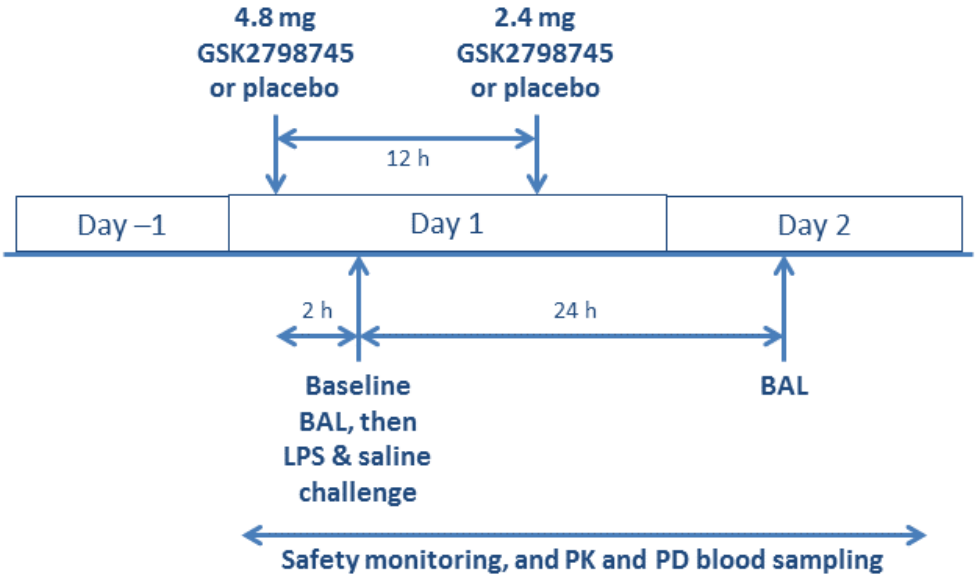
Objectives	Endpoints
• To assess the safety and tolerability of GSK2798745 in LPS-challenged, healthy volunteers.	• Adverse events (AEs), laboratory safety assessments, 12-lead electrocardiogram (ECG), physical examination, vital signs, spirometry (forced expiratory volume in 1 second [FEV1] and forced vital capacity [FVC]), faecal occult blood test (FOBT).
• To assess the pharmacokinetics (PK) of GSK2798745 in plasma in LPS-challenged, healthy volunteers	• PK parameters of GSK2798745 in plasma (AUC (0-24) and Cmax).

Overall Design:

This is a randomised, placebo-controlled, parallel group, double-blind (sponsor-open), segmental LPS challenge study of GSK2798745 in healthy participants.

Participants will be randomised 1:1 to take 2 single doses (3 tablets total): either 4.8 mg GSK2798745, then 2.4 mg GSK2798745 12 h later; or a dose of placebo, then another dose of placebo 12 h later. The first dose will be administered on Day 1 at 2 h before baseline BAL sampling from a segment in the left lower lobe. 4 ng/kg LPS will subsequently be instilled into the right middle segment and saline control into the lingula segment of the contralateral side. The second dose of study treatment will be administered 10 hours after LPS challenge followed by post-dose BAL sampling on Day 2. A schematic of the study is provided in Figure 1.

Figure 1 Study schematic



BAL: bronchoalveolar lavage; LPS: lipopolysaccharide; PD: pharmacodynamic; PK: pharmacokinetic.

Number of Participants:

Sufficient participants will be screened to ensure that a maximum of 60 evaluable participants (defined as all participants for whom results of the primary analysis can be determined) are enrolled onto the study: 30 for GSK2798745 and 30 for placebo. Interim analyses will be conducted, to assess the difference, if any, between treatments in the primary endpoint. Recruitment can continue whilst interim analyses are conducted. Depending on the results of the interim analyses, recruitment may be stopped.

Treatment Groups and Duration:

Each participant will take up to approximately 5 weeks to complete the study. Participants will be screened within 28 days before dosing and remain on site from the day before dosing (Day −1) until the day after dosing (Day 2). Participants will receive a follow up phone call 2 days after discharge, and return to the study site on Day 8 (± 1 day) for a follow-up visit.

2. SCHEDULE OF ACTIVITIES (SOA)

- The timing and number of planned study assessments, including safety, pharmacokinetic, pharmacodynamics and exploratory biomarker assessments may be altered during the course of the study based on newly available data (e.g., to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring.
- Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files, but will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the informed consent form (ICF).

2.1. Screening

	Screening (Days –28 to –2)
Outpatient visit	X
Informed consent	X
Inclusion and exclusion criteria	X
Demography	X
Full physical examination	X
Height and weight	X
Medical history	X
Past and current medical conditions	X
HIV, Hepatitis B and C screening	X
FSH and oestradiol[1]	X
Drug, alcohol and cotinine screen	X
C-SSRS	X
Laboratory assessments[2]	X
12-lead ECG[3]	X
Vital signs[4]	X
Spirometry[5]	X
FOBT[6]	X

C-SSRS: Columbia Suicide Severity Rating Scale; ECG: electrocardiogram; FOBT: Faecal Occult Blood Test; HIV: human immunodeficiency virus.

1. Postmenopausal females whose postmenopausal status is in doubt only.
2. Haematology, clinical chemistry (including liver chemistry), cardiac troponins, urinalysis and coagulation profile.
3. In triplicate.
4. Blood pressure and heart rate in triplicate. Single temperature measurement.
5. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). Triplicate measurement, the highest of which should be recorded in the case report form.
6. May be measured during the screening window or on Day –1. FOBT cards will be provided at screening and must be returned to the laboratory and analysed before dosing.

2.2. Treatment period

		Day 1[1]									Day 2[1]			
	Day –1[2]	Pre-dose	0 h	1 h	2 h	3 h	6 h	8 h	12 h	14 h	25.5 h	26 h	30 h	Day 4
Inpatient stay[2]		←==========											==========→	
Telephone call[3]														X
Drug, alcohol and cotinine screen	X													
Laboratory assessments[4]	X												X	
12-lead ECG[5]	X	X											X	
Blood pressure and heart rate[5]	X	X		X			X		X	X			X	
Temperature[6]	X	X		X	X		X	X	X	X			X	
Spirometry[7]	X	X					X				X		X	
C-SSRS													X	
Randomisation		X												
Study treatment			X						X					
Pulse oximetry[8]					X							X		
Bronchoscopy, baseline BAL and challenge[9]					X									
Bronchoscopy and post-challenge BAL[10]												X		
Blood sample for exploratory biomarkers[11]		X			X[11]	X	X	X	X	X		X[11]		
Blood sample for PK		See footnote 12												
AE review		←==========											==========→	
SAE review	←==========												==========→	
Concomitant medication review	←==========												==========→	

AE: adverse event; BAL: bronchoalveolar lavage; ECG: electrocardiogram; PK: pharmacokinetic; SAE: serious adverse event, C-SSRS: Columbia Suicide Severity Rating Scale.

1. Time points relative to the first dose on Day 1.
2. Admission on Day –1, at a time to allow all Day –1 procedures to be done; discharge on Day 2, at least 4 h after the bronchoscopy.

3. To check for any AEs.
4. Haematology, clinical chemistry (including liver chemistry), cardiac troponins, urinalysis and coagulation profile.
5. Triplicate on Day-1 and pre-dose. Single measurements at time points after dosing.
6. Immediately before BAL sampling/LPS challenge at the 2-h time point.
7. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). Triplicate measurements at each time point, the highest of which should be recorded in the case report form.
8. Pulse oximetry to be measured during bronchoscopy procedures. Only AEs to be recorded.
9. BAL samples for total protein, neutrophil counts, GSK2798745 and exploratory biomarkers. Baseline BAL sample from a segment in the left lower lobe, followed by segmental challenge of the lungs: LPS in right middle segment; saline control in the lingula segment of the contralateral side. Challenges to be done as close as possible to 2 h post-dose.
10. BAL samples for total protein, neutrophil counts, GSK2798745 and exploratory biomarkers. Baseline and post-dose measurements to be done by the same person, where possible.
11. Blood samples for exploratory biomarkers. The 2- and 26-h blood samples should be taken immediately before BAL sampling.
12. PK samples will be taken pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 6, 8, 12, 12.5, 13, 13.5, 14, 15, 24 and 26 h after the first dose. The 2- and 26-h blood sample should be taken immediately before BAL sampling

2.3. Follow-up/Early Withdrawal

	Follow-up/Early Withdrawal (Day 8 [± 1 day])
Outpatient visit	X
Full physical examination	X
Weight	X
Laboratory assessments[1]	X
12-lead ECG[2]	X
Vital signs[3]	X
Spirometry[4]	X
FOBT[5]	X
AE review	X
SAE review	X
Concomitant medication review	X

AE: adverse event; ECG: electrocardiogram; FOBT: Faecal Occult Blood Test; SAE: serious adverse event.

1. Haematology, clinical chemistry (including liver chemistry), cardiac troponins and urinalysis.
2. Single measurement.
3. Blood pressure, heart rate, temperature. Single measurement.
4. Forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). Triplicate measurement, the highest of which should be recorded in the case report form.
5. FOBT cards will be provided upon discharge from the unit and should be returned by the follow-up/early withdrawal visit.

3. INTRODUCTION

GSK2798745 is a potent and selective transient receptor potential vanilloid 4 (TRPV4) channel blocker being investigated for the treatment of acute respiratory distress syndrome (ARDS). TRPV4 blockade is expected to ameliorate the injury inflicted to the alveolar capillary barrier in ARDS patients and reduce the leakage of protein and fluid into the alveolar space. Studies investigating administration of GSK2798745 have been conducted in healthy volunteers and in patients with chronic heart failure.

GSK2798745 has been administered orally to healthy participants as single doses ranging from 0.25 to 12.5 mg. A dosage of 5 mg once daily has been administered for up to 14 days in healthy participants. Further, GSK2798745 at a dose of 2.4 mg has been evaluated as a single dose and subsequently as repeated doses for 7 days in participants with heart failure.

No clinically significant safety concerns were observed with single or repeat administration of GSK2798745 in either healthy volunteers or participants with heart failure.

3.1. Study Rationale

The influx of protein rich fluid into the lung due to damage to the alveolar capillary barrier, with resultant adverse effects on respiratory function is a fundamental underlying defect in ARDS. In this Phase 1 proof of mechanism study, a segmental lipopolysaccharide (LPS) challenge will be used as a surrogate injury model to investigate the effects of TRPV4 channel blockade on alveolar-septal barrier permeability in man. The LPS model has been widely used for the evaluation of anti-inflammatory agents [Zielen, 2015], and will aid in the characterization of TRPV4 blockade on lung permeability and inflammation.

Segmental LPS challenge, in which a small quantity of the endotoxin, LPS, is administered directly to one segment of the lung, is a model of focal and self-limiting lung inflammation. LPS induces pulmonary endothelial cell permeability [Fu, 2009] via calcium dependent cytoskeleton rearrangement [Bannerman, 1998; Gandhirajan, 2013]. LPS may also directly act on endothelial cells to cause permeability by degrading intercellular junctions [Bannerman, 1998]. The upregulation of a pro-inflammatory signalling cascade via NF-kB leading to endothelial activation and pro-inflammatory mediator release [Bosmann, 2012] may be regulated by the influx of calcium [Kandasamy, 2013], and may also induce further calcium influx and endothelial permeability [Lush, 2000; Tiruppathi, 2006].

Under normal conditions, concentrations of total protein in alveolar fluid are low. LPS induced endothelial damage leads to accumulation of protein rich fluid into the lung due to the migration of large molecules, such as albumin, from blood to the alveolar space. Preclinical and clinical studies have shown a positive correlation between amount of protein the bronchoalveolar lavage and extent of lung damage [Holter, 1986; Moazed, 2016; Yu, 2015]. In the lung, TRPV4, a Ca^{2+}-permeable non-selective cation channel, is widely expressed in cells involved in ARDS, namely microvascular endothelium,

alveolar epithelium, alveolar macrophages, and circulating neutrophils and monocytes, and is a known regulator of endothelial permeability [Narita, 2015; Suresh, 2015; Huh, 2012] and pulmonary oedema [Balakrishna, 2014; Hamanaka, 2007]. Therefore, TRPV4 may play an important role in LPS-induced alveolar permeability. The relevance of the TRPV4 channel to LPS induced injury has been confirmed in a mouse sepsis model. In this severe injury model, administration of a TRPV4 inhibitor 1 h prior to LPS injection (i.p.) increased survival by 70%. Although this study was not specifically designed to evaluate bronchoalveolar lavage (BAL) protein levels, total protein concentrations in BAL fluid of mice pre-treated with a TRPV4 inhibitor were reduced by approximately 20% after the LPS challenge in TRPV4 treated mice compared with controls [Dalsgaard, 2016].

It is hypothesised that TRPV4 inhibitors modulate the LPS response by reducing LPS induced calcium influx (either directly [e.g. via Toll-like receptor 4 (TLR4) mediated activation of endothelial cells] or indirectly [e.g. autocrine cytokine activation]).

Given the link to ARDS of endothelial damage and fluid leak, assessment of TRPV4 mediated effects on LPS induced lung barrier damage in healthy volunteers is important to establish whether beneficial effects may be observed in an ARDS patient population.

3.2. Background

ARDS is an acute inflammatory lung injury, associated with increased pulmonary vascular permeability, increased lung weight, and loss of aerated lung tissue [Bellani, 2016]. Treatment options remain limited to supportive care including mechanical ventilation and ARDS represents a significant unmet need accounting for over 10% of intensive care unit (ICU) admissions and a mortality rate of up to 40% [Bellani, 2016; Matthay, 2012]. The syndrome is characterised by acute hypoxemic respiratory failure and non-cardiogenic pulmonary oedema and may be precipitated by direct (e.g. pneumonia and aspiration) and indirect insults (e.g. sepsis and transfusion related acute lung injury ([TRALI]) to the lung. Dysregulated inflammation, inappropriate accumulation and activity of leukocytes and platelets, uncontrolled activation of coagulation pathways, and altered permeability of alveolar endothelial and epithelial barriers are central to the pathophysiology of ARDS [Matthay, 2012]. Damage to the alveolar-capillary membrane leads to increased vascular permeability, and the development of interstitial and alveolar protein rich oedema, leading to reduced gas exchange, ventilation perfusion mismatching and arterial hypoxaemia.

TRPV4 has been implicated as a key regulator of lung endothelial barrier integrity, and specifically, the integrity of the lung alveolar-capillary endothelium, which is most relevant to alveolar flooding associated with acute lung injury. TRPV4 activation by hydrostatic stretch in lung microvessels leads to increased endothelial $Ca2^{+}$ concentration and a diverse set of vascular responses, including an increase in endothelial permeability [Morty, 2014]. The importance of TRPV4 in maintaining pulmonary barrier function has been demonstrated in the settings of elevated pulmonary venous [Thorneloe, 2012] or airway pressure [Hamanaka, 2007], and following treatment with chemical and biological toxins such as Hydrochloric acid (HCl) and platelet activating factor [Balakrishna, 2014; Morty, 2014; Yin, 2016] . In these studies, TRPV4 blockade limited lung damage by

reducing plasma fluid leak into the alveolar space (thus increasing arterial oxygenation), and by modulating neutrophil and macrophage recruitment and activity and reducing overall mortality in response to LPS [Balakrishna, 2014; Morty, 2014; Yin, 2016; Dalsgaard, 2016]. This evidence suggests TRPV4 channel blockade may benefit patients with ARDS where alveolar capillary leak is a primary driver of injury, by reducing fluid leak, reducing ventilation perfusion mismatching, improving oxygenation and reducing the need for mechanical ventilation with potential reductions in mortality.

3.3. Benefit/Risk Assessment

3.3.1. Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of GSK2798745 may be found in the Investigator's Brochure.

All potential risks of GSK2798745 are based on pre-clinical data. No risks have been identified in the clinical studies of GSK2798745 conducted before the effective date of this protocol.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) [GSK2798745]		
Vascular lesions	Dogs (4-week study): at 30 mg/kg/day, 2 males had arterial lesions • One male: Heart – Coronary artery inflammation; Thymus – Arteriole inflammation with fibroplasia • One male: Epididymides – Artery degeneration/necrosis with inflammation Dogs (12-week study): At 10 mg/kg/day, 1 male and 1 female had arterial lesions • One male: Epididymides – Arteriole degeneration/necrosis with lymphocytic inflammation • One female: Bladder – Arteriole degeneration/necrosis with lymphocytic inflammation	Participant Monitoring: The arterial lesions noted in heart, thymus, epididymides, and urinary bladder cannot be monitored directly. There is currently no human translation biomarker or understanding of the underlying mechanism. Participant Exposure: Since these effects cannot be monitored directly in clinical studies, coverage of ≥30 fold will be maintained from the no-effect dose (3 mg/kg/day); exposure will not intentionally exceed the average daily area under concentration-time curve (AUC) of 0.513 hr*µg/mL and/or maximum observed plasma concentration (Cmax) of 0.050 µg/mL on an individual basis.
Myocardial toxicity	Dogs (4-week study): at 30 mg/kg/day, myofibre degeneration/necrosis and inflammation (2 animals)	Participant Selection: Participants with any history or current disease constituting a risk when taking the study treatment, including relevant history of acute coronary syndromes (including unstable angina), coronary angioplasty, or stenting, will be excluded. Participant Monitoring: Cardiac troponin levels will be monitored and ECGs will be done during the study. Participant Exposure: Exposure levels will be maintained below the threshold detailed in the Dose Justification Section (see Section 5.5).

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Mortality/moribund condition; poor viability	Dogs (4-week study): at 30 mg/kg/day, one male terminated early (Day 6) due to poor clinical condition. Another male had transient whole body shaking on Days 8 and 9. Dogs (13-week study): at 10 mg/kg/day one male was terminated early (Day 74) due to welfare reasons. Rats (micronucleus and comet study): mortality occurred following 1 to 3 doses at ≥600mg/kg/day	Participant Monitoring: Weight and adverse events reported by participants will be monitored.
Gastrointestinal (GI) and/or hepatic toxicity	GI toxicity: ≥3 mg/kg/day in dogs and at 30 and 300 mg/kg/day in rats, consisting of mucosal erosion/ulceration in the stomach and/or duodenum. Hepatic Toxicity: Biliary epithelial hypertrophy/hyperplasia and periductal mixed inflammatory cell infiltrate into the liver was observed at 300 mg/kg/day in rat (7-day study) and focal hepatocellular degeneration in 1 male dog at 30 mg/kg/day (4-week study)	Participant Selection: Participants with active ulcer disease or GI bleeding or those who are taking concomitant medications, including nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids, will be excluded. Assessment of faecal occult blood will be performed before and after dosing. Participant Monitoring: Participants will be monitored for GI intolerance (e.g by adverse events such as abdominal discomfort) and sequential clinical chemistry analysis, including liver enzymes. Follow-up faecal occult blood test (FOBT) will be conducted.
Testicular toxicity	Inconsistent finding in rats (4-week study): Spermatid retention at ≥60 mg/kg/day, however no effect observed in 13-week study. The observations in the 4-week study were not associated with degenerative changes in testes or epididymides. No spermatogenic abnormalities were observed in dogs.	Participant Exposure: A safety margin of ≥40 fold will be maintained from the no effect dose (60 mg/kg/day) in rats.
Skeletal muscle toxicity	Rat (4-week study): Myofiber necrosis: myofiber degeneration/regeneration; fibroplasia, at 300 mg/kg/day in the soleus muscle.	Participant Monitoring: Creatinine phosphokinase (CPK) levels will be taken before and after dosing.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Seizures and convulsions	Rats (micronucleus and comet study): convulsions observed at ≥600 mg/kg/day. Convulsions were not related to Cmax, nor occurred at any predictable time from dose administration. Dogs: No central nervous system (CNS) findings at 12 mg/kg/day in the dog 7-day EEG/CV study. In other compounds in the same series, convulsions have been observed.	Participant Selection: Participants with any history or current disease constituting a risk when taking the study treatment, including a history of seizure disorder or stroke within the last 5 years will be excluded from the study.
Low food consumption	Dogs (4-week study): 30 mg/kg/day reduced food consumption. Two males were terminated early (Day 10) due to extremely reduced food consumption. Rats (4-week study): 300 mg/kg/day had decreased food consumption.	Participant Monitoring: Weight will be monitored.
Effects on macrophages (Phospholipid accumulation)	Inconsistent effects observed in Rats (4-week study): ≥60 mg/kg/day in the lung (prominent alveolar macrophages); 300 mg/kg/day in the mesenteric lymph node (increased cellularity of sinus macrophages) and thymus (macrophage vacuolation; increased thymus weight). Consistent with phospholipid accumulation (phospholipidosis) based on ultrastructural appearance of mesenteric lymph nodes at 300 mg/kg/day. Findings were not associated with degenerative changes. In 13-week studies in rats, these effects were not observed.	Participant Exposure: A safety margin of ≥40 fold will be maintained from the no effect dose (60 mg/kg/day) in rats.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Theoretical Risk: Potential effects on vasoregulation .	TRPV4 mediates prostaglandin release from isolated human endothelial cells and in vivo in rats, supporting the potential for TRPV4 blockade to modulate blood pressure via prostaglandin release. No effect of GSK2798745 on blood pressure was observed in preclinical or clinical studies.	Participant Monitoring: Blood pressure will be monitored throughout the study.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Theoretical Risk: Potential effect on hearing.	Genetic deletion of TRPV4 in mice has been shown to effect hearing. TRPV4 knockout (KO) mice at age 8 weeks exhibited normal hearing thresholds, but at age 24 weeks, had delayed-onset hearing loss; additionally, the cochlea was found to be vulnerable to acoustic injury with sound overexposure [Tabuchi, 2005]. Patients with Charcot-Marie-Tooth Disease Type 2C (CMT2C), an autosomal dominant axonal neuropathy related to TRPV4 gene mutations, demonstrate symptoms that include hearing loss caused by nerve damage in the inner ear (sensorineural hearing loss). These are predominantly gain of function TRPV4 abnormalities, in which the hearing loss is sporadic among family members; and relegated to some TRPV4 defects, but not in others. Although the exact mechanism is unclear, it has been suggested that the TRPV4 channel plays an important role in peripheral nerve function and that the alterations in TRPV4 in CMT2C may be due to increased channel activity leading to excessive calcium influx and a calcium overload. There is potential for benefit with GSK2798745, in that with cells (HEK293) expressing the CMT2C mutant channel, inhibitors of the TRPV4 channel were found to block the increased intracellular calcium concentrations and resultant cell death [Landouré, 2010]. In a study evaluating effects on heart failure with similar dosing exposure, a week-long duration and comprising an older participant demographic, audiometry testing was conducted. Although a variety of changes were observed, primarily sporadic, low frequency and single frequency changes in those on treatment as well as placebo, none were assessed as a signal for concern by the reviewing expert audiologist.	Participant Exposure: Dosing will be limited to a short duration of a single day.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Theoretical risk: Potential suicidal ideation	GSK2798745 is considered to be a CNS-active drug. There has been some concern that some CNS-active drugs may be associated with an increased risk of suicidal thinking or behaviour when given to some patients with certain conditions. GSK considers it important to monitor for such events before or during clinical studies with CNS-active compounds. Participants being treated with GSK2798745 should be assessed at screening for suicidality.	Participant Selection: The risk of such an event would be very low in single dose trials in healthy volunteers however participants who, in the investigator/designee's judgement, poses a significant suicide risk, will be excluded from the study. A C-SSRS questionnaire will be completed before and after dosing.
Study procedures-related		
Risk of adverse events following delivery of LPS	Previously reported segmental LPS related adverse events include pleuritic pain, pyrexia, head ache, nausea and alveolitis [Hohlfeld, 2008; Holz, 2015]. It is known that LPS and pro-inflammatory mediators can cause gut permeability [Al-Sadi, 2014; Guo, 2013]. We expect the systemic exposure to LPS and systemic inflammatory response to LPS to be minimal following the segmental topical dose of 4ng/kg LPS. As such, we assess the risk to increased gut permeability, and potential increased bioavailability, with this regimen to be low.	Participant Monitoring: Participants will be monitored with safety assessments, including clinical laboratory tests, physical examination, ECGs, vital signs (including temperature), pulse oximetry and spirometry during the study. Experienced site staff will conduct the procedures. Participants will be carefully monitored and managed with standard procedures in the event of complications.
Risks associated with bronchoscopy and BAL sampling:	Procedure related complications include cough, transient fever, chills and myalgias, transient infiltrates, bronchospasm, transient fall of lung function, transient decrease in baseline PaO2 (partial pressure of oxygen in arterial blood).	

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Risk associated with blood draws	Fainting, mild pain, bruising, irritation or redness.	Experienced site staff will follow standard approaches for managing events related to blood draws.
Risks associated with spirometry	Shortness of breath, coughing, light-headedness or fainting, and/or chest tightness may be induced by spirometry testing.	Participants experiencing any of these symptoms will receive standard medical treatment by the study investigator.

3.3.2. Benefit Assessment

There will be no intended therapeutic clinical benefit to the participants taking part in the study as it is a healthy volunteer study. However, participants will undergo a medical evaluation during screening including physical exams, electrocardiograms (ECGs) and laboratory assessments which may provide important health information.

By taking part in this study, the participant will be contributing to the development of GSK2798745 for the treatment of ARDS, a syndrome with significant unmet need.

3.3.3. Overall Benefit:Risk Conclusion

No benefit to healthy participants is expected. The study activities have risks, however these risks are mitigated by exclusion of participants with identified comorbidities, short (single day) duration of dosing with GSK2798745 and exposure limited to previously approved exposure levels and inclusion of safety margins, as well as safety monitoring by trained staff. The study will provide important information towards future development of TRPV4 blockers that may help patients with ARDS.

The design of the study is considered low risk to the participants and justified based on the safety information from the nonclinical studies and the previous clinical trials carried out on GSK2798745.

4. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
• To investigate the effect of GSK2798745 on alveolar-septal barrier permeability following LPS challenge in healthy volunteers.	• Baseline adjusted total protein concentration in bronchoalveolar lavage (BAL) samples at 24 h after segmental LPS challenge.
Secondary	
• To investigate the effect of GSK2798745 on neutrophil levels in LPS-challenged lungs.	• Baseline adjusted total and differential cell count of neutrophils in BAL samples at 24 h after segmental LPS challenge.
• To assess the safety and tolerability of GSK2798745 in LPS-challenged, healthy volunteers.	• Adverse events (AEs), laboratory safety assessments, 12-lead electrocardiogram (ECG), physical examination, vital signs, spirometry (forced expiratory volume in 1 second [FEV1] and forced vital capacity [FVC]), faecal occult blood test (FOBT).

Objectives	Endpoints
• To assess the pharmacokinetics (PK) of GSK2798745 in plasma in LPS-challenged, healthy volunteers	• PK parameters of GSK2798745 in plasma (AUC (0-24) and Cmax).
Exploratory	
• To assess concentration of GSK2798745 in BAL. • To investigate the effect of GSK2798745 on exploratory markers of endothelial barrier permeability and/or injury, and inflammation, in LPS-challenged lungs. • To assess metabolite GSK3526876 (major metabolite of GSK2798745) in plasma. • To compare the PK of GSK2798745 with the effect of prophylactic dosing on alveolar-septal barrier permeability following LPS challenge in healthy volunteers. • To investigate the effect of GSK2798745 on white blood cell levels (except neutrophils) in LPS-challenged lungs.	• Levels of GSK2798745 in BAL samples at 2 and 26 h after dosing. • Baseline adjusted levels of exploratory biomarkers in blood and/or BAL. • Plasma concentrations of metabolite GSK3526876. • Comparison of PK parameters of GSK2798745 in plasma with baseline corrected total protein concentration in BAL. • Baseline adjusted total and differential cell count of white blood cells (except neutrophils) in BAL samples at 24 h after segmental LPS challenge.

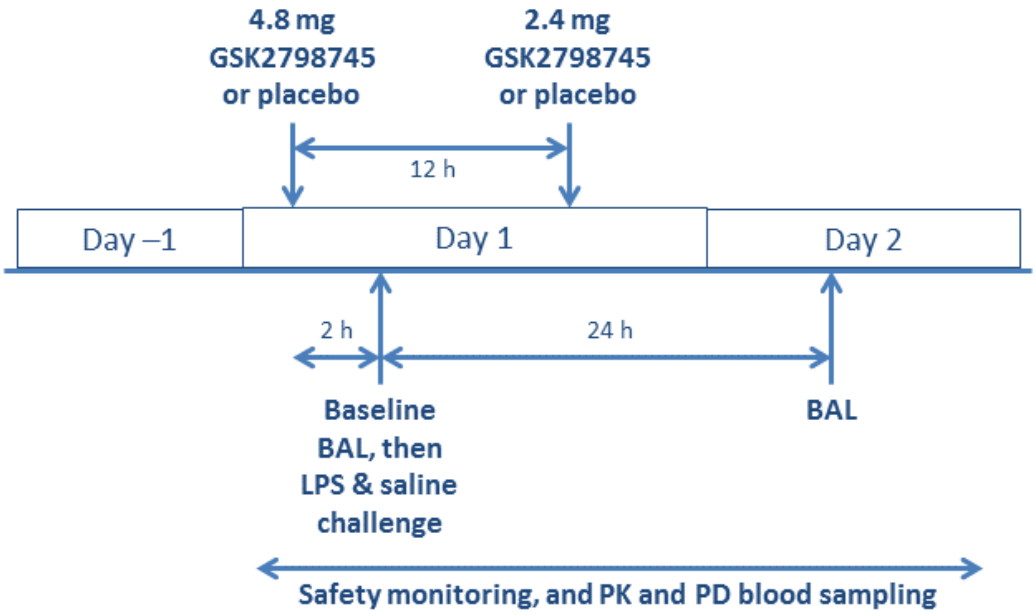
5. STUDY DESIGN

5.1. Overall Design

This is a randomised, placebo-controlled, parallel group, double-blind (sponsor-open), segmental LPS challenge study of GSK2798745 in healthy participants.

Participants will be randomised 1:1 to take 2 single doses (3 tablets total): either 4.8 mg GSK2798745, then 2.4 mg GSK2798745 12 h later; or a dose of placebo, then another dose of placebo 12 h later. Participants will undergo segmental LPS challenge to the lungs at 2 h after the first dose. BAL samples will be taken after dosing: immediately before and at 24 h after the LPS and saline challenge. Blood samples will be taken before and after dosing and the challenge. Safety will be monitored throughout. A schematic of the study is provided in Figure 2.

Figure 2 Study schematic



BAL: bronchoalveolar lavage; LPS: lipopolysaccharide; PD: pharmacodynamic; PK: pharmacokinetic.

Each participant will take up to approximately 5 weeks to complete the study. Participants will be screened within 28 days before dosing and remain on site from the day before dosing (Day −1) until the day after dosing (Day 2). The first dose will be administered on Day 1 at 2 h before baseline BAL sampling from a segment in the left lower lobe. 4 ng/kg LPS will subsequently be instilled into the right middle segment and saline control into the lingula segment of the contralateral side. The second dose of study treatment will be administered 10 hours after LPS challenge followed by post-dose BAL sampling on Day 2. Participants will receive a follow up phone call 2 days after discharge, and return to the study site on Day 8 (± 1 day) for a follow-up visit.

5.2. Number of Participants

Sufficient healthy participants will be screened to ensure that a maximum of 60 evaluable participants (defined as all participants for whom results of the primary analysis can be determined) are enrolled onto the study: 30 for GSK2798745 and 30 for placebo. Interim analyses will be conducted (as described in Section 10.3.4), to assess the difference, if any, between treatments in the primary endpoint. There are two interim analyses planned, depending on the results of the interim analyses, recruitment may be stopped. Participants who prematurely discontinue the study, and whose data are not evaluable (i.e. those for whom results of the primary analysis cannot be determined), may be replaced. Recruitment into the study can continue whilst interim analyses are taking place.

5.3. Participant and Study Completion

A participant is considered to have completed the study if he/she has completed all phases of the study including the follow-up visit.

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

5.4. Scientific Rationale for Study Design

- *Use of a placebo:* The randomised, placebo-controlled study design is a well-established methodology to evaluate potential treatment effects in experimental studies. The use of a placebo arm allows for a valid evaluation of changes in PD markers attributable to treatment versus those independent of treatment. Given that participants are healthy and treatment is not being withheld, the use of placebo is considered acceptable.
- *Parallel group:* The use of a parallel group design is considered to limit the burden of procedures and exposure of LPS to participants. A crossover design only minimally decreases the required number of evaluable participants and owing to the expected dropout rate in a crossover study, and the burden of procedures for each participant, a parallel group design was selected.
- *Blinding:* A double blind design is a standard methodology for randomised, controlled studies to avoid bias.
- *Selection of primary endpoint:* Changes in total protein concentrations to monitor influx of proteinaceous fluid into the lung has been used in several LPS challenge studies. Evidence points to the involvement of TRPV4 in changes to alveolar capillary barrier following LPS administration, with subsequent leak of proteinaceous fluid as measured by total protein. Given the influx of fluid with high concentrations of protein observed in ARDS patients, monitoring total protein changes is considered an acceptable surrogate to assess for target engagement of GSK2798745 and for potential effects in ARDS patients.
- *Timing of primary endpoint:* As previous studies show that total protein concentrations peak at 24 h after segmental LPS challenge [O'Grady, 2001; Holz, 2015], post-challenge BAL samples will be taken at 24 h after the challenge.
- *Timing of challenge:* Baseline BAL sampling prior to administration of study drug is not considered feasible due to the burden of bronchoscopy (i.e. two procedures required – one pre and one post dose to instil LPS). A baseline bronchoscopy is planned 2 h after administration of the first dose, with the aim of maximising exposure to GSK2798745 (in healthy volunteers, the median [range] Time to reach maximum plasma concentration (Tmax) of the tablet formulation of GSK2798745 to be used in this study is 1.5 h [1–3 h]). Drug on board at the time of baseline BAL fluid sampling is expected to have little to no effect on baseline BAL total protein concentrations as TRPV4 channels are quiescent until stimulated and blockade of these channels does affect barrier permeability of 'healthy' endothelial cells [internal data]. The effects of LPS to induce endothelial permeability are believed to occur rapidly. Investigation of LPS induced permeability and markers of cell activation and inflammation over time consistently indicates that the initial events leading to increased barrier permeability and inflammation occur within first 2 h post

intratracheal (i.t.) LPS exposure; where any earlier measurements are limited. LPS induces intracellular stress fibre formation within 5 min in both human dermal microvascular endothelial cells (HMVEC) endothelial and A549 epithelial cells [Ngamsri, 2010]. Increased levels of TNFα following i.t. LPS instillation are observed within 2 h in man [O'Grady, 2001] and other pro-inflammatory mediators within 4 h in a mouse model of acute lung injury (ALI) [Bosmann, 2012]. Permeability across an endothelial monolayer is observed after 0.5 h and peaks at 2 h post-LPS [Bannerman, 1998]. BAL total protein increases following LPS i.t. challenge in man as early as 2 h and reaches significance at 6 h post-LPS [Matthay , 2012] with significant increases in neutrophil counts also observed as early as 2 h. Looking to a systemic LPS model of sepsis with a higher LPS insult, earlier sampling is possible and high levels of inflammatory mediators are measured within 1 h, which reach plateau by 3 h. Therefore, despite our conservative treatment effect prediction (see Section 5.5), we expect that high plasma and/or alveolar fluid concentrations of GSK2798745 will be required at the time of and/or soon after the challenge, in order to inhibit the effects of LPS. Therefore, LPS will be administered shortly after time of Tmax (1.5-hours) to ensure sufficient drug on board.

5.5. Dose Justification

5.5.1. Dose Rationale

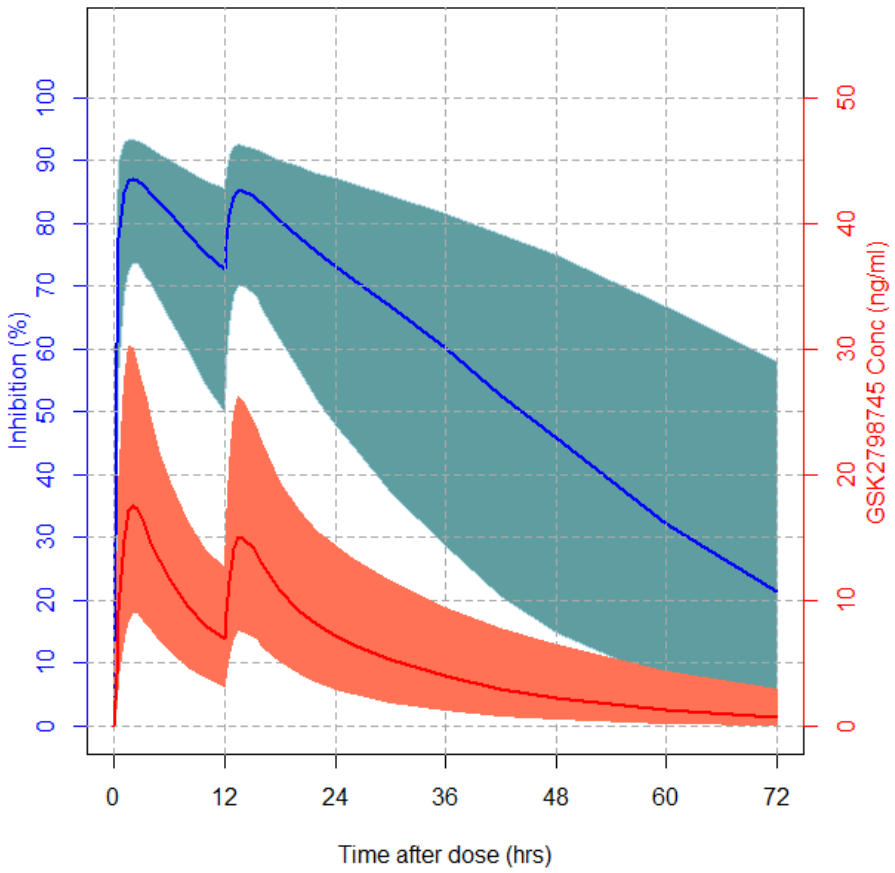
The first time in human study (TR4113787) for GSK2798745 evaluated doses from 0.25 mg to 12.5 mg as single doses, and 5 mg once daily repeat dosing for 14 days in healthy volunteers in cohorts 1–3. Cohorts 4 and 5 of the study were administered once daily 2.4 mg dose of capsules with food for 7 days to stable heart failure participants. Exposure from different tablet formulations administered with or without food was characterised in another clinical study in healthy volunteers (204725). Exposure from all available clinical data to date was analysed with a population pharmacokinetic (POP PK) approach accounting for participant weight, formulation, impact of food amongst other variables. Trial simulations were performed with this POP PK model with differing dosing regimen.

The estimated human IC50 of 2.1–3.2 ng/mL was derived from data from a rat study, which was conducted to assess the ability of different doses of GSK2798745 infusion to reduce the increased lung-to-bodyweight ratio induced by the TRPV4 agonist. The IC50 was also corrected for species differences using protein binding data and TRPV4 potency differences in *invitro* assays. To evaluate drug activity/efficacy at the intended dosing regimen, the percentage pulmonary oedema blockade was estimated using the population model and the corrected potency values derived from the rat pulmonary study.

Based on the simulations, the intended dosing scheme for this study is a 4.8 mg dose followed by a 2.4 mg dose at 12 h. Table 1 lists the predicted average percentage pulmonary oedema blockade over the 24-h period based on this potency range. The schematic in Figure 3 also depicts the range of GSK2798745 systemic exposure and the predicted percent inhibition by TRPV4 with the intended regimen. At 26 h post the first dose, when the primary endpoint sample will be taken, the predicted TRPV4 inhibition is 71.1% (44.4–86.3) [median (95% prediction interval)].

The intended dosing regimen also limits the daily ceiling human exposure at individual participant level to 30-fold of the exposure observed at the no observed adverse effect level (NOAEL) dose of 3 mg/kg from the 3-month dog safety study (D70496G). The current clinical doses are selected so that no participant intentionally exceeds the daily AUC of 513 ng*hr/mL and C_{max} of 50 ng.hr/mL. The likelihood of one or more participants of the 30 participants to be dosed with this regimen exceeding the threshold is listed in Table 1.

Figure 3 Predicted GSK2798745 exposure and corresponding effect on pulmonary oedema after doses of 4.8 mg then 2.4 mg, 12 h apart



Note: percentage inhibition based on the rat, agonist-driven, pulmonary oedema model.

Table 1 Predicted exposure, probability of exceeding threshold and predicted efficacy after doses of 4.8 mg then 2.4 mg, 12 h apart, administered to 30 participants

Percentage pulmonary oedema blockade over 24 h (median [95% PI])	Predicted exposure (median [95% PI])		% Probability that ≥1 of 30 participants will exceed the toxicokinetic limit[1]	
	AUC0–24 h (ng.h/mL)	Cmax (50 ng/mL)	AUC0–24 h (513 ng.h/mL)	Cmax (50 ng/mL)
79.1 [66.5–87.1]	272.4 [176.2–400.9]	23.1 [15.2–34.4]	5.6	1.6

PI: predicted interval.

1. The percentage of the 500 simulated studies (of 30 subjects each) in which ≥ 1 subject exceeds the toxicokinetic limit.

5.5.2. Treatment Effect Rationale

Data from the systemic LPS model described in Section 5.4 [Shyamsundar, 2009] , and from other models of lung injury (chlorine gas exposed mice [Suresh, 2015], pulmonary venous pressure induced injury of isolated mouse lungs [Narita, 2015], and a mouse model of intratracheal instillation of hydrochloric acid [Hamanaka, 2007]) suggest that TRPV4 blockade contributes 20–80% to a reduction in total protein in BAL fluid after the injury. Averaging the effect from those studies, the maximum effect possible in this segmental LPS model is estimated to be approximately 50%, assuming total TRPV4 channel blockade. Therefore, with estimated exposure expected to reduce total protein by approximately 71% in a pure agonist driven challenge, a median treatment effect of 35% might be expected in this LPS challenge. Based on pragmatic reasons and given some of the uncertainty around TRPV4 contribution to proteinaceous fluid influx as measured by total protein in a segmental LPS challenge, an estimated 30% treatment effect was nominally selected for sample size calculations.

6. STUDY POPULATION

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

6.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

AGE
1. Between 18 and 50 years of age inclusive, at the time of signing the informed consent.

TYPE OF PARTICIPANT AND DISEASE CHARACTERISTICS
2. Volunteers who are overtly healthy as determined by medical evaluation including medical history, physical examination, laboratory tests (including a normal coagulation profile), ECGs, vital signs and spirometry.

Note: In the event of out-of-range results of safety tests, the tests may be repeated once within the screening window. If a retest result is again outside the reference range and considered clinically significant by the investigator and GSK medical monitor, the participant will be considered a screen failure.

3. Normal spirometry (FEV1 ≥80% of predicted, FEV1/FVC ratio ≥70%) at screening and before dosing.

WEIGHT
4. Body weight ≥50 kg and body mass index (BMI) within the range 19–29.9 kg/m^2 (inclusive).

SEX
5. Male or female. **a. Male participants:** A male participant must agree to use contraception, as described in Appendix 5, during the treatment period and for at least 7 days after the last dose of study treatment and refrain from donating sperm during this period. **b. Female participants:** A female is eligible to participate if she is not of childbearing potential, as defined in Appendix 5.

INFORMED CONSENT
6. Capable of giving signed informed consent which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

6.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTC INTERVAL)
1. Significant history of or current cardiovascular, respiratory (eg asthma, chronic obstructive pulmonary disorder (COPD), bronchiectasis, active Tuberculosis [TB]), hepatic, renal, gastrointestinal, endocrine, hematological, autoimmune or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; constituting a risk when taking the study treatment; or interfering with the interpretation of data.
2. Participant who, in the investigator/designee's judgement, poses a significant suicide risk. Evidence of serious suicide risk may include any history of suicidal behaviour and/or any evidence of suicidal ideation on any questionnaires e.g., Type 4 or 5 on the Columbia Suicide Severity Rating Scale (C-SSRS) in the last 5 years.

3. Active ulcer disease or gastrointestinal bleeding at the time of screening (positive faecal occult blood test [FOBT] at screening).
4. Abnormal blood pressure as determined by the investigator.
5. ALT or bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
6. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
7. QTc >450 msec

   NOTES: the QTc is the QT interval corrected for heart rate according Fridericia's formula (QTcF).
8. At risk of Torsades de pointes (e.g. a personal history or a family history of sudden unexplained death, long QT, familial cardiac syndrome, or cardiomyopathy).
9. Chronic or acute infection within the 4 weeks before dosing, (e.g. upper and lower respiratory infection within the 4 weeks before dosing).
10. Major (as per investigator judgment) surgery within the last 12 weeks prior to randomisation or planned within 3 months of screening.

PRIOR/CONCOMITANT THERAPY

11. Use of prescription or non-prescription drugs (except paracetamol), including vitamins, herbal and dietary supplements (including St John's Wort) within 7 days or 5 half-lives (whichever is longer) before the first dose of study medication, unless, in the opinion of the investigator and GSK Medical Monitor, the medication will not interfere with the study procedures or compromise participant safety.
12. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator and/or GSK Medical Monitor, contraindicates their participation.

PRIOR/CONCURRENT CLINICAL STUDY EXPERIENCE

13. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within 3 months.
14. The participant has participated in a clinical trial and has received an investigational product within the following time period before the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
15. Exposure to more than four new chemical entities within 12 months before the first dosing day.

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA

16. Presence of hepatitis B surface antigen (HBsAg) at screening

17. Positive hepatitis C antibody test result at screening.

    NOTE: Participants with positive Hepatitis C antibody due to prior resolved disease can be enrolled, only if a confirmatory negative Hepatitis C Ribonucleic acid (RNA) test is obtained.

18. Positive Hepatitis C RNA test result at screening or within 3 months prior to first dose of study treatment.

    NOTE: Test is optional and participants with negative Hepatitis C antibody test are not required to also undergo Hepatitis C RNA testing.

19. A positive pre-study drug/alcohol/cotinine screen.
20. A positive test for immunodeficiency virus (HIV) antibody.
21. Regular use of known drugs of abuse.

OTHER EXCLUSIONS
22. History of regular alcohol consumption within 6 months of the study defined as an average weekly intake of >21 units for males or >14 units for females. One unit is equivalent to 8 g of alcohol: a half-pint (~240 mL) of beer, 1 glass (125 mL) of wine or 1 (25 mL) measure of spirits.
23. Current smoker or a history of smoking within 6 months of screening, or a total pack year history of >5 pack years. [number of pack years = (number of cigarettes per day/20) x number of years smoked].

6.3. Lifestyle Restrictions

6.3.1. Meals and Dietary Restrictions

- Participants are not permitted to consume red wine, Seville oranges, grapefruit or grapefruit juice, and/or pomelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days before the start of study treatment until discharge from the clinical unit.
- Participants will be required to fast from midnight before the bronchoscopies on Day 1 and 2 until after the procedure and for at least 2 hours before dosing.

6.3.2. Caffeine, Alcohol, and Tobacco

- During the Treatment Period, participants will abstain from ingesting caffeine- or xanthine-containing products (eg, coffee, tea, cola drinks, and chocolate) for 24 hours before the start of dosing until after collection of the final pharmacokinetic (PK) and/or pharmacodynamic sample.
- During the Treatment Period, participants will abstain from alcohol for 24 hours before the start of dosing until after collection of the final PK and/or pharmacodynamic sample.
- Only non-smokers may be recruited into this study.

6.3.3. Activity

- Participants will abstain from strenuous exercise for 72 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities during studies (eg, watching television, reading).

6.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomised. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs).

Participants may be rescreened once. If rescreening is preformed, participants must be assigned a different unique participant identification number for the rescreening, and all screening procedures must be repeated. See the study reference manual (SRM) for more details.

7. TREATMENTS

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

7.1. Treatments Administered

Study Treatment Name:	**GSK2798745**	**Matching Placebo**	**Challenge Agent: GMP grade lipopolysaccharide from *Escherichia coli* (*E. Coli* Group 0113:H10:K Negative) for the segmental LPS challenge**
Dosage formulation:	White to slightly coloured, round biconvex tablet. Product: AP, Tab-A	White to slightly coloured, round biconvex tablet. Product code: CET, Tab-A..	LPS is available from stock lyophilized in a 1 microgram vial, formulated in 1% lactose and 0.1% PEG6000. Clear solution.
Unit dose strength(s)/Dosage level(s):	Unit dose strength 2.4 mg. Dosage Levels: 4.8 mg and 2.4 mg	Not applicable	4 ng/kg
Route of Administration	Oral	Oral	Direct application to the lung segment, via bronchoscopy
Dosing instructions:	Two tablets to be taken on the morning of Day 1, then one tablet to be taken 12 hours later.	Two tablets to be taken on the morning of Day 1, then one tablet to be taken 12 hours later.	One single instillation of 10 mL by bronchoscopy 2 hours after dosing with GSK2798745 or placebo on Day 1.
Packaging and Labeling	GSK2798745 tablets will be provided in high-density polyethylene (HDPE) bottles with child-resistant closures that include induction-seal liners. Each bottle will be labelled as required per country requirement.	Placebo tablets will be provided in high-density polyethylene (HDPE) bottles with child-resistant closures that include induction-seal liners. Each bottle will be labelled as required per country requirement.	-
Manufacturer	GSK	GSK	List Laboratories, Inc.

Sterile saline (0.9%) for control challenge will be sourced by the clinical site.

7.1.1. Dose Modification

No dose modifications are permitted without submission of a substantial amendment to the protocol.

7.2. Challenge Agents

Bacterial endotoxin is a component of the cell wall of Gram-negative bacteria present in a variety of occupational and general environmental settings.

The dose of LPS used in this study is 4 ng/kg, which is 100 times lower than doses used in inhalation models of endotoxin-induced lung inflammation. This dose was chosen based on the data published by O'Grady et al [O'Grady, 2001]. In this study, lavage after 24-h in subjects challenged with 4 ng/kg of endotoxin revealed a localised inflammatory response that was neutrophil-predominant. The site performing this study has experience with this model. The LPS challenge agent to be used for the procedure is Good Manufacturing Practice (GMP)-grade product and will be sourced by GSK from List Laboratories, Inc. A certificate of analysis will be provided for each batch of the LPS challenge agent to ensure its quality and safety.

Reconstitution will be done under the responsibility and supervision of the investigator or qualified site staff who performs the bronchoscopy and administers the reconstituted LPS in individual dilution to the subject. The reconstitution will be done on the day of bronchoscopy immediately before the procedure (within 1 hour). If more than one subject per day will be investigated, the first process of reconstitution and administration of LPS has to be completed before a second process is started.

The reconstitution process is described as an example for the nominal dose of 10,000 EU of endotoxin per vial. Each vial of LPS contains a lyophilized solid containing 10,000 EU of endotoxin. Upon reconstitution with 5 mL of sterile saline 0.9%, the vial will contain 2,000 EU/mL = 2 EU/µL. Forty (40) µL/kg body weight will be withdrawn from the vial and transferred into a sterile endotoxin-free 30 mL-sample vial (Acila AG, Weiterstadt, Germany). Sterile saline 0.9% will be added to give a final volume of 20 mL. Ten (10) mL of this solution contains the application dose of 40 EU (4 ng)/kg, and will be filled into a 10 mL-syringe. This individual dilution will be administered to the participant by segmental pulmonary application during the bronchoscopy. The remaining 10 mL will be stored frozen (-20°C) as a retention sample.

The negative control will be performed with 10 mL of sterile saline 0.9% only. The reconstitution process will be adapted to the actual endotoxin content as determined in the Certificate of Analysis. The Investigator Site File will contain the current instructions for reconstitution in order to ensure the application dose of 40 EU/kg.

7.3. Method of Treatment Assignment

All participants will be centrally randomized using an Interactive Web Response System (IWRS). Before the study is initiated, the log in information and instructions for the

IWRS will be provided to each site. Participants will be registered using the IWRS, and assigned a unique number (randomisation number). The randomisation number encodes the participant's treatment (GSK2798745 or placebo), according to the randomization schedule generated prior to the study by the Statistics Department at GSK. Each participant will be dispensed blinded study treatment, labelled with his/her unique randomisation number.

7.4. Blinding

This will be double blind study. All study staff involved in clinical assessments (which includes the investigator, sub-investigators, other site staff) and the participant will be blinded to the treatment allocated to individual participants and to post challenge PD results. Selected sponsor study team members (and delegates if programming activities are outsourced) will be unblinded to perform the interim analysis. This may include the medical monitor, study statistician, study programmer (and delegates) and study pharmacokineticist. Access to unblinded data will be kept to the minimum set of individuals required to implement any interim analyses, but may include GSK management/review committees if alterations to the study conduct are required. Details of who were unblinded to what data and when will be included in the clinical study report. The IWRS will be programmed with blind-breaking instructions. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a participant's treatment assignment is warranted. Participant safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact GSK prior to unblinding a participant's treatment assignment unless this could delay emergency treatment of the participant. If a participant's treatment assignment is unblinded GSK must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and CRF, as applicable.

A participant whose treatment assignment is inadvertently unblinded (either to investigative staff or the participant themselves) will be permitted to remain in the study, although the accidental unblinding will be recorded as a protocol deviation and hence the participant will be subject to review as to their inclusion in analyses.

GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any participant with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the participant's treatment assignment, may be sent to investigators in accordance with local regulations and/or GSK policy.

7.5. Preparation/Handling/Storage/Accountability

1. The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.
2. Only participants enrolled in the study may receive study treatment and only authorised site staff may supply or administer study treatment. All study treatments 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 authorised site staff.

3. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).
4. Further guidance and information for the final disposition of unused study treatment are provided in the SRM.
5. Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff.
6. A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

7.6. Treatment Compliance

- When the individual dose for a participant is prepared from a bulk supply, the preparation of the dose will be confirmed by a second member of the study site staff.
- When participants are dosed at the site, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study treatment and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment. Study site personnel will examine each participant's mouth to ensure that the study treatment was ingested.
- GSK2798745 and the placebo will be orally administered to participants at the site.

7.7. Concomitant Therapy

7.7.1. Permitted Medications

Paracetamol, at doses of ≤2 grams/day, is permitted for use any time during the study. Rescue medication, such as salbutamol, is also permitted after bronchoscopy or spirometry procedure, if required. Other concomitant medication may be considered on a case-by-case basis by the investigator in consultation with the Medical Monitor if required.

7.7.2. Prohibited Medications

Except for the permitted medications noted above (Section 7.7.1), participants must abstain from taking prescription or non-prescription drugs (including vitamins and dietary or herbal supplements) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) before the start of study treatment until completion of the follow-up visit, unless, in the opinion of the investigator and sponsor, the medication will not interfere with the study.

7.8. Treatment after the End of the Study

Participants will not receive any additional treatment from GSK after completion of the study because only healthy volunteers are eligible for study participation.

8. DISCONTINUATION CRITERIA

8.1. Individual Stopping Criteria

8.1.1. Liver Chemistry Stopping Criteria

Increased monitoring criteria have been designed to assure participant safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance). These protocol guidelines are in alignment with FDA premarketing clinical liver safety guidance: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.

Increased monitoring will be performed for a participant if liver chemistry stopping criteria are met.

Liver Safety Required Actions and Follow up Assessments Section can be found in Appendix 6

8.1.2. QTc Stopping Criteria

The QTc should be based on single or averaged QTc values of triplicate electrocardiograms obtained over a brief (e.g., 5-10 minute) recording period.

A participant who meets either bulleted criterion below will be withdrawn from study:

- QTcF >500 msec
- Change from baseline of QTc >60 msec

8.1.3. Symptoms of Cardiac Ischaemia and Cardiac Troponin Stopping/Increased monitoring Criteria

8.1.3.1. Asymptomatic Participant

Cardiac troponin (cTn) will be measured before and at the end of the study. If any cTn assessment is >ULN or >2 times the participant's baseline value (Day −1), the participant should be contacted immediately to assess for symptoms of cardiac ischemia.

8.1.3.2. Symptomatic Participant

If a participant experiences symptoms of cardiac ischaemia (e.g. chest pain, increased shortness of breath, and diaphoresis), cardiology consultation should be obtained immediately. The participant should be evaluated by a cardiologist and undergo any clinically appropriate testing. The participant should be followed up until symptoms are

resolved. If the event occurs after the first dose, but before the second, the participant should not receive the second dose.

8.1.4. Bronchoscopy Stopping Criteria

A participant will be withdrawn from the study if they experience symptomatic bradycardia or tachycardia requiring treatment as a consequence of bronchoscopy, BAL and LPS instillation. In addition, participants who have the following may also be withdrawn at the discretion of the investigator:

- Oxygen saturation <90% (on oxygen)
- Prolonged somnolence following administration of midazolam
- Significant hypertension/hypotension

8.2. Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance or administrative reasons.
- If the participant 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 participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.
- Refer to the SoA (Section 2.3) for data to be collected at the time of withdrawal.

8.3. Study Stopping Criteria

If there is a serious unexpected adverse event considered at least possibly related to the investigational product administration in one participant; or a severe non-serious AEs considered as, at least, possibly related to the investigational product administration in two participants, the study will be temporarily halted until full review of the events. In participants, significant changes from baseline measurements will be reviewed and participants will be followed until resolved.

8.4. Lost to Follow Up

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

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

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the Schedule of Activities (SoA).
- 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 participant should continue or discontinue study treatment.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA.
- The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 500 mL.
- Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

9.1. Bronchoscopy

Bronchoscopy will be performed in accordance with site standard operating procedures (SOPs), at 2 hours and 26 hours. These SOPs will reflect current standards of practice in hospital care and will include (but are not limited to) the following items:

- Oxygen supplementation will be given to all participants.
- Pulmonary function will be monitored before the bronchoscopy and after the bronchoscopy until FEV1 is within 20% of the pre-procedure value.
- All participants will be monitored in a recovery/holding room post bronchoscopy. Participants will be discharged only after approval is obtained from the

supervising physician, and participants will be given a 24-hour contact number. Admission facilities will be available in the event a participant is not deemed fit for discharge or if overnight observation is deemed necessary by the study physician or site-specific policies.

Following bronchoscopies, participants will be monitored for at least 4 hours. However, should the investigator have any concern for participant safety, participants may be requested to remain resident for further observation.

9.2. Segmental lung challenge

Participants will undergo segmental challenge to the lungs, via bronchoscopy, at 2 hours after the first dose of investigational medicinal product (IMP): 10 mL LPS (4 ng/kg) will be instilled into the right middle segment and 10 mL saline control will be instilled into the lingula segment of the contralateral side.

Local SOPs will be followed and further details about the segmental LPS/saline challenge are provided in the SRM.

9.3. Pharmacodynamic Assessments

BAL samples will be taken, via bronchoscopy, to measure total protein and neutrophils counts. Baseline samples will be taken immediately before the LPS and saline challenges, from a segment in the left lower lobe, and post-challenge samples will be taken at 24 h after the LPS and saline challenges, from the challenged segments.

Details of BAL sample collection, processing, storage and shipping procedures are provided in the SRM.

9.4. Pharmacokinetics

Blood and BAL samples for assay of GSK2798745 will be collected at the time points indicated in the SoA (Section 2.2). Details of sample collection, processing, storage and shipping procedures are provided in the SRM.

PK analysis will be performed under the control of Platform Technologies and Science-IVIVT (PTS-IVIVT)/GlaxoSmithKline. Plasma and BAL concentrations of GSK2798745 will be determined using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Plasma samples will also be analysed for the metabolite GSK3526876, GSK will store the remaining plasma and/or BAL for future possible metabolite investigations. Analysis of compound-related metabolites may be reported under a separate protocol.

9.5. Adverse Events

The definitions of an AE or SAE can be found in Appendix 4.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the participant to discontinue the study (see Section 8).

9.5.1. Time Period and Frequency for Collecting AE and SAE Information

- All SAEs will be collected from the start of treatment until the follow-up visit. However, any SAEs assessed as related to study participation (e.g. study treatment, protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product, will be recorded from the time a participant consents to participate in the study.
- All AEs will be collected from the start of treatment until the follow-up visit.
- Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee within 24 hours, as indicated in Appendix 4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment 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 4.

9.5.2. Method of Detecting AEs and SAEs

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

9.5.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs, will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 8.4). Further information on follow-up procedures is given in Appendix 4.

9.5.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment 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 treatment 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 a SAE or other specific safety information (eg: summary or listing of SAE) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

9.5.5. Pregnancy

- Details of all pregnancies in female partners of male participants will be collected after the start of study treatment and until the follow-up visit.
- If a pregnancy is reported, the investigator should inform GSK within 24 hours of learning of the pregnancy and should follow the procedures outlined in Section 12.5.

9.6. Treatment of Overdose

GSK does not recommend specific treatment for an overdose. The investigator (or physician in charge of the participant at the time) will use clinical judgment to treat any overdose.

In the event of an overdose, the investigator or treating physician should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the participant for AE/SAE and laboratory abnormalities until GSK2798745 can no longer be detected systemically (at least 5 days).
3. Obtain a plasma sample for PK analysis within 1 day from the date of the last dose of study treatment if requested by the Medical Monitor (determined on a case-by-case basis).
4. Document the quantity of the excess dose as well as the duration of the overdosing in the CRF.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the Medical Monitor based on the clinical evaluation of the participant.

9.7. Safety Assessments

Planned time points for all safety assessments are provided in the Schedule of Activities (SoA).

9.7.1. Physical Examinations

- A complete physical examination will include, at a minimum, measuring weight and assessments of the Skin, Cardiovascular, Respiratory, Gastrointestinal and Neurological systems. Height will be measured at screening only.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

9.7.2. Vital Signs

- Vital signs will be measured in a semi-supine position after 5 minutes' rest and will include temperature, systolic and diastolic blood pressure and heart rate
- At each time point before dosing, 3 readings of blood pressure and heart rate will be taken. The first reading should be rejected. The second and third readings should be averaged to give the measurement to be recorded in the CRF. At all other time points, single measurements will be taken.

9.7.3. Pulse Oximetry

- Pulse oximetry will be monitored during each bronchoscopy. Clinically significant results will be recorded as AEs.

9.7.4. Electrocardiograms

- Triplicate 12-lead ECGs will be obtained at each time point before dosing. At all other time-points a single 12-lead ECG will be obtained as outlined in the SoA (see Section 2) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to Section 8.1.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- ECG to be measured in a semi-supine position after 5 minutes' rest.

9.7.5. Spirometry

Spirometry assessments will be performed from screening through the final visit as indicated in the SoA (Section 2). The following parameters will be assessed:

- Forced expiratory volume in one second (FEV_1)
- Forced vital capacity (FVC)

Measurements will be made in triplicate and the best recording (i.e. the highest FEV_1 and the highest FVC) from 3 technically acceptable manoeuvres will be recorded in the CRF. To fulfill the entry criteria, FEV_1 should be ≥80% of predicted and FEV1/FVC ratio ≥70%) at screening and pre-dose.

Details on performing the spirometry assessments are provided in the SRM.

9.7.6. Clinical Safety Laboratory Assessments

- Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- 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 CRF. The laboratory reports must be filed with the source documents.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study (until the follow-up visit) should be repeated until the values return to normal or baseline or are no longer considered significantly abnormal 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 aetiology should be identified and the sponsor notified.
- If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE or AE), then the results must be recorded in the CRF.

9.7.7. Faecal Occult Blood Test (FOBT)

Based on the preclinical finding of gastric erosions, FOBT will be performed before and after dosing (See Section 2). Details on FOBT are provided in the SRM.

9.7.8. Columbia Suicide Severity Rating Scale (C-SSRS)

Based on preclinical studies that have been conducted, GSK2798745 is considered to be a central nervous system (CNS)-active drug. There has been some concern that some CNS-active drugs may be associated with an increased risk of suicidal thinking or behaviour when given to some patients with certain conditions. Although GSK2798745 has not been shown to be associated with an increased risk of suicidal thinking or behaviour, GSK considers it important to monitor for such events.

The C-SSRS is a measure of suicidal ideation and behaviour, with demonstrated predictive validity and reliability. Sections of the C-SSRS include suicidal ideation, intensity of ideation, suicidal behaviour, and actual suicide attempt(s). The C-SSRS assesses lifetime and current suicidal thoughts and behaviours across these categories based on an increasing severity of a 1- to 5-rating scale. The semi-structured questionnaire is completed by a trained and experienced neurologist, psychiatrist, or

neuropsychologist, or another trained and experienced person approved by the Sponsor, who may be the Principal Investigator or a sub-investigator for the study. See SRM for details of the scale.

The C-SSRS will be performed at screening and after dosing before discharge (See Section 2).

9.8. Exploratory biomarkers

Blood and BAL samples for exploratory biomarker analysis of endothelial barrier permeability and/or injury and inflammation will be collected at the time points indicated in the SoA (Section 2).

Samples may also be used for research to develop methods or support identification of prognostic/diagnostic biomarkers associated with clinical outcomes in ARDS and related diseases.

Details of sample collection, processing, storage and shipping procedures are provided in the SRM.

Exploratory biomarker results may be entered into the clinical study database before or after database lock and unblinding, or may be reported separately.

9.9. Genetics

Genetics are not evaluated in this study.

10. STATISTICAL CONSIDERATIONS

The primary study objective is to investigate the effect of GSK2798745 relative to placebo on BAL total protein at 24 hours after segmental LPS challenge. In this study a Bayesian framework will be used (with non-informative priors) to estimate the posterior probability of any percentage reduction in mean 24 h post-LPS BAL protein level (GSK2798745 relative to placebo). It is anticipated that BAL total protein will be $\log_e$ transformed to improve normality before model fitting. Following back-transformation treatment comparisons will be expressed as percentage changes.

10.1. Sample Size Determination

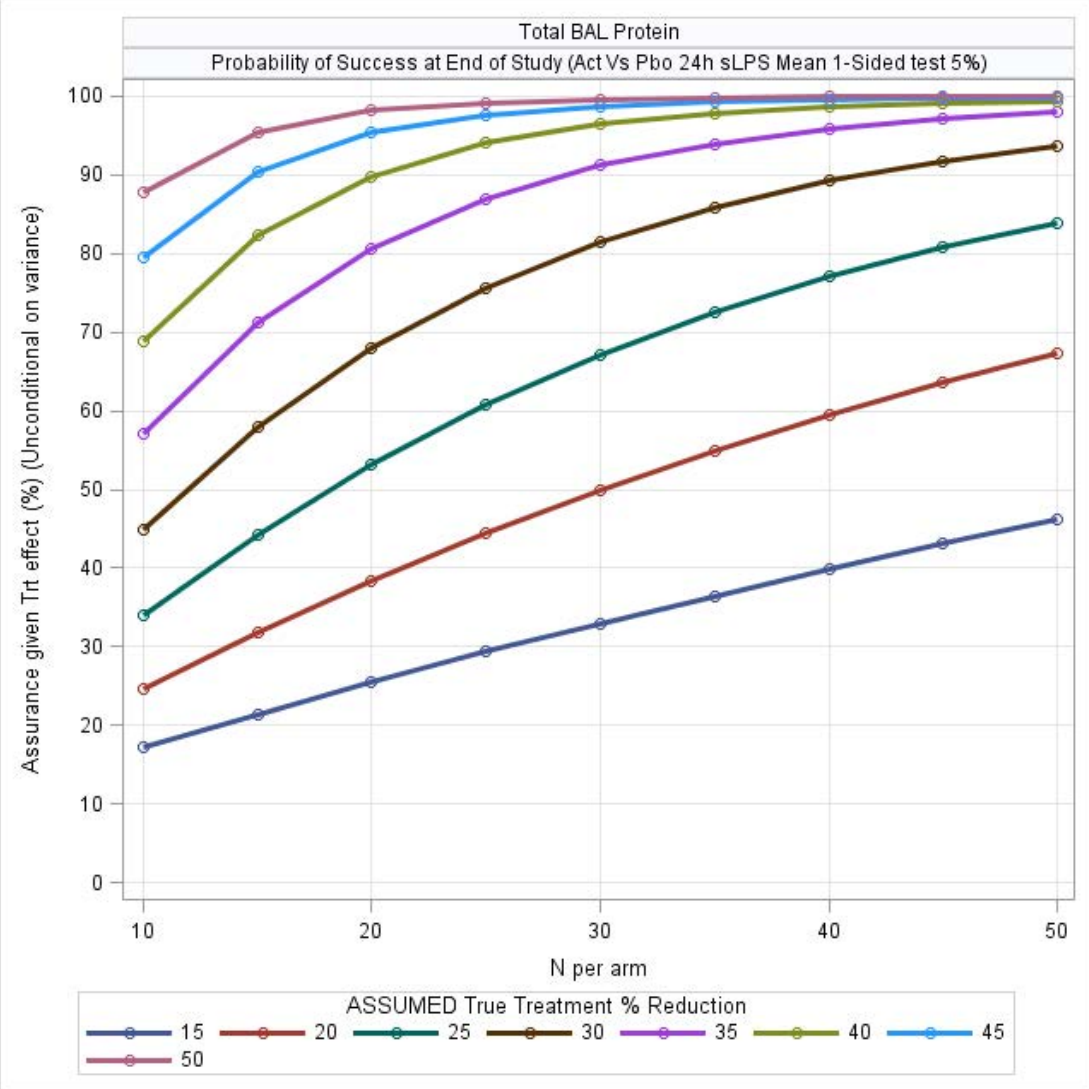
The sample size of 30 participants per arm (1:1 allocation) is based on an upper limit of feasibility. Figure 4 illustrates the probability of study success for a range of sample sizes and assumed true treatment effects unconditional on variability.

Assuming a true treatment effect of a 30% reduction in mean BAL total protein in participants receiving GSK2798745 relative to placebo at 24 h after segmental LPS challenge to the lung, the probability of study success for the study is 82% (this excludes the possibility of success from a review of secondary data), unconditional on the variability of BAL total protein in segmental LPS challenged lungs. Outright end of study success is defined as at least a 95% posterior probability that the percentage reduction in

the mean 24 h post-LPS BAL total protein level (GSK2798745 relative to placebo) exceeds zero.

Despite limited historical information for guidance on the true treatment effect for GSK2798745, 30% is believed to be a rational estimate. However, probability of study success is sensitive to the true treatment effect – for example, the probability of study success reduces to 50% if the true treatment effect is 20% (GSK2798745 relative to placebo) with a sample size of 30 per arm but increases to 96% if the true treatment effect is 40%.

Figure 4 Probability of Study Success (Unconditional on Variance) for a Range of Treatment Profiles and Sample Sizes



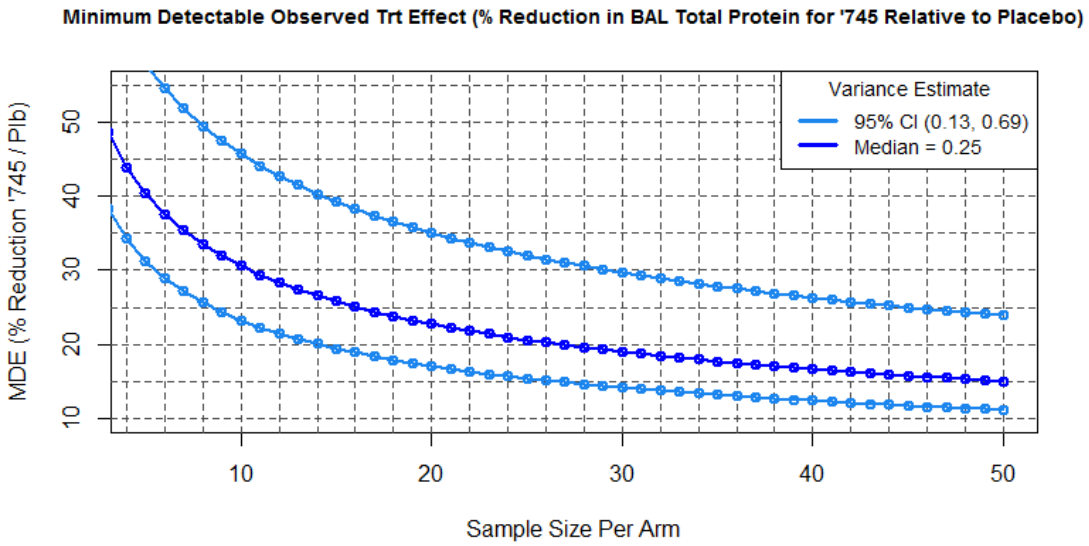
Estimates for the mean and variance of BAL total protein in segmental LPS challenged lungs were obtained from data in an observational study assessing the variability of the inflammatory response to segmental LPS challenge by the Fraunhofer Institute of

Toxicology and Experimental Medicine, Germany [Holz, 2015] using a random effects model with total protein levels from 11 participants measured at baseline and 24 h over 2 study periods. The data were assumed to be representative of future segmental-LPS challenged lungs to be observed in this study.

The estimate of the mean BAL protein in 24 h post-LPS challenged lungs on the $\log_e$ scale is 5.47 (95% CI 5.23, 5.72). The variance estimate on the $\log_e$ scale is 0.25 (95% CI 0.13, 0.69). A posterior distribution was formed for the standard deviation and for each possible value of standard deviation from this distribution the power of the study was calculated and multiplied by its probability. The resulting values were all summed to give probability of study success unconditional on variance, which is a more accurate representation of the probability of study success rather than assuming a single variance estimate with no uncertainty.

Figure 5 shows the minimum observed treatment effect in the study that would achieve success (expressed as a percentage reduction in BAL total protein for GSK2798745 relative to placebo) for a number of sample sizes. Assuming the variance in the study is the median obtained from the historical data, the minimum treatment effect to trigger success is 19% for a sample size of 30 participants per arm. As the sample size increases, the minimum observed treatment effect that would trigger success decreases due to the decreasing standard error (greater confidence in the observed treatment effect). This figure gives rationale for including the data review region since it is probable that moderate observed treatment effects will not achieve success with a sample size of 30 per arm.

Figure 5 Minimum Detectable Effect for Range of Sample Sizes



10.2. Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All participants who sign the ICF.
Evaluable	All participants for whom results of the primary analysis can be determined.
Safety	All randomized participants who take at least 1 dose of study treatment. Participants will be analysed according to the treatment they actually received.

Other populations, such as Per-Protocol population, may be defined in the RAP.

10.3. Statistical Analyses

10.3.1. Efficacy Analyses

Endpoint	Statistical Analysis Methods
Primary	The mean percentage reduction in BAL total protein (µg/mL) in segmental LPS challenged lungs, at 24 h post-challenge, for GSK2798745 relative to placebo, will be assessed using an analysis of covariance (ANCOVA) model (fitted in a Bayesian framework with non-informative priors for model parameters) testing that there is at least 95% posterior probability that the percentage reduction in 24 hour post-challenge mean BAL total protein (GSK2798745 relative to placebo) exceeds zero , adjusting for baseline BAL total protein. A sensitivity analysis will include 24 h BAL total protein in the saline segment (saline challenge will be administered to the contralateral lobe) as a covariate. Additional factors may be included as covariates if deemed appropriate.
Secondary	See Section 10.3.2 and Section 10.3.3
Exploratory	Will be described in the reporting and analysis plan.

10.3.2. Safety Analyses

All safety analyses will be performed on the Safety Population.

Endpoint	Statistical Analysis Methods
Secondary	For the safety data, no formal hypotheses will be tested. Safety data such as adverse events, electrocardiogram (ECG), physical examinations, vital signs, spirometry, FEV1 and FVC will be displayed in the form of listings, frequencies, summary statistics and graphs. Interpretation will be aided by clinical expertise. Full details, including example outputs, will be documented in the RAP.

10.3.3. Other Analyses

PK, pharmacodynamic, and biomarker exploratory analyses will be described in the reporting and analysis plan. The population PK analysis and pharmacodynamic analyses will be presented separately from the main clinical study report (CSR).

10.3.4. Interim Analyses

An interim analysis will occur once approximately 10 participants per arm have completed the treatment period to assess if the study should stop for futility or continue. Subsequently, if the decision at the first interim is to continue, an interim analysis will occur once approximately 20 participants per arm have completed the treatment period to assess whether the study should continue or stop for success or futility. If at this point the study continues, the maximum 30 participants per arm will be recruited. The study then either meets the success criteria, undergoes analysis of secondary data (review) or is declared a failure. Enrolment into the study will continue whilst interim analyses are taking place.

This interim framework is being used to allow the possibility to reduce the number of participants enrolled into the study, either in the case of an overwhelming observed treatment effect or no/poor treatment effect. The interim stopping criteria for success is based on the probability of the study going on to meet the success criteria (predictive probability that the posterior probability is at least 0.95) whilst the stopping criteria for futility is based on the probability of the study going on to declare success or review (predictive probability that the posterior probability is at least 0.75, see Table 2).

Table 2 Interim Decision Criteria

Interim Analysis	Success	Futility
10 subjects per arm	N/A – cannot stop for success at the first interim.	Predictive Prob (PP $\geq$ 0.75) $\leq$ 0.075
20 subjects per arm	Predictive Prob (PP $\geq$ 0.95) $\geq$ 0.85	Predictive Prob (PP $\geq$ 0.75) $\leq$ 0.15

At each interim, the probability of the study going on to meet these criteria will be calculated. This probability value may be used to assist in determining whether there is sufficient rationale to continue/discontinue the study. If the probability of study success is sufficiently high, then the study may stop for success. Conversely, if the probability of study success or review is sufficiently low then the study may stop for futility. The probability thresholds for success and futility have been calibrated to balance the risk of declaring success despite a placebo-like drug (type 1 error) and declaring failure despite a positive true underlying treatment effect for GSK2798745 relative to placebo (type 2 error).

The predictive probability at each interim is calculated using predictive inference methods. The observed data at the interim is used to obtain estimates of the mean and standard error of the treatment effect for GSK2798745 relative to placebo. These estimates are then used to predict the treatment effect for participants in the remainder of the study using the predictive interim distribution. These two parts of data (observed and predicted) are combined to create a complete study. The end of study success criteria is then applied to this complete data set made up of observed and predicted participants. This process is performed 10,000 times to account for the uncertainty in the estimated treatment effect at the interim – i.e. the same observed data is combined with thousands of permutations for the remainder of the study, and each time the end of study success criteria is applied. The proportion of complete studies that meet the pre-defined success criteria gives the predicted probability of success if the study was to continue at the interim.

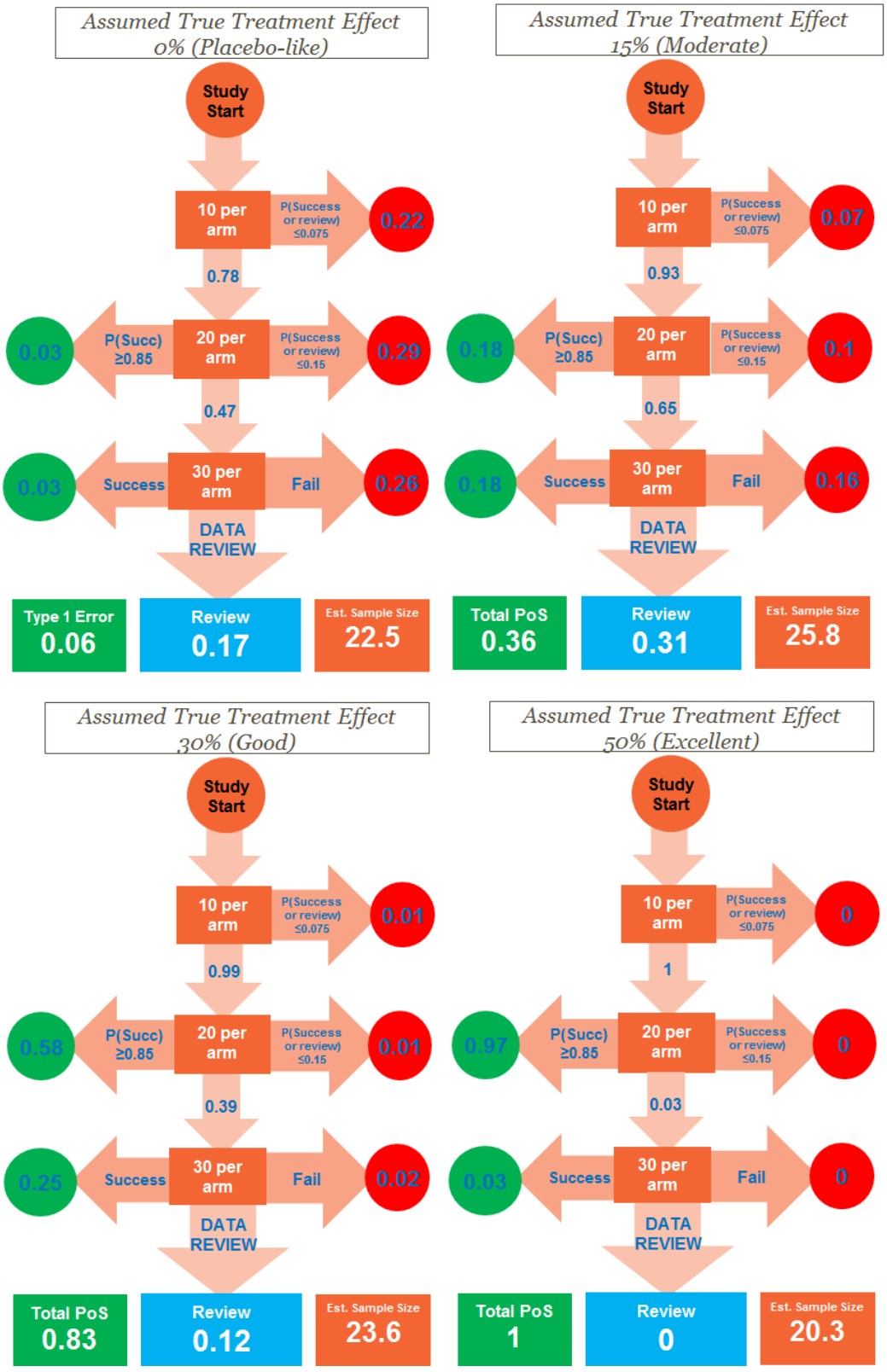
To inform the study design, a range of scenarios were reviewed to assess the operating characteristics when changing the total sample size, interim decision rules, interim analysis time points and assumed true treatment effects. Characteristics such as the overall probability of study success/failure, probability of success/failure at each interim, and the expected number of participants to be recruited were reviewed under a variety of design options.

Figure 6 show the operating characteristics of the chosen study design under true percentage reductions in total BAL protein for GSK2798745 relative to placebo of 0%, 15%, 30% and 50%, respectively, unconditional on variability. The schematic demonstrates all possible decision pathways the study may take. To obtain the operating characteristics of a given study design, 10,000 studies were simulated with each one taking a particular pathway and finishing in one of the pockets on the left or right of the diagram, or indeterminate (additional data review). The overall proportion of simulated studies reaching a particular conclusion gives the probability of a single study reaching that conclusion.

A stricter posterior probability futility threshold is implemented at the first interim to reduce the probability of incorrectly declaring futility. Furthermore, a minimum of 20 participants per arm must be recruited before declaring success. These rules are shown within the left (success) and right (futile) arrows. Under this design, assuming a true treatment effect of 30%, the overall probability of study success is approximately 83%, unconditional on variability. In addition, the probability of requiring a maximum of 20 participants per arm is approximately 61% (under the same assumption of true treatment effect of 30%). In the case of a placebo-like drug the probability of incorrectly declaring success is approximately 6% (akin to type 1 error rate). The blue boxes at the bottom of the charts show the probability that a study goes to data review.

The Reporting and Analysis Plan will describe the planned interim analyses in greater detail.

Figure 6 Interim Analysis Framework for Range of True Treatment Effects



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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase
AUC	Area under concentration-time curve
$AUC_{0–24}$	Area under the curve during 24 hours
BAL	Bronchoalveolar Lavage
BMI	Body mass index
BUN	Blood urea nitrogen
Ca^{2+}	Calcium
C_{max}	Maximum observed plasma concentration
CMT2C	Charcot-Marie-Tooth Disease Type 2C
CNS	Central Nervous System
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disorder
CPK	Creatinine phosphokinase
CRF	Case Report Form
CV	Cardiovascular
CSSRS	Columbia Suicide Severity Rating Scale
ECG	Electrocardiogram
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
EU	Endotoxin Unit
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in One Second
FVC	Forced vital capacity
FOBT	Faecal Occult Blood Test
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilance
GI	Gastrointestinal
GMP	Good Manufacturing Practice
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen
HCl	Hydrochloric acid
HDPE	High-density polyethylene
HIV	Human Immunodeficiency Virus
HMVEC	Human Dermal Microvascular Endothelial Cells
HPLC	High performance liquid chromatography
IB	Investigator's Brochure

IC_{50}	50% maximal inhibitory concentration
ICF	Informed consent form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IMP	Investigational medicinal product
IP	Investigational Product
i.t.	Intratracheal
IRB	Institutional Review Board
IWRS	Interactive Web Response System
Kg	Kilogram
KO	Knockout
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
MCH	Mean corpuscular haemogloin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligrams
mL	Milliliter
MSDS	Material Safety Data Sheet
Msec	Milliseconds
mSV	MilliSievert
Ng	Nanogram
NOAEL	No observed adverse effect level
PK	Pharmacokinetic
POP PK	Population Pharmacokinetic
PTS-DMPK	Platform Technologies and Science-Drug Metabolism and Pharmacokinetics
QC	Quality control
QTcF	QT duration corrected for heart rate by Fridericia's formula
RAP	Reporting and Analysis Plan
RBC	Red blood cells
RNA	Ribonucleic acid
SAE	Serious adverse event(s)
SOA	Schedule of Activities
SRM	Study Reference Manual
SUSAR	Suspected Unexpected Serious Adverse Reactions
TB	Tuberculosis
TLR 4	Toll-like receptor 4
Tmax	Time to reach maximum plasma concentration
TRALI	Transfusion Related Acute Lung Injury
TRPV4	Transient receptor potential vanilloid 4
ULN	Upper Limit of Normal

WBC	White blood cells
WOCBP	Women of Child Bearing Potential

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
None

12.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed in Table 3 will be performed by the local laboratory.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 3 Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Haematology	Platelet Count RBC Count Haemoglobin Haematocrit	RBC Indices: MCV MCH %Reticulocytes	WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[1]	BUN CRP	Potassium	Aspartate Aminotransferase (AST)/ Serum Glutamic-Oxaloacetic Transaminase (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	Alanine Aminotransferase (ALT)/ Serum Glutamic-Pyruvic Transaminase (SGPT)	Total Protein
	Glucose (fasting not required)	Calcium	Alkaline phosphatase	CPK
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Tests	• Cardiac troponin (cTn) • Coagulation profile (Quick/INR, PTT and thrombocytes) • Faecal Occult Blood Test (FOBT)			
Other Screening Tests	• Follicle-stimulating hormone and estradiol (as needed in women of non-childbearing potential only) • Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and			

Laboratory Assessments	Parameters
	hepatitis C virus antibody) • Alcohol, cotinine and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines).

NOTES :

1. Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 8.1.1 and Appendix 6 All events of ALT ≥3 × upper limit of normal (ULN) and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).

12.3. Appendix 3: Study Governance Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
  - 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 ICH Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Substantial amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- 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/EC
  - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

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.

Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative 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 participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.
- Participants who are rescreened are required to sign a new ICF.

Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant 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 participant.
- The participant 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.

Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Dissemination of Clinical Study Data

- Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.
- GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study participants, as appropriate.
- GSK will provide the investigator with the randomization codes for their site only after completion of the full statistical analysis.
- The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- 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 CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants 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 ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final Clinical Study Report (CSR)/ equivalent summary 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

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.
- Data reported on the CRF 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 the source document agreement (to be signed by the investigator (or delegate) at the site).

Study and Site Closure

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

The investigator may initiate study-site 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 site 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 participants by the investigator
- Discontinuation of further study treatment development

12.4. Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition

- An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of a study treatment, whether or not considered related to the study treatment.
- 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 a study treatment.

Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study treatment 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 study treatment 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.
-

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 participant'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 participant's condition.
- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that

leads to the procedure is the AE.

- 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).

A 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 participant 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 participant 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 AE. 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, diarrhoea, 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 participant 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 AE and SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to GSK.
- 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.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficiently 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 an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- 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 study treatment administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, 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 GSK. 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** GSK**.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK 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 participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool
• The primary mechanism for reporting SAE to GSK will be the electronic data collection tool. • If the electronic system is unavailable for more than 24 hours, then the site will use the paper SAE data collection tool (see next section). • The site will enter the SAE data into the electronic system as soon as it becomes available. • The investigator or medically-qualified sub-investigator must show evidence within the eCRF (e.g., check review box, signature, etc.) of review and verification of the relationship of each SAE to IP/study participation (causality) within 72 hours of SAE entry into the eCRF. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the medical monitor (who is also the SAE coordinator) by telephone. • Contacts for SAE reporting can be found in SRM.

SAE Reporting to GSK via Paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the medical monitor (who is also the SAE coordinator)**.** • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. • 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 SRM.

12.5. Appendix 5: Contraceptive Guidance and Collection of Pregnancy Information

Definitions

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with ONE of the following:

- Documented hysterectomy
- Documented bilateral salpingectomy
- Documented bilateral oophorectomy

Note: Documentation can come from the site personnel's: review of participant's medical records, medical examination, or medical history interview.

3. Postmenopausal female

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
- Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance

Male participants

- Male participants with female partners of child-bearing potential are eligible to participate if they agree to ONE of the following during the protocol-defined time frame in Section 6.1:
- 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 an additional method of contraception with a failure rate of <1% per year as described in Table 4 when having penile-vaginal intercourse with a woman of childbearing potential

- Men 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 protocol-defined time frame.
- In addition, male participants must refrain from donating sperm from the time of first dose of study treatment until 2 weeks after last dose of study treatment.

Table 4 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 • oral • intravaginal • transdermal
Progestogen-only hormonal contraception associated with inhibition of ovulation • injectable
Highly Effective Methods That Are User Independent
• Implantable progestogen-only hormonal contraception associated with inhibition of ovulation • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • bilateral tubal occlusion
Vasectomized partner *(A vasectomized partner is a highly effective contraception 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 drug. 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 participant.)*

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 participants in clinical studies.

Collection of Pregnancy Information

Male participants with partners who become pregnant

- Investigator will attempt to collect pregnancy information on any male participant's female partner of a male study participant who becomes pregnant while participating in this study. This applies only to participants who receive study treatment.
- 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 GSK within 24 hours of learning of the partner's pregnancy.
- 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 GSK
- Generally, 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 foetal status (presence or absence of anomalies) or indication for procedure.

12.6. Appendix 6: Liver Safety: Required Actions and Follow-up Assessments

Phase I liver chemistry increased monitoring criteria and required follow up assessments

Liver Chemistry Increased Monitoring Criteria	
ALT-absolute	ALT≥3xULN If ALT≥3xULN **AND bilirubin**[1,2] ≥ 2xULN (>35% direct bilirubin) or **INR** >1.5, Report as an SAE. See additional Actions and Follow Up Assessments listed below

Required Actions and Follow up Assessments	
Actions	**Follow Up Assessments**
• Report the event to GSK **within 24 hours** • Complete the liver event CRF, and complete an SAE data collection tool if the event also meets the criteria for an SAE[2] • Perform liver event follow up assessments • Monitor the participant until liver chemistries resolve, stabilise, or return to within baseline (see **MONITORING** below) **MONITORING:** **If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24 hrs** • Monitor participants twice weekly until liver chemistries resolve, stabilise or return to within baseline • A specialist or haepatology consultation is recommended **If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24-**	• Viral hepatitis serology[3] • Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend • Obtain blood sample for pharmacokinetic (PK) analysis, obtained within 48hrs of last dose[4] • Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). • Fractionate bilirubin, if total bilirubin≥2xULN • Obtain complete blood count with differential to assess eosinophilia • Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE report form • Record use of concomitant medications on the concomitant medications report form including acetaminophen, herbal remedies, other over the counter medications. • Record alcohol use on the liver event alcohol intake case report form **If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5:**

Liver Chemistry Increased Monitoring Criteria	
72 hrs • Monitor participants weekly until liver chemistries resolve, stabilize or return to within baseline	• Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG) or gamma globulins. • Serum acetaminophen adduct high performance liquid chromatography (HPLC) assay (quantifies potential acetaminophen contribution to liver injury in participants with definite or likely acetaminophen use in the preceding week [James, 2009]. • Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease; complete Liver Imaging and/or Liver Biopsy CRF forms.

1. Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study treatment for that participant if ALT ≥ 3xULN and bilirubin ≥ 2xULN. Additionally, if serum bilirubin fractionation testing is unavailable, record presence of detectable urinary bilirubin on dipstick, indicating direct bilirubin elevations and suggesting liver injury.
2. All events of ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin) or ALT ≥ 3xULN and INR>1.5, if INR measured, which may indicate severe liver injury (possible 'Hy's Law'), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis); INR measurement is not required and the threshold value stated will not apply to participants receiving anticoagulants
3. Includes: 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