Division: Worldwide Development
Information Type: Protocol Amendment

Title:	A Placebo Controlled, Double-blind, Multi-centre, Single Dose, Parallel Group, Randomised Clinical Trial of GSK2862277 in Patients undergoing Oesophagectomy Surgery

Compound Number: GSK2862277

Development Phase: IIA

Effective Date: 23-AUG-2016

Protocol Amendment Number:05

SYNOPSIS: GSK2862277 is a fully-human domain antibody (dAb) that potently and selectively binds to TNF Receptor 1 (TNFR1) and inhibits signalling. The aims of this placebo-controlled study are to evaluate the, safety, tolerability, pharmacokinetics and pharmacodynamics of GSK2862277 in patients scheduled to undergo surgical resection of oesophageal cancer. There will be two treatment groups comprising one active and one placebo arm with approximately 40 patients per arm.

The primary objective of the study will be to evaluate the effect of GSK2862277 on the pulmonary vascular permeability in oesophagectomy patients following surgery. A number of key secondary endpoints are also included in the study to evaluate the effect of GSK2862277 on tissue dysfunction in the lung that may also occur after surgery. A single nebulised dose will be used in order to achieve optimal exposure in the lung, the initial site of injury.

Subject: Infection, autoantibodies, TNFR1; dAb; Oesophagectomy; Acute Respiratory Distress Syndrome; Acute Lung Injury.

Author (s): PPD (Fibrosis DPU); PPD (VEO); PPD (Clin Immunology); PPD (CPSSO); PPD (Discovery Medicine); PPD (Clin Development) PPD (Clinical Statistics); PPD (BPS); PPD (CPMS); PPD (Biopharm Research)

Revision Chronology

GlaxoSmithKline Document Number	**Date**	**Version**
2014N197251_00	2014-MAY-23	Original
2014N197251_01	2014-JUN-02	Amendment No. 1
The amendment was made to correct an error in the study title and to revise the limits on exclusion criteria for alcohol consumption.		
2014N197251_02	2014-JUL-23	Amendment No. 2
The amendment was made to reduce the upper threshold for AST & ALT liver enzyme values for inclusion in the study, as per instruction from the UK competent authority (Medicines and Healthcare Regulatory Authority [MHRA]). A change to the QTc inclusion criteria was also made to correct an error not previously identified.		
2014N197251_03	2014-NOV-16	Amendment No. 3
The amendment was made to account for the possibility of inoperable cases during the initial stages of oesophagectomy surgery, where subjects would have to be withdrawn; additional time window for dosing; amending randomisation window to up-to 72 hours prior to day of surgery. Blood sample aliquots for a translational sub-study clarified, materials and methods of dose preparation added as an appendix. Additional minor/typographical updates and typographical changes have also been made.		
2014N197251_04	2015-FEB-16	Amendment No. 4
The amendment was made to remove the mandatory 1 hour post-dose ECG due to concerns over logistics prior to surgery (on the condition that alternative cardiac monitoring is in place). Also, a 24 hour window has been added for the pre-dose assessments and a typographical error in exclusion criteria #6 has been amended. A PK sample has also been included if a liver event occurs as this had been omitted in error. The opportunity has also been taken to make some other minor amendments.		
2014N197251_05	2016-AUG-23	Amendment No. 5
The amendment was made to detail the inclusion of an external expert to the safety review team. The opportunity has also been taken to make some other minor amendments.		

SPONSOR SIGNATORY

PPD

23 August 2016

Director, Discovery Medicine

Dr Richard Marshall Date

Head, Fibrosis DPU

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Regulatory Agency Identifying Number(s): 2014-000643-33

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol number TFR116341

I confirm agreement to conduct the study in compliance with the protocol, as amended by this protocol amendment.

I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described study.

I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

Investigator Name:	
Investigator Address:	
Investigator Phone Number:	

Investigator Signature	Date

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LIST OF ABBREVIATIONS

µg	Microgram
AE	Adverse Event
ALI	Acute Lung Injury
ALT	Alanine aminotransferase (SGPT)
Anti-HA	Anti-hemagglutinin
ARDS	Acute respiratory distress syndrome
AST	Aspartate aminotransferase (SGOT)
AUC	Area under concentration-time curve
AUC(0-t)	Area under the concentration-time curve from time zero (pre-dose) to last time of quantifiable concentration within a subject across all treatments
BAL	Broncho-alveolar lavage
BP	Blood pressure
BUN	Blood urea nitrogen
CI	Confidence Interval
Cmax	Maximum observed concentration
CO_2	Carbon dioxide
CPK	Creatine phosphokinase
CPMS	Clinical Pharmacokinetics Modelling & Simulation
CPSSO	Clinical Pharmacology Science and Study Operations
CRF	Case Report Form
CRS	Cytokine Release Syndrome
CV	Coefficient of variance
dAb	Domain antibody
DNA	Deoxyribonucleic acid
DPU	Discovery Performance Unit
DRE	Disease-related event
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELF	Epithelial Lining Fluid
EVLW	Extra Vascular Lung Water
EVLWI	Extra Vascular Lung Water Index
FDA	Food and Drug Administration
FEV1	Forced expiratory volume in 1 second
FiO_2	Oxygen Requirement
FSH	Follicle Stimulating Hormone
FTIH	First time in humans
GCP	Good Clinical Practice
GCS	Glasgow Coma Score
GCSP	Global Clinical Safety and Pharmacovigilence
GEDV	Global end-diastolic volume
GGT	Gamma glutamyltransferase
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen

HIV	Human Immunodeficiency Virus
HR	Heart rate
HRT	Hormone replacement therapy
IA	Interim analysis
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	Intensive Care Unit
IDSL	Integrated Data Standards Library
IEC	Independent Ethics Committee
IH	Inhalation
IL	Interleukin
IM	Intramuscular
IRB	Institutional Review Board
ITU	Intensive therapy unit
IV	Intravenous
IVRS	Interactive voice response system
Kg	Kilogram
L	Litre
LPS	Lipopolysaccharide
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligrams
mL	Milliliter
Msec	Milliseconds
NF-κB	Nuclear factor κB
OI	Oxygenation Index
OLV	One Lung Ventilation
PaO_2	Partial Pressure of Oxygen in arterial blood
Paw	Mean airway pressure
PD	Pharmacodynamic
PEEP	Positive and expiratory pressure
PGx	Pharmacogenetics
PiCCO	Pulse Contour Cardiac Output
PK	Pharmacokinetic
POD	Post operative day
PTS-DMPK	Platform Technologies and Science - Drug Metabolism and Pharmacokinetics
PVPI	Pulmonary vascular permeability index
QTcB	QT duration corrected for heart rate by Bazett's formula
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)

SD	Standard deviation
SDS	Safety Data Sheet
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SOFA	Sequential Organ Failure Assessment
SRT	Safety review team
SPM	Study Procedures Manual
sRAGE	Soluble receptor for advanced glycation end-products
SVR	Systemic vascular resistance
SVV	Stroke volume variation
TA	Technical Agreement
TB	Tuberculosis
tmax	Time of occurrence of Cmax
TNF	Tumor necrosis factor
TNFR1	Tumor necrosis factor receptor 1
TNFR2	Tumor necrosis factor receptor 2
Tregs	Regulatory T cells
UK	United Kingdom
US	United States
VILI	Ventilator Induced Lung Injury
vWF	Von Willebrand Factor
WBC	White blood cells

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1. INTRODUCTION

GSK2862277 is a fully-human domain antibody (dAb) that potently and selectively binds to TNF Receptor 1 (TNFR1) and inhibits signalling. GSK2862277 is being investigated for potential utility in prevention and treatment of Acute Respiratory Distress Syndrome (ARDS) and other acute inflammatory conditions by either inhalation (IH) or intravenous (IV) routes.

Current experience with selective TNFR1-targetting therapies in humans is limited to GSK-sponsored Phase I trials in healthy subjects with GSK2862277 and a predecessor molecule known as GSK1995057. Therapeutic antibodies that bind to TNF-α are well established in the clinical management of certain chronic inflammatory conditions and have also been evaluated in patients with sepsis. Since sepsis is a common risk factor for development of ARDS, experience from these trials is informative to the development of TNFR1 antagonists in ARDS patients. The results of TNF-α antibody trials have been highly variable with many failing to demonstrate a significant survival benefit [Abraham, 1997; Abraham, 1998; Cohen, 1996; Bernard, 2014]. In one study (with Etanercept) a dose related increase in mortality was observed [Fisher, 1996], whereas others have demonstrated a reduction in 28-day all-cause mortality and organ dysfunction [Panacek, 2004]. Recently, a meta-analysis of fifteen trials evaluating anti-TNF-α antibodies in sepsis patients [Qiu, 2013] concluded that as a class, anti-TNF-α agents produce a significant survival benefit in patients with sepsis. We anticipate that by selectively antagonising TNFR1, GSK2862277 will mitigate the detrimental effects of TNF-α whilst sparing or potentiating the beneficial effects of TNF-α signalling through the other cognate receptor, TNFR2. Therefore, in contrast to existing pan-TNF signalling inhibitors, selective TNFR1 inhibition with GSK2862277 may deliver improved safety and efficacy in acute inflammatory conditions such as ARDS.

Multiple experimental approaches have revealed that TNFR1 mediates most of the well characterised biological activities of TNF-α. The binding of TNF-α to TNFR1 triggers the activation of important transcription factors, NF-κB and AP-1 and caspase proteins that mediate inflammation and cellular apoptosis, respectively [Mori, 2006].

Signalling of TNF-α through TNFR2 is less well characterised and conflicting observations in different tissues hamper a clear understanding of this pathway. More recently it is has been suggested that TNFR2 signalling is important in regulating the apoptotic activity of TNF-α and in the propagation of survival/proliferation signals in neuronal cells [Marchetti, 2004], epithelial cells [Al-Lamki, 2005], and other mesenchymal cells [Bluml, 2010]. TNFR2 may also be important in the control of inflammation since TNFR2 is expressed on a highly suppressive subset of regulatory T-cells (Tregs) [Chen, 2008] and adoptive transfer of TNFR2-deficient CD4+ T-cells results in more severe inflammation and tissue destruction in the CD4+ T-cell transfer model of colitis in mice [Dayer, 2009].

TNFR1 deficient mice are protected against ventilator-induced lung injury (VILI) and acid-induced lung injury due to the absence of TNFR1-mediated death signals that trigger alveolar epithelial dysfunction [Patel, 2013], and inflammatory signals that lead to

endothelial injury and neutrophil migration [Bertok, 2011]. Interestingly, mice genetically deficient in TNFR2 succumb to VILI-induced ARDS and death more quickly than wild-type mice, or mice deficient in both receptors

When administered directly to the lungs of mice challenged with acid instillation or ventilation with large tidal volumes, mouse TNFR1-targeting dAbs attenuate the subsequent development of pulmonary oedema, arterial hypoxemia, and inflammation [Bertok, 2012; Wakabayashi, 2013]. Human TNFR1-targetting dAbs attenuate activation of human endothelial cells and inhibit migration and priming of human neutrophils *in vitro*. When administered directly to the lungs of *Cynomolgus* monkeys and healthy human patients via nebulisation, TNFR1-targetting dAbs attenuate the *in vivo* pulmonary inflammation, epithelial injury, and endothelial injury associated with pulmonary endotoxin (LPS) challenge [Investigator Brochure GlaxoSmithKline Document Number 2012N147005_02 ; Proudfoot, 2014].

ARDS is a serious condition associated with significant morbidity, mortality and healthcare utilisation, for which no effective pharmacologic treatment is available. Selectively antagonising TNFR1 with GSK2862277 and sparing or potentiating TNFR2 signalling may attenuate the inflammation and tissue injury associated with ARDS and encourage repair and resolution. If acute treatment with GSK2862277 is well tolerated and improves pulmonary physiology, it may lead to improved outcomes and survival in ARDS patients.

An initial Phase I study in healthy volunteers has been completed (TFR116343). GSK2862277 was administered as single intravenous doses from 0.002 mg/kg to 2.0 mg/kg or single inhaled doses of 26.0 mg to healthy subjects. In addition, repeat dosing with 2.0 mg/kg IV and 26.0 mg IH in healthy subjects was also performed in this study. One subject, who was later shown to have high levels of pre-existing anti-drug antibodies, developed symptoms consistent with cytokine release syndrome (CRS), which was self-limiting and mild in severity. No SAEs or deaths were reported during the study and there were no clinically significant changes in ECGs or vital signs. The terminal half-life was approximately 5 hours in serum and 9 hours following IV dosing; as expected for a domain antibody. Engagement of pharmacology in humans was demonstrated using an *ex vivo* whole blood TNF-α stimulation assay that measured the inhibition of induced IL-8 release. GSK2862277 was shown to be potent in this assay with an IC_{90} of 38,000 pg/mL. Further details can be found in the Investigator Brochure (GlaxoSmithKline Document Number 2012N147005_02).

Overall GSK2862277 was considered to be well-tolerated, with an acceptable PK/PD profile to merit further investigation in patients.

1.1. Study Rationale

Respiratory complications remain a relatively frequent and significant complication following oesophagectomy surgery and are associated with a significant risk of mortality. The reported incidence of ARDS after oesophagectomy varies substantially by region with a range of 13-33% in studies that used the consensus definition for ARDS [Schilling, 1998; Schilling, 2001; Tandon, 2001; Paul, 2011]. Analysis of large case

series and recent trials in patients undergoing oesophagectomy in the UK indicate that between 20-25% of patients develop ARDS post-operatively [Park, 2009; Perkins, 2014].

The aetiological heterogeneity and physiological flux often encountered in cohorts of ARDS patients makes the conduct of small experimental medicine studies challenging. In contrast, patients undergoing oesophagectomy represent a single aetiology where injury is more controlled with respect to timing and severity. Numerous physiological and biochemical assessments of lung injury can be made during the immediate post operative period, some of which are associated with clinical outcome in this cohort [Park, 2009].

The most common surgical approach in patients undergoing resection of oesophageal cancer is to perform a transthoracic oesophagectomy. Access to the oesophagus is achieved through the deflation of one of the lungs, whilst gas exchange is maintained in the contralateral lung using one-lung ventilation (OLV). Lung deflation may illicit ischemic injury and/or reperfusion injury upon re-inflation, whilst the high-inspired oxygen concentrations and inflation pressures associated with OLV risk the development of VILI [Michelet, 2006]. Lung injury associated with mechanical ventilation is an established concept involving direct injury to the alveolar epithelium and pulmonary vascular endothelium leading to inflammation, permeability dysfunction and pulmonary oedema [Slutsky, 2013]. As a result of these insults, systemic inflammation, increased pulmonary vascular permeability, and hypoxaemia can develop in the period immediately following OLV [Tsai, 2009; D'Journo, 2010; Perkins, 2014]. High levels of systemic inflammation and severe hypoxaemia immediately post-operatively predict progression to severe respiratory complications [D'Journo, 2010; Park, 2009] following oesophagectomy.

This study will be the first investigation of GSK2862277 in patients and is primarily designed to investigate the impact of pre-operative administration of GSK2862277 on biological and physiological markers of lung injury in patients undergoing surgical resection of oesophageal cancer. A single nebulised dose of GSK2862277 will be administered prior to surgery in order to achieve optimal exposure at the site of injury following OLV and lung deflation. This study will test the hypothesis that inhibition of TNFR1 signalling during surgery will reduce tissue damage and inflammation in the lungs and reduce the severity of both systemic inflammation and post-operative complications. It is anticipated that selective blockade of TNFR1 using GSK2862277 will attenuate the inflammation and alveolar epithelial and endothelial tissue injury observed post-surgery in these patients, whilst encouraging normal resolution and repair processes.

This study will also investigate the safety and tolerability of inhaled GSK2862277 in this patient population, and provides for exploration of pharmacokinetics, pharmacodynamics and immunogenicity. The primary endpoint for this study is the change in pulmonary vascular permeability index (PVPI) from pre-surgical levels to the end of surgery. This physiological endpoint will be measured by single indicator transpulmonary thermodilution, using the PiCCO haemodynamic analyser. This technique allows an indirect determination of extra vascular lung water (EVLW; mL/kg) that can either be indexed to predicted body weight (EVLW Index (EVLWI)), or, for a more specific measure of vascular permeability, to pulmonary blood volume (via PVPI).

Determination of EVLW by single indicator thermodilution correlates closely to actual lung water levels in humans and large animal models, is an independent predictor of mortality in ARDS and sepsis patients, and has previously been used to assess pulmonary oedema in oesophagectomy and other thoracic surgery patients [Brown, 2009; Perkins, 2014]. Recent clinical investigations into the diagnostic utility of both EVLWI and PVPI in patients with ARDS-related pulmonary oedema and cardiogenic pulmonary oedema suggests PVPI may be a useful means with which to discriminate between permeability oedema (due to lung injury) and hydrostatic oedema caused by left-sided heart failure [Monnet, 2007]. This is reinforced by clinical trials that show that EVLWI and PVPI measures are strongly correlated in ARDS patients and weakly correlated in patients with cardiogenic pulmonary oedema [Kushimoto, 2012].

A reduction in PVPI of 15% or more is considered to be meaningful based on the known precision of the PiCCO instrument [Monnet, 2011].

A key secondary objective of the trial will be to evaluate the effect of GSK2862277 on post-operative hypoxaemia using the ratio of partial pressure of arterial oxygen and fraction of inspired oxygen (PaO_2/FiO_2) at the end of surgery. PaO_2/FiO_2 is a measure of the arterial hypoxaemia that may occur in oesophagectomy patients post-surgery as a result of lung injury following OLV and/or lung deflation.

Previous clinical trials suggest that both PaO_2/FiO_2 and EVLW measures are sufficiently sensitive to reflect changes in underlying pulmonary pathophysiology in response to pharmacological interventions. For example, nitric oxide transiently corrects arterial hypoxaemia when delivered directly to the lungs of ARDS patients, which is reflected in an approximately 3 kPa increase (20mmHg) in PaO_2/FiO_2 [Afshari, 2011]. Similarly, a single inhaled dose of salbutamol was shown to decrease post-operative EVLW by 2.3 mL/kg and PVPI by 0.5 units in a thoracic surgery population [Licker, 2008].

The expression profiles of biomarkers of inflammation and tissue injury in the ventilated versus the collapsed lung could be different at the end of surgery, therefore, subjects will be randomised to have broncho-alveolar lavage (BAL) samples taken from either the ventilated or collapsed lung to explore the effect of GSK2862277 on biomarkers measured in BAL from both ventilated and collapsed lungs.

Finally, patients will be followed up during the rest of their hospital stay to explore whether GSK2862277 treatment impacts the development of respiratory and non-respiratory complications.

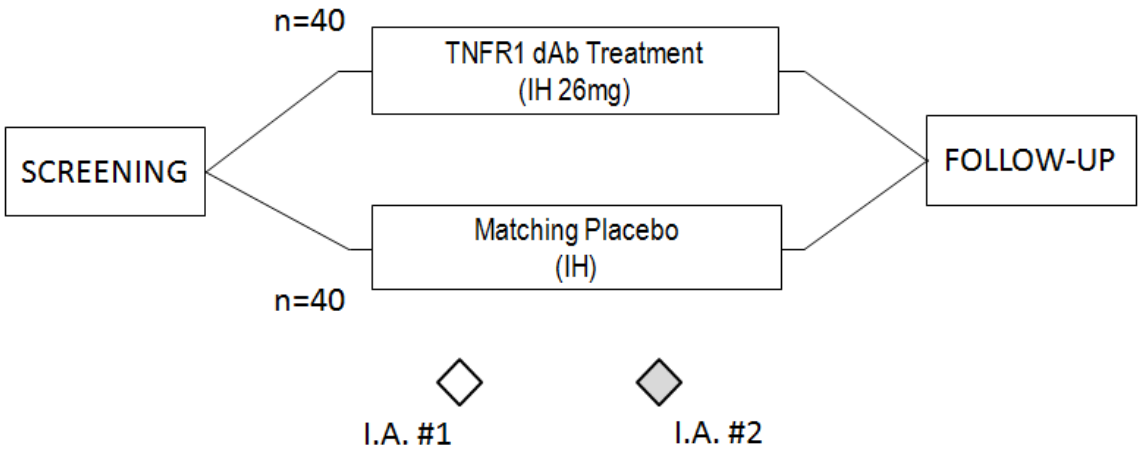
2. OBJECTIVE(S) AND ENDPOINT(S)

	Objectives	Endpoints
1.1	**Primary**: To evaluate whether a single nebulised dose of GSK2862277 prevents peri-operative lung injury compared to placebo, as assessed by measurement of pulmonary vascular permeability.	• Baseline adjusted change in PVPI on completion of surgery
2.1	**Secondary:** To evaluate whether a single nebulised dose of GSK2862277 prevents peri-operative lung injury compared to placebo, as assessed by measurement of pulmonary oedema.	• Baseline adjusted change in EVLWI on completion of surgery
2.2	**Secondary:** To evaluate the safety and tolerability of GSK2862277 administered pre-operatively.	• AEs • Clinical laboratory safety data. • ECG readings • Vital signs
2.3	**Secondary**: To evaluate whether a single nebulised dose of GSK2862277 prevents peri-operative lung injury compared to placebo, as assessed by degree of hypoxaemia	• Baseline adjusted change in $PaO_2/FiO2$ on completion of surgery
2.4	**Secondary:** To evaluate differences in expression profile of BAL biomarkers from both ventilated and collapsed lungs immediately after surgery	• Levels of BAL biomarkers (e.g. IL-6, sRAGE, protein levels) on completion of surgery.
2.5	**Secondary**: To evaluate the effect of a single IH dose of GSK2862277 compared to placebo, on attenuating lung and distal organ injury over the post-operative period	• Change over time in PaO_2/FiO_2 post-operatively on Day 2 through to Day 4 (as available; SpO_2/FiO_2 when arterial line removed) • Change over time in PVPI and EVLWI post-operatively on Day 2 through to Day 4 • Daily SOFA scores on Day 2 through to Day 4
2.6	**Secondary**: To describe plasma pharmacokinetics of a single inhaled dose of GSK2862277.	• Plasma concentrations of GSK2862277 and derived pharmacokinetic parameters.
2.7	**Secondary:** To quantify the concentration of GSK2862277 in BAL and to compare to plasma levels of GSK2862277.	• BAL concentrations of GSK2862277 and derived pharmacokinetic parameters • Ratio of BAL concentration to plasma concentration.
2.8	**Secondary**: To evaluate the levels and specificity of any anti-drug antibodies formed following dosing with GSK2862277.	• Incidence and titers of serum anti-GSK2862277 antibodies post dosing

	Objectives	**Endpoints**
3.1	**Exploratory**: To evaluate the effect of GSK2862277 compared to placebo on clinical and patient outcomes.	Further exploratory evaluation of effectiveness may be conducted where data permit. Endpoints may include, but are not limited to: • Diagnosis of ARDS out to day 28; • 28 day survival; • Ventilator Free Days; ICU & hospital length of stay; • Organ Failure Free Days; • Haemodynamic assessments (e.g. Cardiac Index, Global End Diastolic Volume, etc) • Oxygenation Index
3.2	**Exploratory:** To evaluate changes in TNFR-related and disease biology biomarkers in the blood of patients who have been treated with GSK2862277 compared to placebo, on completion of surgery and in the immediate post-operative period.	• Difference from placebo in levels of blood and cellular, biomarkers (e.g. IL-6 sRAGE) on completion of surgery and through to Day 4.

3. STUDY DESIGN

3.1. Study Schematic

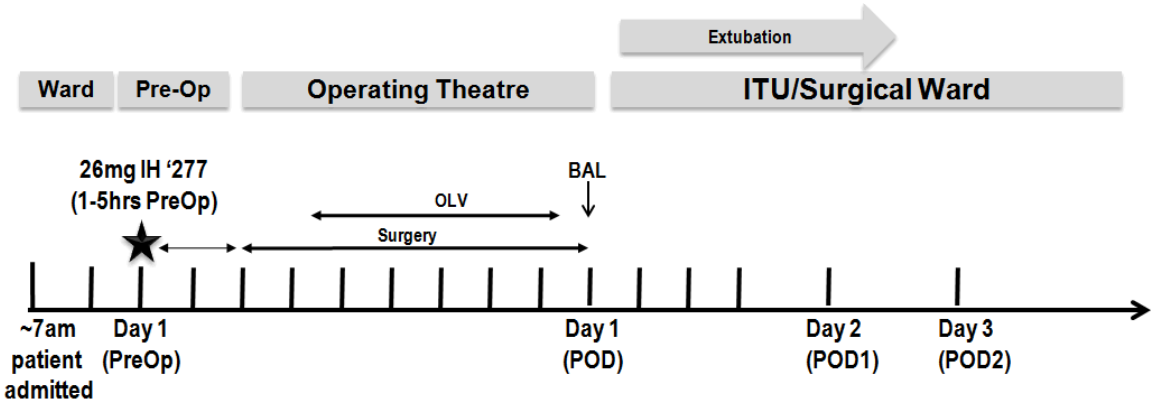


◇ I.A. #1 - Safety data look after approx 10 patients complete Day 7.

◇ I.A #2 – Interim Analysis for efficacy/futility and safety after approximately n=40 patients complete Day 7.

3.2. Peri-operative schematic

To aid understanding of the flow of events around the time of surgery for each patient, a schematic representing an example timeline with key study events is included below:



★ Indicates dosing event
BAL = Bronchoalveolar lavage
OLV = One Lung Ventilation
IH = Inhaled
POD = Post-Operative Day

Timing of events indicated above and location of patients relative to surgery start/finish, and dosing events are subject to reasonable deviation as clinically indicated, and may differ slightly across participating study centres. See the Time and Events Tables Section 6.1 for detailed planned procedure and assessment time points.

3.3. Discussion of Study Design

The proposed study will be a randomised placebo controlled, double-blind, multi-centre, single dose parallel group, design. There will be two treatment groups comprising one active and one placebo arm with approximately 40 patients per group. Patients enrolled in the study will be scheduled to undergo planned/elective trans-thoracic surgery for oesophagectomy. In the previous GSK2862277 FTIH study, one subject who was subsequently shown to have high levels of pre-existing anti-HAVH antibodies developed a mild infusion reaction following intravenous dosing of GSK2862277. To further mitigate the risk to patients in this trial, all subjects will be pre-screened prior to dosing to ensure the absence of any pre-existing antibodies capable of binding to GSK2862277. The production of anti-drug antibodies post-dosing will be assessed during the study but is not expected to occur after a single inhaled dose of GSK2862277.

The primary objective of the study will be to evaluate the effect of GSK2862277 on the development of pulmonary vascular permeability in oesophagectomy patients following surgery. A number of key secondary endpoints are also included in the study to evaluate the effect of GSK2862277 on tissue dysfunction in the lung that may also occur after surgery. A range of biomarkers will be measured to demonstrate GSK2862277 pharmacology in this patient population in addition to measuring biological responses to

injury. Patients will be followed up to explore the effect of GSK2862277 treatment on the occurrence of post-operative complications and healthcare utilisation.

3.3.1. Design Rationale

Delivery of GSK2862277 directly to the lungs of oesophagectomy patients prior to surgery may prevent the alveolar epithelial dysfunction brought about by intraoperative VILI. It is considered that this method of delivery will ensure optimal drug exposure in the lung to ensure a robust test of whether local inhibition of TNFR1 is able to attenuate the early pathological changes which lead to progression of lung injury.

Patients will be randomized via a central allocation system to receive either GSK2862277 or placebo as an inhaled (via nebulisation) dose. A single inhaled dose of GSK2862277 or placebo to match will be administered approximately 1-5 hours prior to the subject's start of (knife to skin) surgery, before the initiation of pre-operative procedures.

Regular assessments will be conducted until the time of patient discharge. Patients will be followed up as outpatients at Day 28 if they have been discharged from hospital before that time.

3.3.2. Dose Rationale

Data from prior clinical pharmacology studies in healthy human patients conducted with GSK2862277 and the closely related and pharmacologically comparable molecule GSK1995057, establish dose: exposure relationships following inhaled dosing, and development of a robust human PK/PD model to support dose selection in patients.

Lung injury in patients undergoing oesophagectomy may occur during surgery (peri-operatively) as a result of OLV and/or during the immediate post-operative period when patients receive intensive care. This is reinforced by the observation that physiological markers of lung injury (e.g. PVPI and PaO_2/FiO_2) are most elevated immediately after completion of surgery, and the development of clinical ARDS occurs immediately post-operatively (within 72 hours of surgery), with the majority of cases reported 24-48 hours after completion of surgery. Increases in systemic inflammation also peak immediately after completion of surgery, with a suggestion that TNFR1 signalling (as reflected by IL-6 and IL-8 levels) begin to wane after this time point [Perkins, 2014]. It is anticipated that attenuation of TNFR1 signalling in the lungs of patients peri-operatively will prevent damage to the lung during OLV and reduce subsequent systemic inflammation in the post operative phase.

Given the potential for VILI and alveolar epithelial dysfunction peri-operatively, achieving sufficient exposure at the alveolar epithelial lining of the lung during this period (first 24 hours) is considered crucial to protecting against peri-operative lung injury. Delivering GSK2862277 directly to the lungs is also preferred in this patient population given the potential for blood loss, haemodilution, and volume expansion during surgery. Experience with healthy subjects suggests that GSK2862277 can be efficiently delivered to human lungs by nebulising an aerosol optimised for delivery to peripheral airways.

In summary, this study will investigate the effect of a single nebulised dose on peri-operative lung injury, and the development of systemic inflammation and other complications in the post-operative period. GSK2862277 will be administered as an orally inhaled aerosol over approximately 3 to 5 minutes.

The proposed dose for this study is 26 mg via nebulisation. The resulting exposure within the lung compartment is expected to be maintained at greater than that required to inhibit 90% of TNFR1 signalling (IC_{90} based on IL-8 inhibition in various human cell systems). In a previous study in healthy volunteers who also received nebulised endotoxin (LPS), lung levels (derived from lavage) 7 hours post dose were approximately 40 µg/mL in the epithelial lining fluid (ELF). Therefore with elimination half-life estimated to be in the region of 5-9 hours it is predicted that concentrations above IC_{90} will be maintained for at least 24 hours. Although peak exposures will be significantly above that predicted to be required for efficacy ($>IC_{90}$), consideration also needs to be given to the significant variability between individual exposures (40% CV on AUC between patients at 26 mg), increased levels of TNF-α that may alter the potency of GSK2862277, and also the potential for dilution due to fluid in the lungs (oedema). This single inhaled dose is expected to achieve and maintain sufficient inhibition to enable exploration of the effects of TNFR1 inhibition within the lungs taking all these pharmacological and practical considerations into account.

3.4. Risk Management

Table 1 Summary of Key Issues, Their Impact and Strategy to Mitigate Risk

Potential risk	Summary of data	Impact- eligibility criteria	Strategy- monitoring/stopping criteria
Pre-existing anti-framework antibodies present in humans may interact with GSK2862277 causing TNFR1 activation and cytokine release in vivo.	One subject enrolled in the FTIH trial experienced mild, self-limiting symptoms consistent with cytokine release syndrome after receiving 2mg/kg IV dosing GSK2862277 for 5 days. The subject was found to have high titres of pre-existing anti-framework antibodies.	Exclusion of patients with pre-existing antibodies to GSK2862277.	Screening for pre-existing antibodies.
Based on known pharmacological class effects, potential for impact on host immunity.	No infections attributed to treatment with GSK2862277 noted in clinical trials to date.	Exclusion of patients with a positive QuantiFERON-TB Gold test at screening for mycobacterium tuberculosis. Exclusion of patients who screen positive for Hepatitis B or Hepatitis C. Exclusion of patients who are known to be HIV positive.	Patients will be monitored for signs of infection during dosing and throughout follow-up within the hospital.
Risk of impact to vaccine efficacy and safety of live or attenuated vaccines.	No data available.	Patients having received any type of vaccination within 3 weeks of scheduled surgery or are expected to be vaccinated before the end of the study (Day 28).	Ensure no actual or planned vaccine administration as part of routine study visit assessments.

4. STUDY POPULATION

4.1. Number of Subjects

Sufficient patients will be enrolled such that approximately 80 patients complete dosing and critical assessments.

4.2. Eligibility Criteria

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in the Investigator Brochure (GlaxoSmithKline Document Number 2012N147005_02).

Deviations from inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

4.2.1. Inclusion Criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

1. Subject has a planned elective transthoracic oesophagectomy
2. Male or female between 18 and 80 years of age inclusive, at the time of signing the informed consent.
3. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form.
4. A female subject is eligible to participate if she is of:

    - Non-childbearing potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) > 40 MlU/ml and estradiol < 40 pg/ml (<147 pmol/L) is confirmatory]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the contraception methods listed in the appendix of this concept protocol if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrollment. For most forms of HRT, at least 2-4 weeks should elapse between the cessation of therapy and the blood draw; this interval depends on the type and dosage of HRT. Following confirmation of their post-menopausal status, they can resume use of HRT during the study without use of a contraceptive method.

5. Liver parameters according to the thresholds below: AST and ALT < 3xULN; alkaline phosphatase and bilirubin ≤ 1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
6. QTcB or QTcF ≦ 450 msec at screening
    - Either QTcB or QTcF, machine or manual over-read can be used. This applies to both males and females. The QT correction formula used to determine inclusion and discontinuation for an individual subject should be the same throughout the study.

- Based on average QTc value of triplicate ECGs obtained over a brief recording period.

It is recognised that in some subjects the QTc may be variable over time (e.g. intermittent use of a pacemaker; junctional rhythm). Inclusion of these subjects should be discussed with the medical monitor prior to enrolment.

4.2.2. Exclusion Criteria

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

1. Positive screening test for pre-existing antibodies that bind GSK2862277.
2. Current evidence or history of pneumonia within 14 days before dosing.
3. Diagnosis of chronic respiratory disease with a forced expiratory volume in one second (FEV1) less than 50% predicted or resting oxygen saturations of less 92%.
4. 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 or GSK Medical Monitor, contraindicates their participation.
5. The subject has received an investigational product within the following time period prior to 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).
6. Use of corticosteroids (IV, oral or IM) at a dose of > 10 mg/day prednisolone (or equivalent) within 14 days prior to dosing, or anti-Tumor Necrosis Factor (anti-TNF) or anti-Interleukin-1 (anti-IL1) within 60 days prior to dosing.

4.2.2.1. Criteria Based Upon Medical Histories

7. History or current evidence of clinically significant renal disease, diabetes mellitus/metabolic syndrome, hypertension, peripheral vascular disease or any other clinically significant respiratory, cardiovascular, neurological, endocrine, or hematological abnormalities that are uncontrolled on permitted therapy. Significant is defined as any disease that, in the opinion of the Investigator, would put the safety of the patients at risk through study participation, or which would affect the safety analysis or other analysis if the disease/condition exacerbated during the study.

8. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
9. History of regular alcohol consumption within 6 months of the study, defined as:
    - an average weekly intake of >28 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.

4.2.2.2. Criteria Based Upon Diagnostic Assessments

10. Screens positive for Hepatitis B surface antigen, Hepatitis C antibody
11. Known Human Immunodeficiency Virus (HIV) positive; testing will be conducted in accordance with local procedures
12. Tests positive for Mycobacterium tuberculosis using QuantiFERON Gold Test.

4.2.2.3. Other Criteria

13. Subject has received a live attenuated vaccine(s) within 3 weeks of randomisation or will require vaccination with a live attenuated vaccine prior to the end of the study (Day 28).
14. Unwillingness or inability to follow the procedures outlined in the protocol.
15. Subject is mentally or legally incapacitated.

4.3. Screen and Baseline Failures

Data for screen and baseline failures will be collected in source documentation and will be transmitted to GSK.

A screen failure is defined as any subject who has been assigned a subject identifier, but does not continue in the study beyond Visit 1 (screening) or any subject who completes Visit 1 but is subsequently found to be ineligible for the study based on findings from laboratory or any other screening test conducted at Visit 1.

Additionally, if a subject completes written informed consent and experiences a serious adverse event (SAE) in the time period between completing written informed consent and the planned Visit 1 date, the subject will be assigned a subject identifier and classified as a screen failure.

The interactive voice response system (IVRS) used to track study enrolment will be notified and at a minimum the following information will be collected in the eCRF for screen failures:

- Date of screening visit
- Subject identifier
- Demographic information, including race, age and gender

- Inclusion/exclusion criteria
- Reason subject failed screening Serious Adverse Events information, if applicable, for any SAE that occurred in the time period between completing written informed consent and planned Visit 1 date.

4.4. Withdrawal Criteria and Procedures

A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons.

In some cases, a subject may be randomised to the study and surgery initiated, but subsequently halted due to a decision being made that the case is in fact inoperable. In the event of an open and shut case subjects will be withdrawn from the study by the investigator. As the subjects will have received a dose of study dose prior to surgery, every effort should be made to complete as many safety and PK procedures as possible prior to hospital discharge and the Day 28 assessments.

Should a subject fail to attend the clinic for their final Day 28 visit, the site should attempt to contact the subject and re-schedule the missed visit as soon as possible. In cases where the subject does not return for the rescheduled visit or cannot be reached to reschedule the missed visit, the site should make every effort to regain contact with the subject (3 telephone calls and if necessary a certified letter to the subject's last known mailing address) so that they can appropriately be withdrawn from the study.

These contact attempts should be documented in the subject's medical record. Should the subject continue to be unreachable, then and only then will he/she be considered to have withdrawn from the study with a primary reason of "Lost to Follow-up". For all other patients withdrawing from the study, an alternative reason for discontinuation should be recorded in the eCRF.

4.5. Subject Completion

A completed subject is one who has completed all phases of the study including the follow-up visit.

The end of the study is defined as the last subject's last visit.

Once subjects have completed the study they will return to normal standard of care treatment for a patient with oesophageal cancer.

5. STUDY TREATMENT

5.1. Investigational Product and Other Study Treatment

	Study Treatment		
Product name:	GSK2862277	Placebo/Diluent to Match IH GSK2862277	Reconstitution Fluid for IH GSK2862277
Formulation description:	The GSK2862277 drug product is formulated with sucrose, glycine, sodium dihydrogen phosphate, and polysorbate 80[1]	Placebo/Diluent to Match IH GSK2862277is formulated with sucrose, glycine, sodium dihydrogen phosphate, and polysorbate 80 in Water for Injection[1]	Reconstitution Fluid for IH GSK2862277is formulated with polysorbate 80 in Water for Injection
Dosage form:	Lyophile for reconstitution for inhalation	Solution for inhalation	Not applicable
Unit dose strength(s)/ Dosage level(s):	40 mg/vial. 26 mg.	Volume to match active dose.	Not applicable
Route/ Administration Duration:	Inhalation Nebulised solution. Duration of nebulisation will be approximately 3-5 min.	To match active	Not applicable
Dosing instructions:	Patients single dosed.	To match active	Not applicable
Physical description:	White to off-white, uniform lyophilized cake.	A clear, colorless to pale yellow liquid.	A clear, colorless to pale yellow liquid.
Device:	Pari eFlow with s30 mesh (article number 678G2003)	Pari eFlow with s30 mesh (article number 678G2003)	Not applicable
Manufacturer/ source of procurement:	Drug product – GSK, Parma Device – Pari Pharma	Placebo/Diluent to Match IH GSK2862277 – GSK, Parma Device – Pari Pharma	GSK, Parma

[1]In addition to the excipients listed, sodium hydroxide is added to adjust the pH

5.2. Treatment Assignment

Patients will be assigned to receive either ventilated or collapsed lung BAL procedure and either active drug or placebo via nebulisation in accordance with the randomization schedule generated by Clinical Statistics, prior to the start of the study, using validated internal software.

As data emerge during the interim analyses, flexibility to change remaining subject randomisation of the BAL procedure to only either the collapsed or ventilated lung will be retained.

5.3. Study Stopping Criteria

If any trends or unexpected increases in safety signals are observed, which in the Investigator's opinion, are of greater intensity, frequency, or duration than expected for the patient group under investigation, the GSK Medical Monitor should be notified as soon as possible. If the Investigator and GSK Medical Monitor consider that there is a reasonable possibility that the event was related to treatment with the investigational product, the study may be put on-hold until all available safety data from the study has been reviewed.

Two interim reviews are planned during the course of the study at which emerging safety data will be formally reviewed:

Interim analysis #1: Unblinded safety review after approx 10 patients have completed Day 7 visit; roughly 5 per arm.

The principal objective will be to determine whether there are any clear safety signals that would preclude continuation of the study.

Unless there are safety concerns recruitment into the study may continue whilst interim analysis 1 is being performed.

Interim analysis #2: Unblinded safety review and futility analysis after approx (40 patients have completed Day 7 visit; roughly 20 per arm.)

Although interim analysis #2 is scheduled to occur after approximately 20 patients per arm have been recruited and completed the Day 7 study visit, it is possible that recruitment rates may be lower than anticipated in which case the interim analysis may occur after a fixed time with whatever data are available.

The same set of safety outputs as interim analysis #1 will also be reviewed.

Unless there are safety concerns, recruitment may continue whilst interim analysis #2 data are collected and the analysis performed. At completion of interim #2 the study may be stopped for futility if there is no evidence for a positive effect of GSK2862277 across study endpoints. . The RAP will contain further details and operating characteristics of the futility component of Interim analysis #2.

5.4. Blinding

This will be a double-blind (sponsor unblind) study. The investigator and subject will be blinded. GSK staff (the sponsor) will not be blinded. At least one site pharmacist (at each site) will not be blinded to study treatment in order to enable preparation of each patient's dose. See Section 5.6 for further details.

The investigator or treating physician may unblind a subject's treatment assignment **only in the case of an emergency**, or in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject as judged by the investigator. Investigators have direct access to the subject's individual study treatment. It is preferred (but not required) that the investigator first contact the GSK Medical Monitor or appropriate GSK study personnel to discuss options before unblinding the subject's treatment assignment. If GSK personnel are not contacted before the unblinding, the investigator must notify GSK as soon as possible after unblinding, but without revealing the treatment assignment of the unblinded subject, unless that information is important for the safety of patients currently in the study. The date and reason for the unblinding must be fully documented in the appropriate data collection tool.

GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any subject 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 subject's treatment assignment, may be sent to clinical investigators in accordance with local regulations and/or GSK policy.

5.5. Packaging and Labelling

The contents of the label will be in accordance with all applicable regulatory requirements.

5.6. Preparation/Handling/Storage/Accountability

A description of the methods and materials required for preparation of GSK2862277 and reconstitution will be detailed in Section 12.6 and the Study Procedures Manual (SPM).

Unblinded pharmacists at each clinical site are a necessary requirement to enable preparation of study treatment, but other site staff including the investigator and study patients will remain blinded to study treatment.

Study treatment must be dispensed or administered according to procedures described in the TA. Only patients enrolled in the study may receive study treatment. Only authorized site staff may supply or administer study treatment. All study treatment must be stored in a secure area with access limited to the investigator and authorized site staff. Storage instructions for all study treatments supplied by GSK (including active drug product as well as prepared solutions) are provided in the TA. Maintenance of a temperature log (manual or automated) is required.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance. The investigator or the head of the medical institution (where applicable), or designated site staff (e.g., storage manager, where applicable) must maintain study treatment accountability records throughout the course of the study. The responsible person(s) will document the amount of study treatment received from and returned to GSK, or destroyed locally, as appropriate and the amount administered to patients. The required

accountability unit for this study will be per individual vial. Discrepancies are to be reconciled or resolved. Procedures for final disposition of unused study treatment are listed in the SPM.

Investigational product is not expected to pose significant occupational safety risk to site staff under normal conditions of use and administration. Take adequate precautions to avoid unintentional occupational exposure. In the event of unintentional occupational exposure notify the monitor, medical monitor and/or study manager. A Safety Data Sheet (SDS)/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.

5.7. Assessment of Compliance

When patients are dosed at the study 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.

5.8. Treatment of Study Treatment Overdose

For this study, any dose of GSK2862277 over and above the initial 26 mg IH dose will be considered an overdose.

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

5.9. Concomitant Medications and Non-Drug Therapies

5.9.1. Permitted Medications

All medications that are required by the patient will be allowed. All concomitant medications should be recorded in the CRF.

5.9.2. Prohibited Medications and Non-Drug Therapies

No medications will be prohibited.

6. STUDY ASSESSMENTS AND PROCEDURES

This section lists the procedures and parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Table Section 6.1. Whenever vital signs, 12-lead ECGs and blood draws are scheduled for the same nominal time, the assessments should occur in the following order: 12-lead ECG, vital signs, blood draws. The timing of the assessments should allow the blood draw to occur at the exact nominal time.

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential and required for study conduct.

The timing and number of planned study assessments, including safety, pharmacokinetic, pharmacodynamic/biomarker, Serum/plasma biomarkers, immunogenicity assessments may be altered during the course of the study based on newly available data to ensure appropriate monitoring. The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment. The IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form. No more than 500 mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

It is recognised that assessments and procedures not specifically listed as part of this protocol may be performed as part of standard of care according to local practices.

6.1. Time and Events Table

Table 2 Time and Events Table (Screening)

Procedures	Screening (7 to 28 days inclusive prior to surgery)[4]
Informed Consent	X
Demography	X
Medical History	X
Smoking Status & History	X
Quantiferon-GOLD for TB[3]	X
HepB, HepC, HIV testing[2]	X
Inclusion/Exclusion Criteria	X
Concomitant Medication	X
Physical Examination	X
Vital Signs	X
12-lead ECG[1]	X
Serious Adverse Events	X
Clinical Labs including Haematology and Chemistry	X
Pre-existing ADA screening[3]	X
Pharmacogenetics (PGx) sample[5]	X
IVRS	X

1. Rest the subject in a supine or semi-recumbent position for at least 5 min and then take three measurements 5 minutes apart.
2. HIV testing dependent on local practice
3. If repeat screening is required previous results from ADA screening and Quantiferon-GOLD testing can be used for up to 60 days.
4. In case of repeat screening, screening can occur less than 7 days prior to dosing if ADA and Quantiferon results are available.
5. Can occur any time during the study after eligibility is confirmed and consent provided

Table 3 Time and Events Table Day 1

	Pre-dose[4]	0 h[1]	1 h	Immediately prior or at start of surgery	On completion of surgery
Randomisation	X[2]				
Brief Physical Examination	X				
Concomitant Medication	X				
IVRS	X[2]				
Vital Signs	X		X		
12-lead ECG	X		X[5]		
Adverse Events	X				
Serious Adverse Events	X				
Clinical Labs including Haematology and Chemistry	X				
Blood sample for immunogenicity	X				
Blood sample for PK	X		X		X
Blood sample for urea and total protein					X
Blood samples for translational sub-study[3]	X				X
Sample for Urinalysis	X				
Blood sample for serum biomarkers	X				X
Nebulised (IH) Study Treatment		X			
Transpulmonary thermodilution				X	X
P/F Ratio: • Arterial blood sample • Record FiO_2				X	X
Measure SpO_2 using pulse oximetry				X	X
BAL Sampling					X

1. Dosing may occur 1-5 hours prior to surgery
2. Randomisation & associated IVRS activities may be conducted up-to 72 hours prior to administration of study treatment (can be longer on agreement with the sponsor for extenuating circumstances e.g. bank holiday weekends).
3. Only applicable at designated sites. Can be taken at screening if required.
4. Can be taken up to 24 hours prior to dosing
5. If 12 lead ECG is not available, monitoring by 3 lead ECG/telemetry (or similar) is acceptable

NB: Time points (except pre-dose) refer to post-start of drug administration on Day 1.
Pre-dose is defined as the time from admission to hospital up to the administration of study treatment.

Table 4 Time and Events Table (Days 2 to 28)

Procedures	Day 2	Day 3	Day 4	Daily until discharge	Day 8[1]	Day of discharge	Day 28 (FU) ±3 days
Concomitant Medication	X	X	X	X		X	X
IVRS						X	X
Transpulmonary thermodilution[2]	X	X	X				
P/F Ratio • Arterial blood sample (as available) • Record FiO_2	X	X	X				
Measure SpO_2 using pulse oximetry	X	X	X				
Additional SOFA Components[3] • Glasgow Coma Score (GCS) • Record Mean Arterial Pressure[4] or administration of vasopressors required • Bilirubin • Platelets • Creatinine (or urine output)	X	X	X				
Vital Signs			X			X	X
12-lead ECG	X		X			X	
Adverse Events	X	X	X	X	X	X	X
Serious Adverse Events	X	X	X	X	X	X	X
Clinical Labs including Haematology and Chemistry	X	X	X		X		
Blood sample for serum biomarkers	X	X	X		X		
Blood samples for translational sub-study[5]	X						
Blood sample for Immunogenicity					X		X
Blood sample for PK	X[6]	X[7]					
Sample for Urinalysis					X		

1. If discharged before Day 8 take blood and urine samples on day of discharge
2. PVPI will be measured via single-indicator transpulmonary thermodilution as long as the subject remains in the ICU with a patent indwelling PiCCO catheter, up to Day
3. Sequential Organ Failure Assessment (SOFA) Score will only be calculated up to Day 4.
4. If no arterial blood sample calculate MAP using equation: 2X diastolic blood pressure + systolic blood pressure. Then divide by 3.
5. Only applicable at designated sites
6. Sample to be taken 24 to 26 hours post-dose of study treatment.
7. Sample to be taken 46 to 50 hours post-dose of study treatment.

Procedures	Day 2	Day 3	Day 4	Daily until discharge	Day 8[1]	Day of discharge	Day 28 (FU) ±3 days	8. Organ Failure Free Days needs to be recorded every day until Day 28 (See SPM).
Organ Failure Free Days							X[8]	
ARDS diagnosis status						X		
Ventilator Free Days							X	
ICU & Hospital Length of Stay						X		
Survival							X	

6.2. Demographic/Medical History Assessments

The following demographic parameters will be captured: year of birth, gender, race and ethnicity.

Medical/medication/alcohol history will be assessed as related to the eligibility criteria listed in Section 4.2. Cardiovascular medical history/risk factors including height, weight, blood pressure, smoking history (pack-years), medical conditions will also be assessed at baseline. More information will be provided in the SPM.

6.3. Safety

Planned time points for all safety assessments are listed in the Time and Events Table (Section 6.1). Additional time points for safety tests (such as vital signs, physical exams and laboratory safety tests) may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

Information regarding surgery and blood product administration will also be captured if applicable. More information will be provided in the SPM.

The Safety Review Team (SRT) is a GSK cross-functional team reviewing all available safety data related to the project, including in-stream data from this study, in an ongoing manner. The SRT is an internal GSK requirement put in place to ensure holistic evaluation of the safety profile of an investigational product with systematic, periodic and documented reviews of available safety data, with the appropriate communication and escalation of new findings that have the potential to impact patient safety.

The SRT for this project will review data from this study in collaboration with an independent external (to GSK) expert reviewer.

6.3.1. Physical Exams

A complete physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities. Height and weight will also be measured and recorded.

A brief physical examination will include assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

6.3.2. Vital Signs

Vital sign measurements to be measured in a semi-recumbent or supine position (after 5 minutes rest if time point is an outpatient visit) will include systolic and diastolic blood pressure, pulse rate, temperature and respiratory rate.

6.3.3. Electrocardiogram (ECG)

Triplicate 12-lead ECGs will be obtained at screening and single 12-lead ECGs will be obtained thereafter during the study, using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, RR and QTc intervals.

At the 1 hour post-dose time point monitoring by 3 lead ECG/telemetry (or similar) is acceptable if 12 lead ECG is not available.

6.3.4. GSK2862277 Antibodies (Immunogenicity)

Blood samples for testing antibodies against GSK2862277 will be collected at the time points indicated in the Time and Events Tables. The actual date and time of each blood sample collection will be recorded.

6.3.5. Clinical Laboratory Assessments

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed below. Details for the preparation and shipment of samples will be provided by Quest. Reference ranges for all safety parameters will be provided to the site by the laboratory.

If additional non-protocol specified laboratory assessments are performed at the site's local laboratory and result in a change in subject management or are considered clinically significant by the Investigator (for example SAE or AE or dose modification) the results must be captured and sent to GSK along with other study data as defined in Section 12.4.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed below:

Haematology

Platelet Count	*RBC Indices*:	*Automated WBC Differential:*
RBC Count	MCV	Neutrophils
WBC Count (absolute)	MCH	Lymphocytes
Reticulocyte Count	MCHC	Monocytes
Haemoglobin		Eosinophils
Haematocrit		Basophils

Clinical Chemistry

BUN	Potassium	AST (SGOT)	Total and direct bilirubin
Creatinine	Chloride	ALT (SGPT)	Uric Acid
Glucose	Total CO_2	GGT	Albumin
Sodium	Calcium	Alkaline phosphatase	

Routine Urinalysis

Specific gravity
pH, glucose, protein, blood and ketones by dipstick
Microscopic examination (if blood or protein is abnormal)

Other screening tests

HIV (tested in accordance with local practise)
Hepatitis B (HBsAg)
Hepatitis C (Hep C antibody -- if second generation Hepatitis C antibody positive, a hepatitis C antibody Chiron RIBA immunoblot assay (or other third generation immunoassay) should be reflexively performed **on the same sample** to confirm the result)
FSH and estradiol (as needed in women of non-child bearing potential only)
Quantiferon GOLD for assessment of TB

All laboratory tests with values that are significantly abnormal during participation in the study or within 72 hours after the last dose of study treatment should be repeated until the values return to normal or baseline. If such values do not return to normal within a period judged reasonable by the investigator, the aetiology should be identified and the sponsor notified.

6.4. Pharmacokinetics

6.4.1. Blood Sample Collection

Blood samples for pharmacokinetic analysis of GSK2862277 will be collected at the time points indicated in Section 6.1, Time and Events Tables. The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

Processing, storage and shipping procedures are provided in the Study Procedures Manual (SPM).

6.4.2. BAL Sample Collection

BAL samples for PK analysis of GSK2862277 will be collected at the time points listed in the Time and Events Table. Details of BAL sample collection, processing, storage and shipping procedures are provided in the SPM.

GSK2862277 concentration in BAL will need to be corrected for dilution using urea concentration as a correction factor to estimate the volume of ELF removed via the lavage. A plasma sample taken at the same time as the BAL, or as soon as practically possible, will be used to determine the plasma urea concentration which will then be used with the BAL urea concentration to determine the dilution factor.

Where: Dilution factor = urea(plasma)/urea(BAL).

6.4.3. Sample Analysis

Sample analysis will be performed under the control of PTS-DMPK/Scinovo, GlaxoSmithKline, the details of which will be included in the Study Procedures Manual. Concentrations of GSK2862277 will be determined in samples using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SPM).

Once the sample has been analyzed for GSK2862277 any remaining sample volume may be analyzed for other compound-related metabolites and the results reported under a separate PTS-DMPK, GlaxoSmithKline protocol.

6.5. Biomarker(s)/Pharmacodynamic Markers

6.5.1. BAL collection

Bronchoscopy and brochoalveolar lavage (BAL) will be performed in accordance with the site standard procedures at times outlined in Section 6.1, Time and Events Tables. These standard procedures will reflect current standards of practice in hospital care and will include (but are not limited to) the following items:

- Bronchoscopy will only be performed by an appropriately qualified member of staff.
- The Bronchoscopist will be assisted by suitably qualified staff.
- Oxygen supplementation will be given to all subjects. Further details on these procedures are in the SPM.
- Low volumes of fluid will be used compared to a standard lavage (See SPM for details).

6.5.2. Pharmacodynamic Markers in Serum and BAL

Samples will be collected to determine concentrations of biomarkers in serum and BAL (these may include but are not limited to sTNFR1 and IL-6) using an appropriately validated assay. Samples will be collected at times outlined in Section 6.1, Time and Events Tables. The timing of the collections may be adjusted on the basis of emerging PK or PD data from this study or other new information in order to ensure optimal evaluation of the PD endpoints. Details on sample preparation, storage and analysis will be given in the SPM.

6.5.3. Disease Biomarkers

Blood and BAL sample(s) will be collected during this study and may be used for the purposes of measuring biomarkers to identify factors that may influence the development of ARDS in patients undergoing surgery for oesophageal cancer, and/or medically related conditions, as well as the biological and clinical responses to GSK2862277. If relevant, this approach will be extended to include the identification of biomarkers associated with adverse events.

Samples will be collected at the time points indicated in Section 6.1, Time and Events Tables. The timing of the collections may be adjusted on the basis of emerging PK or PD data from this study or other new information in order to ensure optimal evaluation of the biomarker endpoints.

Candidate biomarkers and subsequently discovered biomarkers of the biological response associated with the development of ARDS or medically related conditions and/or the action of GSK2862277 may be identified by application of:

- Detection of protein endpoints in serum and BAL by immunoassay and/or equivalent alternative technologies (these may include but are not limited to vWF and sRAGE).
- Total protein determination in plasma for protein permeability analysis
- Cellular assays and RNA analysis

All samples will be retained for a maximum of 15 years after the last subject completes the trial.

6.6. Pharmacogenetics

With the subject's consent, blood samples will be obtained for pharmacogenetics (PGx) analysis. Information regarding PGx research is included in Section 12.2. The IEC and, where required, the applicable regulatory agency must approve the PGx assessments before these can be conducted at the site. In some cases, approval of the PGx assessments can occur after approval is obtained for the rest of the study. If so, then the written approval will clearly indicate approval of the PGx assessments is being deferred and in most cases, the study, except for PGx assessments, can be initiated. When PGx assessments will not be approved, then the approval for the rest of the study will clearly indicate this and therefore, PGx assessments will not be conducted.

6.7. Efficacy

6.7.1. Transpulmonary Thermodilution

Measurement of PVPI and EVLWI

Extravascular lung water (EVLW) refers to the fluid within the lung but outside the vascular compartment. It includes extravasated plasma, intracellular water, lymphatic fluid, and surfactant.

EVLW will be measured by trans-pulmonary thermodilution via a PiCCO haemodynamic monitor.

PVPI is a derived value from EVLW, and is considered to be less variable than EVLWI alone. Further details can be found in the SPM.

Other supportive parameters from the PiCCO monitor that are calculated at the same time as EVLW and PVPI, may also be databased e.g. GEDV, SVV, SVR, HR etc.

6.7.2. PaO_2 / FiO_2 Ratio

Oxygenation and function of gas exchange will be assessed by the comparison of partial pressure of oxygen arterially (PaO_2) divided by the fraction of oxygen that is being inspired (FiO_2), sometimes referred to simply as the 'P to F ratio'. The P to F ratio will be assessed at time points during the period of intubation and mechanical ventilation.

An arterial blood sample is required for determination of the partial pressure of oxygen and the percentage of O_2 which is being inspired should be recorded at the corresponding time point. Further details can be found in the SPM.

6.7.3. Pulse Oximetry

SpO2 will be collected using pulse oximetry. This will be used to calculate SOFA score when a patient no longer has an arterial line to calculate PaO_2.

6.7.4. Oxygenation Index

Oxygenation index (OI) (sometimes termed *oxygenation factor*) is another measure of gas exchange and pulmonary efficiency which factors in the mean airway pressure (Paw) term in the calculation step [El-Khatib, 2004].

It is calculated using the equation:

Oxygenation Index = (F_iO_2 * Mean Airway Pressure) / P_aO_2

Further details can be found in the SPM.

6.7.5. Sequential Organ Failure Assessment (SOFA) score

The SOFA score is a way of grading organ dysfunction. Scores range from 0 to 4 and represent the extent of dysfunction or failure [Vincent, 1996].

The SOFA score is made up of the following components:

- PaO_2/FiO_2 (mmHg) or SpO_2/FiO_2 (mm/Hg)
- Glasgow Coma Score (GCS)
- Mean Arterial Pressure or administration of vasopressors required
- Bilirubin
- Platelets
- Creatinine (or urine output)

Please refer to Section 12.3 for further details. SOFA scores will be generated at the time points stipulated in Section 6.1, Time and Events Tables.

6.8. Clinical assessments of disease progression

The following data will be collected:

- Diagnosis of ARDS
- ARDS in the post-operative period (including date & time of diagnosis)
- SOFA score post operatively
- 28 day survival
- Ventilator Free Days; ICU & hospital length of stay
- Organ Failure Free Days

Other post-operative complications; e.g. anastamotic leak e.t.c.

7. ADVERSE EVENTS, SERIOUS ADVERSE EVENTS, PREGNANCY AND MEDICAL DEVICE INCIDENTS

7.1. Adverse Events (AE) and Serious Adverse Events (SAEs)

The investigator or site staff are responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

7.1.1. Time period for collecting AE and SAE information

AEs will be collected from the start of Study Treatment and until the follow-up contact. Medical occurrences that begin prior to the start of study treatment but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions CRF.

SAEs will be collected over the same time period as stated above for AEs. However, any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact. All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Section 12.4.

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 subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator would promptly notify GSK.

NOTE: The method of, recording, evaluating and follow-up of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Section 12.4.

7.1.2. Method of Detecting AEs and SAEs

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

- "How are you feeling?"
- "Have you had any (other) medical problems since your last visit/contact?"
- "Have you taken any new medicines, other than those provided in this study, since your last visit/contact?"

7.1.3. Definition of Adverse Events

An AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

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 medicinal product.

Events meeting the definition of an AE **include**:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator.
- 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 prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected 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 this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae.).
- "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE.

Events that **do not** meet the definition of an AE include:

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.

- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where 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.

7.1.4. Definition of Serious Adverse Events

If an event is not an AE per Section 7.1.3, 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, etc).

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

a. Results in death

b. Is life-threatening

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

c. Requires hospitalization or prolongation of existing hospitalization

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

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

d. Results in disability/incapacity, or

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

e. Is a congenital anomaly/birth defect

f. Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be

immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are 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.

g. Is associated with liver injury **and** impaired liver function that in the judgement of the investigator and medical monitor causes prolongation of hospitalization out of what would be expected for normal clinical course:

- ALT ≥ 5xULN and bilirubin ≥ 2xULN (>35% direct)

NOTES:

Bilirubin fractionation should be performed if testing is available. If fractionation is unavailable, urinary bilirubin is to be measured via dipstick (a measurement of direct bilirubin, which would suggest liver injury).

Refer to Section 12.1 for the required liver chemistry follow-up instructions.

7.1.5. Death Events

In addition, all deaths, whether or not they are considered SAEs, will require a specific death data collection tool to be completed. The death data collection tool includes questions regarding cardiovascular (including sudden cardiac death) and noncardiovascular death.

This information should be recorded in the specific death eCRF within one week of when the death is first reported.

7.1.6. Prompt Reporting of SAEs to GSK

Once the investigator determines that an event meets the protocol definition of an SAE, the SAE will be reported to GSK within 24 hours. Any follow-up information on a previously reported SAE will also be reported to GSK within 24 hours.

If the investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before notifying GSK of the event and completing the appropriate data collection tool. The investigator will always provide an assessment of causality at the time of the initial report as described in Appendix 4.

7.1.7. Regulatory Reporting Requirements For SAEs

Prompt notification of SAEs by the investigator to GSK is essential so that legal obligations and ethical responsibilities towards the safety of patients are met.

GSK has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. GSK will

comply with country specific regulatory requirements relating to safety reporting to regulatory authorities, IRBs/IECs and investigators.

Investigator safety reports are prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and GSK policy and are forwarded to investigators as necessary. An investigator who receives an investigator safety report describing an SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from GSK will file it with the Investigator Brochure (GlaxoSmithKline Document Number 2012N147005_02) and will notify the IRB/IEC, if appropriate according to local requirements.

8. DATA MANAGEMENT

For this study subject data will be entered into GSK defined electronic case report forms (eCRFs), transmitted electronically to GSK and combined with data provided from other sources in a validated data system.

Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data. Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug. eCRFs (including queries and audit trails) will be retained by GSK, and copies will be sent to the investigator to maintain as the investigator copy. Subject initials will not be collected or transmitted to GSK according to GSK policy.

9. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

9.1. Hypotheses and Treatment Comparisons

Precision Estimation

No formal statistical hypotheses will be tested in this study. Safety related objectives will be assessed via summary tables, listings and figures.

In addition to summary tables, listings and figures, study objectives relating to efficacy (for example, lung physiology and biomarkers) may be evaluated using posterior distribution(s) for the effect of GSK2862277 relative to Placebo. Point estimates and corresponding 95% credible intervals will be constructed for the difference between the mean of the test treatment and the mean of the reference treatment, μ(test) - μ(reference)

No adjustment for multiplicity will be performed due to the Bayesian analysis framework.

Note: If data require log transformation prior to analysis then point estimates and corresponding 95% credible intervals will be constructed for the ratio of test treatment to placebo treatment μ (test) / μ (reference) in place of those for differences in means.

9.2. Sample Size Considerations

9.2.1. Sample Size Assumptions

The number of patients described in Section 4.1 is based upon:

- Operating characteristics from computer simulations.
- A balance of feasibility considerations (e.g. anticipated duration of study under predicted recruitment rates)

The two endpoints selected for the computer simulations were chosen because they reflect two key physiological parameters that are anticipated will be impacted by administration of GSK2862277. The endpoints are

- Baseline adjusted change in PVPI on completion of surgery
- Baseline adjusted change in PaO2/FiO2 on completion of surgery.

However, many other factors and study endpoints also influence the final progression decision, so the simulation work is for guidance only and the criteria listed in Figure 1 are **non-binding** but are intended to provide a point of reference for a successful outcome.

The success thresholds in Figure 1 should be achievable using this study design when the true drug effects are larger than the minimum desired profile (this statement is based upon estimates of minimum detectable differences derived from access to data from the BALTI prevention trial (Perkins, 2014).

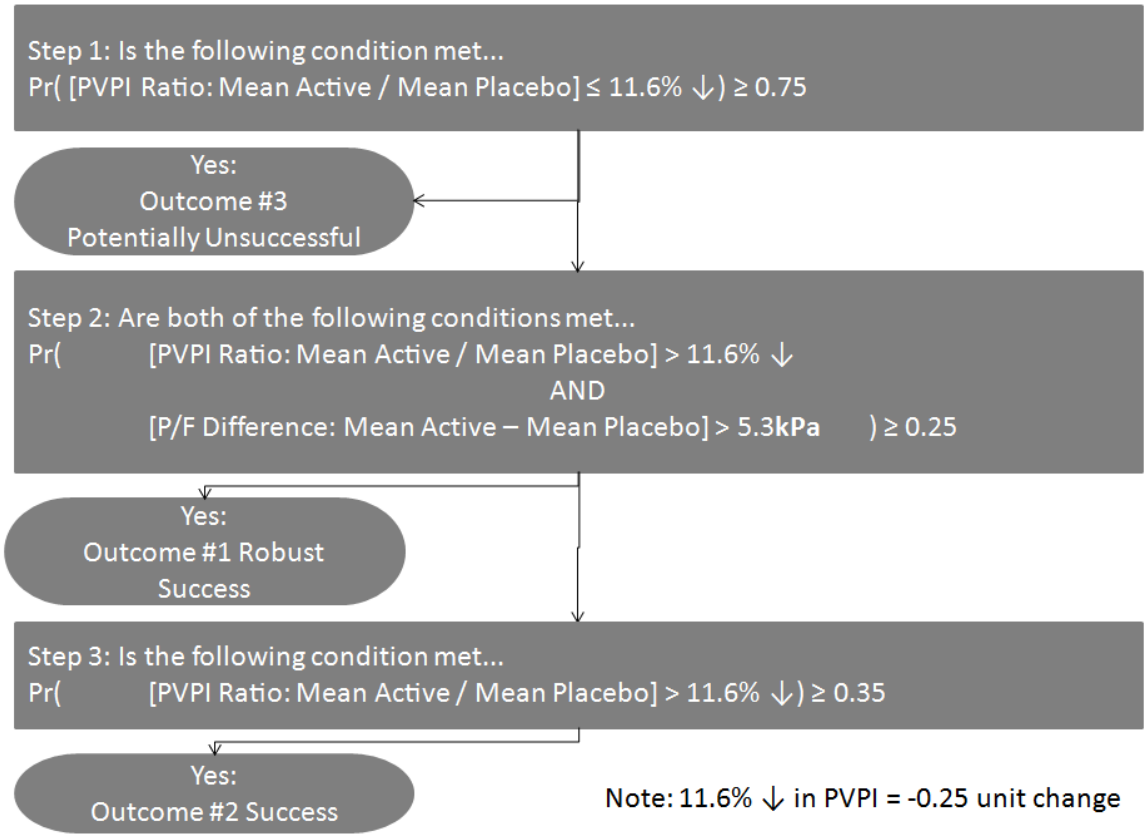
Various confidence levels were examined in the simulation exercise but only the final choices that gave acceptable operating characteristics are presented (Figure 1).

The posterior probability of each outcome will be derived and, if it exceeds its respective confidence level, that outcome will be deemed to have occurred. Combinations of occurring outcomes imply the following four end-of-study states:

Overall success	≥1 of the "Success" outcomes	AND	0 "Failures"
Potentially Unsuccessful	0 "Success" outcomes	AND	1 "Failure"
Inconclusive	0 "Success" outcomes	AND	0 "Failures"
Inconsistent	≥1 of the "Success" outcomes	AND	1 "Failure"

Full details of the computer simulation exercise and its results will be described in the RAP.

Figure 1 Proposed end of study decision pathway



9.2.2. Sample Size Sensitivity

Table 5 Selected results from Sensitivity analysis

Hypothetical Treatment Profile	% of simulated studies with outcome			
	Overall Success	Potentially Unsuccessful	Inconclusive	Inconsistent
Null	4.65%	92.81%	2.54%	0%
Minimum desired profile	80.64%	13.63%	5.73%	0%

Table 5 shows a sample size of 40 p.a. would have an equivalent of a Type I error rate of 4.65% using the decision pathway (Figure 1) and has a high probability (80.64%) of detecting plausible effect sizes. This is deemed acceptable given the exploratory early phase status of this study.

The data in Table 5 were obtained from computer simulations utilising subject level historical information from the BALTI prevention trial [Perkins, 2014]. These data were used to estimate change from baseline response post surgery and the variances and correlations between the two endpoints (see Table 6).

The BALTI prevention trial means were assumed to represent future placebo data and several hypothetical GSK2862277 effect profiles were evaluated (although only 2x profiles are presented in Table 6).

Ten thousand sets of studies (40 patients per arm) were simulated using the multivariate normal distributions implied by Table 6. Each of the 10,000 simulated studies was put through the process shown in Figure 1 and the end of study status recorded (Table 5).

Table 6 Example sets of Scenarios to evaluate operating characteristics

Hypothetical Treatment Profile	**Placebo Vector of mean responses**	**Active Vector of mean responses and Implied treatment effect relative to Placebo**
Null	$\begin{bmatrix} P/F(kPa) \\ LN(PVPI) \end{bmatrix} : \mu_p = \begin{bmatrix} 42.27 \\ 0.765 \end{bmatrix}$	$\mu_a = \begin{bmatrix} 42.27 \\ 0.765 \end{bmatrix} \mu_{dif\,\&rat} = \begin{bmatrix} 0 \\ 0 \end{bmatrix} \& \begin{bmatrix} 1 \\ 1 \end{bmatrix} = NOCHANGE$
Minimum desired profile	$\begin{bmatrix} P/F \\ LN(PVPI) \end{bmatrix} : \mu_p = \begin{bmatrix} 42.27 \\ 0.765 \end{bmatrix}$	$\mu_a = \begin{bmatrix} 48.6105 \\ 0.602481 \end{bmatrix} \mu_{dif\,\&rat} = \begin{bmatrix} 6.3405 \\ -0.16252 \end{bmatrix} \& \begin{bmatrix} 1.15 \\ 0.85 \end{bmatrix} = \begin{matrix} 15\% \uparrow \\ 15\% \downarrow \end{matrix}$
Note: Variance covariance matrix for an individual's Change from Baseline on completion of surgery endpoints (derived from data Perkins, 2014)): $\begin{bmatrix} 143.17249 & \\ -0.467621 & 0.0785513 \end{bmatrix}$, Correlation $\begin{bmatrix} 1 & \\ -0.14313 & 1 \end{bmatrix}$		

9.2.3. Sample Size Re-estimation

No sample size re-estimation is currently planned for this study. However, if during the course of the study, new information becomes available about clinically meaningful differences or variability estimates, sample size re-estimation may be considered. Full details of the procedure used would be specified in the RAP, and any subsequent change to the target sample size would be documented.

An example scenario might be if Interim analysis #2 data suggest a robust treatment effect can be demonstrated using a smaller sample size than stated in Section 3.1 then an appropriate reduction in the recruitment target would be justified.

9.3. Data Analysis Considerations

9.3.1. Interim Analysis

Interim analysis #1: Unblinded safety review after approx 10 patients have completed Day 7 visit; roughly 5 per arm.

The principal objective will be to determine whether there are any emerging safety trends / signals which might require modifications to be made and/or suspension of recruitment pending further data review.

Unless there are safety concerns recruitment into the study may continue whilst interim analysis 1 is being performed.

Further details of the contents of the safety outputs will be provided in the RAP.

Interim analysis #2: Unblinded safety review and futility analysis after approx 40 patients have completed Day 7 visit; roughly 20 per arm.

The outcome of interim analysis #2 will be communicated by GSK to the sites as either "Continue", or "Pause and Review".

Upon a "Pause and Review" communication the available data will be reviewed by the study team (un-blinded) and additional data may be requested from the sites to determine whether to:

- Stop the study early for futility
- Modify aspects of its design
- Resume and continue unaltered

Although interim analysis #2 is scheduled to occur after approximately 20 patients p.a. have available data up to Day 7, it is possible that recruitment rates may be lower than anticipated in which case the interim analysis may occur after a fixed time with whatever data are available.

The futility analysis component of Interim analysis #2 will use the same endpoints as Figure 1 but will utilise predictive inference and a different decision pathway and confidence thresholds; as follows:

- For each treatment arm the available data will be used to obtain the hyper-parameters of a multivariate normal distribution (i.e. obtain a Normal-Inverse-Wishart distribution for each treatment)
- Sample a variance covariance matrix and a mean vector (conditional on the sampled variance covariance matrix) from each Normal-Inverse-Wishart distribution
- Use this newly defined multivariate normal distribution to simulate enough subjects to achieve an overall total of 40 per arm

- Use this hybrid dataset (observed and simulated subjects) to determine whether the probability of observing **Any** decrease in P/F AND **Any** increase in PVPI is greater than 0.1.
  IF so THEN record that simulated study as being futile
- Repeat the previous 3x steps 1000 times.
- Determine the proportion of the 1000 simulated studies which met the futility threshold.
  IF this proportion is larger than 0.3 then the recommendation is to "Pause and Review", otherwise the recommendation is to "Continue"

Note: Expert judgement may be used by the GSK study team if deemed appropriate to override the recommendation from the above statistical analysis (for example, to account for other clinically relevant factors).

The same set of safety outputs as interim analysis #1 will also be reviewed.

Unless there are safety concerns recruitment may continue whilst interim analysis #2 data are collected and the analysis performed.

The RAP will contain further details and operating characteristics of the futility component of Interim analysis #2.

9.4. Final Analyses

9.4.1. Safety Analyses

Safety data will be presented in tabular and/or graphical format and summarized descriptively according to GSK's Integrated Data Standards Library (IDSL) standards.

9.4.2. Pharmacokinetic Analyses

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacokinetics Modeling & Simulation department within GlaxoSmithKline. Plasma GSK2862277 concentration-time data will be analyzed by non-compartmental methods with WinNonlin Phoenix. Calculations will be based on the actual sampling times recorded during the study. From the plasma concentration-time data, the following pharmacokinetic parameters will be determined, as data permit: maximum observed plasma concentration (Cmax), time to Cmax (tmax), area under the plasma concentration-time curve [AUC(0-t)]. Since the proposed sampling regimen is sparse, definitive pharmacokinetics will not be derived from this data and instead data will be compared against previously collected definitive data from healthy volunteers for informal comparability. AUC's or Cmax values will be calculated as appropriate for each dosing day and possibly over the PK sampling window (0-72 hours) if appropriate.

Pharmacokinetic data will be listed and may be presented in graphical form and will be summarized descriptively. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals, R&D.

Statistical analyses of the pharmacokinetic parameter data will be the responsibility of Clinical Statistics, GlaxoSmithKline.

Derived pharmacokinetic parameters will be listed and summarised descriptively by treatment. Descriptive statistics (n, arithmetic mean, standard deviation, 90%CI, minimum, median and maximum,) will be calculated for all pharmacokinetic parameters by treatment.

In addition, for loge-transformed variables geometric mean, 90% confidence interval and %CVb (100 * √ (exp(SD2) -1)) will be provided, where the SD is the standard deviation of log-transformed data.

9.4.3. Pharmacokinetic/Pharmacodynamic Analyses

No formal PK/PD analysis will be conducted.

9.4.4. Pharmacodynamic/Biomarker Analyses

Full details of the endpoints and statistical analysis models not described here will be provided in the RAP.

10. STUDY GOVERNANCE CONSIDERATIONS

10.1. Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of patients begins.

10.2. Regulatory and Ethical Considerations, Including the Informed Consent Process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and, the guiding principles of the 2008 Declaration of Helsinki. This includes, but is not limited to, the following:

- IEC review and favorable opinion/approval to conduct the study and of any subsequent relevant amended documents
- Written informed consent (and any amendments) to be obtained for each subject before participation in the study

- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IEC)

Information regarding pharmacogenetic research is included in Section 12.2. In approving the clinical protocol the IEC/IRB and, where required, the applicable regulatory agency must also approve the PGx assessments (i.e., approval of Section 12.2), unless otherwise indicated. Where permitted by regulatory authorities, approval of the PGx assessments can occur after approval is obtained for the rest of the study. If so, then the written approval will clearly indicate approval of the PGx assessments is being deferred and the study, except for PGx assessments, can be initiated. When PGx assessments are not approved, then the approval for the rest of the study will clearly indicate this and therefore, PGx assessments will not be conducted.

10.2.1. Urgent Safety Measures

If an event occurs that is related to the conduct of the study or the development of the study treatment, and this new event is likely to affect the safety of patients, the sponsor and the investigator will take appropriate urgent safety measures to protect patients against any immediate hazard.

The sponsor will work with the investigator to ensure the IEC/IRB is notified.

10.3. Quality Control (Study Monitoring)

In accordance with applicable regulations including GCP, and GSK procedures, GSK monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements. When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the CRF will serve as the source document.

GSK will monitor the study and site activity to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of patients are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents

10.4. Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance assessment and/or audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study. In the event of an assessment, audit or inspection, the investigator (and

institution) must agree to grant the advisor(s), auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss the conduct of the study, any findings/relevant issues and to implement any corrective and/or preventative actions to address any findings/issues identified.

10.5. Study and Site Closure

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK procedures.

In addition, GSK reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. For multicenter studies, this can occur at one or more or at all sites. If GSK determines such action is needed, GSK will discuss this with the investigator or the head of the medical institution (where applicable), including the reasons for taking such action. When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action prior to it taking effect.

If the study is suspended or prematurely discontinued for safety reasons, GSK will promptly inform investigators or the head of the medical institution (where applicable) and the regulatory authorities of the suspension or premature discontinuation of the study and the reason(s) for the action. If required by applicable regulations, the investigator or the head of the medical institution (where applicable) must inform the IRB/IEC promptly and provide the reason for the suspension or premature discontinuation.

10.6. Records Retention

Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records, except for those required by local regulations to be maintained by someone else, in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must assure that all reproductions are legible and are a true and accurate copy of the original, and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional

requirements or local laws or regulations, or GSK standards/procedures; otherwise, the retention period will default to 15 years.

The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator leaves the site.

10.7. Provision of Study Results to Investigators, Posting of Information on Publically Available Clinical Trials Registers and Publication

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 patients, as appropriate.

GSK will provide the investigator with the randomization codes for their site only after completion of the full statistical analysis.

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12. APPENDICES

12.1. Appendix 1: Liver Safety Process

The procedures listed below are to be followed if a subject has ALT, bilirubin and/or INR elevations that meet the definition of an SAE (as defined in Section 7.1.4):

- Notify the GSK medical monitor within 24 hours of learning of the abnormality to confirm follow-up.
- Complete the liver event case report forms.
- Upon completion of the safety follow-up withdraw the subject from the study unless further safety follow up is required.
- Make every reasonable attempt to have subjects return to the clinic within 24 hours for repeat liver chemistries, additional testing, and close monitoring (with specialist or hepatology consultation recommended).
- Monitor subjects twice weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.
- Obtain viral hepatitis serology including:
    - Hepatitis A IgM antibody.
    - Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM).
    - Hepatitis C RNA.
    - Cytomegalovirus IgM antibody.
    - Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing).
    - Hepatitis E IgM antibody.
- Blood sample for pharmacokinetic (PK) analysis, obtained within 72 hours from last dose. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, **do not obtain a PK sample**. Instructions for sample handling and shipping are included in the SPM.
- Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
- Assess eosinophilia
- Record the appearance or worsening of clinical symptoms of hepatitis (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia) on the AE CRF.

- Record use of concomitant medications, acetaminophen, herbal remedies, other over the counter medications, or putative hepatotoxins on the Concomitant Medications CRF.
- Record alcohol use on the Liver Events CRF.
- 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 HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week (James, 2009).
- Liver imaging (ultrasound, magnetic resonance, or computerized tomography) to evaluate liver disease.
- The Liver Imaging and/or Liver Biopsy CRFs are also to be completed if these tests are performed.

12.2. Appendix 2: Pharmacogenetic research

Pharmacogenetics – Background

Pharmacogenetics (PGx) is the study of variability in drug response due to hereditary factors in populations. There is increasing evidence that an individual's genetic background (i.e., genotype) may impact the pharmacokinetics (absorption, distribution, metabolism, elimination), pharmacodynamics (relationship between concentrations and pharmacologic effects or the time course of pharmacologic effects) and/or clinical outcome (in terms of efficacy and/or safety and tolerability). Some reported examples of PGx associations with safety/adverse events include:

Drug	Disease	Gene Variant	Outcome
Abacavir	HIV [Hetherington, 2002; Mallal, 2002; Mallal, 2008]	*HLA-B* 57:01 (Human Leukocyte Antigen B)*	Carriage of the *HLA-B*57:01* variant has been shown to increase a patient's risk for experiencing hypersensitivity to abacavir. Prospective *HLA-B*57:01* screening and exclusion of *HLA-B*57:01* positive patients from abacavir treatment significantly decreased the incidence of abacavir hypersensitivity. Treatment guidelines and abacavir product labeling in the United States and Europe now recommend (US) or require (EU) prospective *HLA-B*57:01* screening prior to initiation of abacavir to reduce the incidence of abacavir hypersensitivity. *HLA-B*57:01* screening should supplement but must never replace clinical risk management strategies for abacavir hypersensitivity.
Carbamazepine	Seizure, Bipolar disorders & Analgesia Chung, 2010; Ferrell, 2008	*HLA-B*15:02*	Independent studies indicated that patients of East Asian ancestry who carry *HLA-B*15:02* are at higher risk of Stevens-Johnson Syndrome and toxic epidermal necrolysis. Regulators, including the US FDA and the Taiwanese TFDA, have updated the carbamazepine drug label to indicate that patients with ancestry in genetically at risk populations should be screened for the presence of *HLA-B*15:02* prior to initiating treatment with carbamazepine.

Drug	Disease	Gene Variant	Outcome
Irinotecan	Cancer [Innocenti, 2004; Liu, 2008; Schulz, 2009]	*UGT1A1*28*	Variations in the *UGT1A1* gene can influence a patient's ability to break down irinotecan, which can lead to increased blood levels of the drug and a higher risk of side effects. A dose of irinotecan that is safe for one patient with a particular *UGT1A1* gene variation might be too high for another patient without this variation, raising the risk of certain side-effects that include neutropenia following initiation of Irinotecan treatment. The irinotecan drug label indicates that individuals who have two copies of the *UGT1A1*28* variant are at increased risk of neutropenia. A genetic blood test is available that can detect variations in the gene.

A key component to successful PGx research is the collection of samples during the conduct of clinical studies.

Collection of whole blood samples, even when no *a priori* hypothesis has been identified, may enable PGx analysis to be conducted if at any time it appears that there is a potential unexpected or unexplained variation in response to GSK2862277.

Pharmacogenetic Research Objectives

The objective of the PGx research (if there is a potential unexpected or unexplained variation) is to investigate a relationship between genetic factors and response to GSK2862277. If at any time it appears there is potential variability in response in this clinical study or in a series of clinical studies with GSK2862277, the following objectives may be investigated – the relationship between genetic variants and study treatment with respect to:

- Pharmacokinetics and/or pharmacodynamics of study treatment
- Safety and/or tolerability

Study Population

Any subject who is enrolled in the clinical study, can participate in PGx research. Any subject who has received an allogeneic bone marrow transplant must be excluded from the PGx research.

Subject participation in the PGx research is voluntary and refusal to participate will not indicate withdrawal from the clinical study or result in any penalty or loss of benefits to which the subject would otherwise be entitled.

Study Assessments and Procedures

Blood samples can be taken for Deoxyribonucleic acid (DNA) extraction and used in PGx assessments.

If taking blood samples: in addition to any blood samples taken for the clinical study, a whole blood sample (~6ml) will be collected for the PGx research using a tube containing EDTA. It is recommended that the blood sample be taken at the first opportunity after a subject has been randomized and provided informed consent for PGx research, but may be taken at any time while the subject is participating in the clinical study.

- The PGx sample is labelled (or "coded") with a study specific number that can be traced or linked back to the subject by the investigator or site staff. Coded samples do not carry personal identifiers (such as name or social security number). The blood sample is taken on a single occasion unless a duplicate sample is required due to inability to utilize the original sample.

The DNA extracted from the blood sample may be subjected to sample quality control analysis. This analysis will involve the genotyping of several genetic markers to confirm the integrity of individual samples. If inconsistencies are noted in the analysis, then those samples may be destroyed.

The need to conduct PGx analysis may be identified after a study (or a set of studies) of GSK2862277 has been completed and the clinical study data reviewed. In some cases, the samples may not be studied. e.g., no questions are raised about how people respond to GSK2862277.

Samples will be stored securely and may be kept for up to 15 years after the last subject completes the study or GSK may destroy the samples sooner. GSK or those working with GSK (for example, other researchers) will use samples collected from the study for the purpose stated in this protocol and in the informed consent form.

Patients can request their sample to be destroyed at any time.

Subject Withdrawal from Study

If a subject who has consented to participate in PGx research withdraws from the clinical study for any reason other than being lost to follow-up, the subject will be given a choice of one of the following options concerning the PGx sample, if already collected:

- Continue to participate in the PGx research with the PGx sample retained for analysis
- Withdraw from the PGx research and destroy the PGx sample

If a subject withdraws consent for PGx research or requests sample destruction for any reason, the investigator must complete the appropriate documentation to request sample destruction within the timeframe specified by GSK and maintain the documentation in the site study records. The investigator should forward the Pharmacogenetic Sample Destruction Request Form to GSK as directed on the form. This can be done at any time

when a subject wishes to withdraw from the PGx research or have their sample destroyed whether during the study or during the retention period following close of the main study.

Screen and Baseline Failures

If a blood sample for PGx research has been collected and it is determined that the subject does not meet the entry criteria for participation in the clinical study, then the investigator should instruct the participant that their PGx sample will be destroyed. No forms are required to complete this process as it will be completed as part of the consent and sample reconciliation process. In this instance a sample destruction form will not be available to include in the site files.

Pharmacogenetics Analyses

Pharmacogenetics Analyses

1. Specific genes may be studied that encode the drug targets, or drug mechanism of action pathways, drug metabolizing enzymes, drug transporters or which may underpin adverse events, disease risk or drug response. These candidate genes may include a common set of ADME (Absorption, Distribution, Metabolism and Excretion) genes that are studied to determine the relationship between gene variants or treatment response and/or tolerance.

In addition, continuing research may identify other enzymes, transporters, proteins or receptors that may be involved in response to GSK2862277. The genes that may code for these proteins may also be studied.

2. Genome-wide scans involving a large number of polymorphic markers (e.g., single nucleotide polymorphisms) at defined locations in the genome, often correlated with a candidate gene, may be studied to determine the relationship between genetic variants and treatment response or tolerance. This approach is often employed when a definitive candidate gene(s) does not exist and/or the potential genetic effects are not well understood.

If applicable and PGx research is conducted, appropriate statistical analysis methods will be used to evaluate pharmacogenetic data in the context of the other clinical data. Results of PGx investigations will be reported either as part of the main clinical study report or as a separate report. Endpoints of interest from all comparisons will be descriptively and/or graphically summarized as appropriate to the data. A detailed description of the analysis to be performed will be documented in the study reporting and analysis plan (RAP) or in a separate pharmacogenetics RAP, as appropriate.

Informed Consent

Patients who do not wish to participate in the PGx research may still participate in the clinical study. PGx informed consent must be obtained prior to any blood being taken for PGx research.

Provision of Study Results and Confidentiality of Subject's PGx Data

GSK may summarize the PGx research results in the clinical study report, or separately, or may publish the results in scientific journals.

GSK does not inform the investigator, subject, or anyone else (e.g., family members, study investigators, primary care physicians, insurers, or employers) of individual genotyping results that are not known to be relevant to the subject's medical care at the time of the study, unless required by law. This is due to the fact that the information generated from PGx studies is generally preliminary in nature, and therefore the significance and scientific validity of the results are undetermined.

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12.3. Appendix 3: SOFA Score Table

	SOFA Score				
	0	1	2	3	4
Respiration					
Pao_2/Fio_2 (torr)	>400	≤400	≤300	≤200 With respiratory support	≤100 With respiratory support
Coagulation					
Platelets ($\times 10^3/mm^3$)	>150	≤150	≤100	≤50	≤20
Liver					
Bilirubin (mg/dL)	<1.2	1.2–1.9	2.0–5.9	6.0–11.9	>12.0
(μmol/L)	<20	20–32	33–101	102–204	>204
Cardiovascular					
Hypotension	No hypotension	MAP <70 mm Hg	Dopamine ≤5 or dobutamine (any dose)[a]	Dopamine >5 or epi ≤0.1 or norepi ≤0.1[a]	Dopamine >15 or epi >0.1 or norepi >0.1[a]
Central Nervous System					
Glasgow Coma Score	15	13–14	10–12	6–9	<6
Renal					
Creatinine (mg/dL)	<1.2	1.2–1.9	2.0–3.4	3.5–4.9	>5.0
(μmol/L)	<110	110–170	171–299	300–440 or <500 mL/day	>440 or <200 mL/day
or urine output					

epi, epinephrine; norepi, norepinephrine.
[a]Adrenergic agents administered for at least 1 hr (doses given are in μg/kg/min).
To convert torr to kPa, multiply the value by 0.1333.

[Vincent, 1996]

12.4. Appendix 4: Procedures for Detection, Evaluation, Follow-Up and Reporting of Adverse Events and Medical Device Incidents

Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in the appropriate data collection tool.

It is not acceptable for the investigator to send photocopies of the subject's medical records to GSK in lieu of completion of the GSK, AE/SAE data collection tool. However, there may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.

Evaluating AEs and SAEs

Assessment of Intensity

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

Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: An event that prevents normal everyday activities.

An AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality

The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE. A "reasonable possibility" is meant to convey that there are facts/evidence 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 natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated. The investigator will also consult the Investigator Brochure GlaxoSmithKline Document Number 2012N147005_02 and/or Product Information, for marketed products, in the determination of 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 when 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 prior to the initial transmission of the SAE data to GSK.** The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE data collection tool accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up.

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals.

New or updated information will be recorded in the originally completed data collection tool. The investigator will submit any updated SAE data to GSK within the designated reporting time frames.

Reporting of SAEs to GSK

The primary mechanism for reporting SAEs to GSK will be the electronic data collection tool.. If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the GSK Medical Monitor. Then the site will enter the serious adverse event data into the electronic system as soon as it becomes available.

After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) 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, the site can report this information on a paper SAE form or to their GSK protocol contact by telephone.

GSK contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

Medical Devices

Documenting Medical Device Incidents

Any medical device incident occurring during the study will be documented in the subject's medical records, in accordance with the investigator's normal clinical practice, and on the appropriate form. **In addition, for incidents fulfilling the definition of an AE or an SAE, the appropriate AE/SAE data collection tool will be completed as previously described.**

The form will be completed as thoroughly as possible and signed by the investigator before transmittal to GSK. It is **very important that the investigator provides his/her assessment of causality to the medical device provided by GSK at the time of the initial report**, and describes any corrective or remedial actions taken to prevent recurrence of the incident. A remedial action is any action other than routine maintenance or servicing of a device where such action is necessary to prevent recurrence of an incident. This includes any amendment to the design to prevent recurrence.

Follow-up of Medical Device Incidents

All medical device incidents involving an AE, will be followed until resolution of the event, until the condition stabilizes, until the condition is otherwise explained, or until the subject is lost to follow-up. This applies to all patients, including those withdrawn prematurely. The investigator is responsible for ensuring that follow-up includes any supplemental investigations as may be indicated to elucidate as completely as practical the nature and/or causality of the incident.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

12.5. Appendix 5: Protocol Amendment Changes

Amendment 5

Summary of Amendment Changes with Rationale

This amendment was produced to include information regarding inclusion of an external expert to the SRT. The opportunity has also been taken to include minor changes that had previously been documented in File Notes.

List of Specific Changes

CHANGE 1

This text has been updated to clearly reflect the intentions for open/shut cases.

Section 4.4 Withdrawal Criteria and Procedures

REVISED TEXT

In some cases, a subject may be randomised to the study and surgery initiated, but subsequently halted due to a decision being made that the case is in fact inoperable. In the event of an open and shut case subjects will be withdrawn from the study by the investigator. As the subjects will have received a dose of study dose prior to surgery, every effort should be made to complete as many **safety and PK procedures** ~~of the Day 28 follow-up assessments and procedures~~ as possible prior to hospital discharge **and the Day 28 assessments**.

CHANGE 2

Footnote #3 added to clarify screening window for patients that have repeat screening.

Footnote #4 was removed in error in a previous amendment.

Corresponding footnotes have been added to the table.

Section 6.1 Time and Events Table, Table 2

ADDED TEXT

3. **In case of repeat screening, screening can occur less than 7 days prior to dosing if ADA and Quantiferon results are available.**
4. **Can occur any time during the study after eligibility is confirmed and consent provided**

CHANGE 3

Footnote #2 revised to allow flexibility in extenuating circumstances

Footnote #3 revised to allow flexibility for translational samples

Section 6.1 Time and Events Table, Table 3

REVISED TEXT

2. Randomisation & associated IVRS activities may be conducted up-to 72 hours prior to administration of study treatment **(can be longer on agreement with the sponsor for extenuating circumstances e.g. bank holiday weekends).**
3. Only applicable at designated sites. **Can be taken at screening if required.**

CHANGE 4

Added text to provide more detail regarding the safety review team that was already in place and to detail that an external expert has been added to that team.

Section 6.3 Safety

ADDED TEXT

The Safety Review Team (SRT) is a GSK cross-functional team reviewing all available safety data related to the project, including in-stream data from this study, in an ongoing manner. The SRT is an internal GSK requirement put in place to ensure holistic evaluation of the safety profile of an investigational product with systematic, periodic and documented reviews of available safety data, with the appropriate communication and escalation of new findings that have the potential to impact patient safety.

The SRT for this project will review data from this study in collaboration with an independent external (to GSK) expert reviewer.

CHANGE 5

Section has been removed as all SAEs are being recorded for this study and therefore this section is not required.

Section 7.1.6 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as SAEs

DELETED TEXT

~~Certain events are typically associated with the disease under study and are recognised, and common, post-operative problems with Oesphagectomy. These need not be reported according to the standard process for expedited reporting of SAEs to GSK (even though the event may meet the definition of a serious adverse event). These events will be recorded on the DRE page in the subject's CRF within 5 working days. These DREs will be monitored by the Safety Review Team (SRT) on routine basis.~~

~~*NOTE: However, if either of the following conditions apply, then the event must be recorded and reported as an SAE (instead of a DRE):*~~

- ~~*The event is, in the Investigator's opinion, of greater intensity, frequency, or duration than expected for the individual subject, or*~~
- ~~*The Investigator considers that there is a reasonable possibility that the event was related to treatment with the investigational product*~~

CHANGE 6

Section removed as it is not relevant to this study.

Appendix 4: Procedures for Detection, Evaluation, Follow-Up and Reporting of Adverse Events and Medical Device Incidents

DELETED TEXT

~~Subject-completed health outcomes questionnaires and the collection of AE data are independent components of the study. Responses to each question in the health outcomes questionnaire will be treated in accordance with standard scoring and statistical procedures detailed by the scale's developer. The use of a single question from a multidimensional health survey to designate a cause-effect relationship to an AE is inappropriate.~~

Amendment 4

Summary of Amendment Changes with Rationale

The amendment was produced after discussions concerning logistical set-up of the study. Changes include: removal of mandatory 1 hour post-dose ECG (on the condition that alternative cardiac monitoring is in place); 24 hour window has been added for the pre-dose assessments. A typographical error in exclusion criteria #6 has been amended. A PK sample has also been included if a liver event occurs as this had been omitted in error. The opportunity has also been taken to make some other minor amendments.

List of Specific Changes

CHANGE 1

Correction of a typographical error.

Section 4.2.2 Exclusion Criteria #6

PREVIOUS TEXT

Use of corticosteroids (IV, oral or IM) at a dose of $\geq$ 10 mg/day prednisolone (or equivalent) within 14 days prior to dosing, or anti-Tumor Necrosis Factor (anti-TNF) or anti-Interleukin-1 (anti-IL1) within 60 days prior to dosing.

REVISED TEXT

Use of corticosteroids (IV, oral or IM) at a dose of ~~≥~~ > 10 mg/day prednisolone (or equivalent) within 14 days prior to dosing, or anti-Tumor Necrosis Factor (anti-TNF) or anti-Interleukin-1 (anti-IL1) within 60 days prior to dosing.

CHANGE 2

This change is to allow ADA screening and TB testing results to be re-used if repeat screening is required. Also to remove an unclear footnote. Corresponding footnote numbering has been updated.

Section 6.1 Time and Events Table; Table 2

DELETED TEXT

~~3. Can occur any time during the study after eligibility is confirmed and consent provided.~~

ADDED TEXT

3. If repeat screening is required previous results from ADA screening and Quantiferon-GOLD testing can be used for up to 60 days.

CHANGE 3

Removal of mandatory 1 hour post-dose 12-lead ECG to help with the logistical requirements prior to surgery. Cardiac monitoring will be ongoing during this time as standard of care. OI has been removed as it is a derived value and replaced with SpO_2.

Section 6.1 Time and Events Table; Table 3

PREVIOUS TEXT

	Pre-dose	0[1] h	1 h	Immediately prior or at start of surgery	On completion of surgery	
12-lead ECG	X		X			1.Dosing may occur 1-5 hours prior to surgery 2.Randomisation & associated IVRS activities may be conducted up-to 72 hours prior to administration of study treatment. 3.Only applicable at designated sites NB: Time points (except pre-dose) refer to post-start of drug administration on Day 1. Pre-dose is defined as the time from admission to hospital up to the administration of study treatment.
Oxygenation Index				X	X	

REVISED TEXT

	Pre-dose[4]	0[1] h	1 h	Immediately prior or at start of surgery	On completion of surgery	
12-lead ECG	X		X[5]			1. Dosing may occur 1-5 hours prior to surgery 2. Randomisation & associated IVRS activities may be conducted up-to 72 hours prior to administration of study treatment. 3. Only applicable at designated sites **4. Can be taken up to 24 hours prior to dosing** **5. If 12 lead ECG not available, monitoring by 3 lead ECG/telemetry (or similar) is acceptable** NB: Time points (except pre-dose) refer to post-start of drug administration on Day 1. Pre-dose is defined as the time from admission to hospital up to the administration of study treatment.
~~Oxygenation Index~~ **Measure SpO_2 using pulse oximetry**				X	X	

CHANGE 4

Changes have been made to add clarity and to correct errors.

PREVIOUS TEXT

Procedures	Day 2	Day 3	Day 4	Daily until discharge	Day 8[1]	Day of discharge	Day 28 (FU) ±3 days	1. If discharged before Day 8 take blood and urine samples on day of discharge 2. PVPI will be measured via single-indicator transpulmonary thermodilution as long as the subject remains in the ICU with a patent indwelling PiCCO catheter, up to Day 4. 3. Sequential Organ Failure Assessment (SOFA) Score will only be calculated up to Day 4. 4. Sample to be taken 24 to 26 hours post-dose of study treatment. 5. Sample to be taken 46 to 50 hours post-dose of study treatment. 6. Only applicable at designated sites
P/F Ratio (as available) • Arterial blood sample • Record FiO_2	X	X	X					
SOFA Components[3] • PaO2/FiO2 (mmHg) (as available) • Glasgow Coma Score (GCS) • Mean Arterial Pressure or administration of vasopressors required • Bilirubin • Platelets • Creatinine (or urine output)	X	X	X					
SpO_2/FiO_2 ratio: • Pulse Oximetry • Record FiO_2	X	X	X					
Blood samples for translational sub-study[6]		X						
Organ Failure Free Days							X	

REVISED TEXT

Procedures	Day 2	Day 3	Day 4	Daily until discharge	Day 8[1]	Day of discharge	Day 28 (FU) ±3 days	1. If discharged before Day 8 take blood and urine samples on day of discharge 2. PVPI will be measured via single-indicator transpulmonary thermodilution as long as the subject remains in the ICU with a patent indwelling PiCCO catheter, up to Day 4. 3. Sequential Organ Failure Assessment (SOFA) Score will only be calculated up to Day 4. 4**. If no arterial blood sample calculate MAP using equation: 2X diastolic blood pressure + systolic blood pressure. Then divide by 3.** 5. Only applicable at designated sites 6. Sample to be taken 24 to 26 hours post-dose of study treatment. 7. Sample to be taken 46 to 50 hours post-dose of study treatment. **8. Organ Failure Free Days needs to be recorded every day until Day 28 (See SPM).**
P/F Ratio ~~(as available)~~ • Arterial blood sample **(as available)** • Record FiO_2	X	X	X					
Measure SpO_2 using pulse oximetry	**X**	**X**	**X**					
Additional SOFA Components[3] • ~~PaO2/FiO2 (mmHg) (as available)~~ • Glasgow Coma Score (GCS) • **Record** Mean Arterial Pressure[4] or administration of vasopressors required • Bilirubin • Platelets • Creatinine (or urine output)	X	X	X					
~~SpO_2/FiO_2 ratio:~~ • ~~Pulse Oximetry~~ • ~~Record FiO_2~~	~~X~~	~~X~~	~~X~~					
Blood samples for translational sub-study[5]	**X**	~~X~~						
Organ Failure Free Days							X[8]	

CHANGE 5

Change made to correct inaccuracy and to add clarity regarding requirements for cardiac monitoring at 1 hour post-dose.

Section 6.3.3 Electrocardiogram (ECG)

PREVIOUS TEXT

Single 12-lead ECGs will be obtained at each time point during the study using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, RR and QTc intervals.

REVISED TEXT

Triplicate 12-lead ECGs will be obtained at screening and single 12-lead ECGs will be obtained ~~at each time point~~ **thereafter** during the study, using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, RR and QTc intervals.

At the 1 hour post-dose time point monitoring by 3 lead ECG/telemetry (or similar) is acceptable if 12 lead ECG is not available.

CHANGE 6

Changes made for clarity.

Section 6.7 Efficacy

ADDED TEXT

6.7.1 Transpulmonary Thermodilution

Measurement of PVPI and EVLWI

Other supportive parameters from the PiCCO monitor that are calculated at the same time as EVLW and PVPI, may also be databased e.g. GEDV, SVV, SVR, HR etc.

ADDED TEXT

6.7.3 Pulse Oximetry

SpO2 will be collected using pulse oximetry. This will be used to calculate SOFA score when a patient no longer has an arterial line to calculate PaO_2.

PREVIOUS TEXT

6.7.4 Oxygenation Index

Oxygenation index (OI) (sometimes termed *oxygenation factor*) is another measure of gas exchange and pulmonary efficiency which is also reflective of changes in intrapulmonary shunt. OI calculation takes into account the P to F assessment but crucially also takes into account important variables governing mechanical ventilation such as inspiratory time, Positive End Expiratory Pressure (PEEP) and tidal volume via the addition of the mean airway pressure (Paw) term in the calculation step [El-Khatib, 2004].

Further details can be found in the SPM.

REVISED TEXT

6.7.4 Oxygenation Index

Oxygenation index (OI) (sometimes termed *oxygenation factor*) is another measure of gas exchange and pulmonary efficiency which ~~is also reflective of changes in intrapulmonary shunt. OI calculation takes into account the P to F assessment but crucially also takes into account important variables governing mechanical ventilation such as inspiratory time, Positive End Expiratory Pressure (PEEP) and tidal volume via the addition of the~~ **factors in the** mean airway pressure (Paw) term in the calculation step [El-Khatib, 2004].

It is calculated using the equation:

Oxygenation Index = (F_iO_2 * Mean Airway Pressure) / P_aO_2

Further details can be found in the SPM.

PREVIOUS TEXT

6.7.5 The Sequential Organ Failure Assessment (SOFA) score

The SOFA score is made up of the following components:

- PaO_2/FiO_2 (mmHg)

REVISED TEXT

6.7.5 ~~The~~ Sequential Organ Failure Assessment (SOFA) score

The SOFA score is made up of the following components:

- PaO_2/FiO_2 (mmHg) **or SpO_2/FiO_2 (mm/Hg)**

CHANGE 7

Added wording to collect a PK blood sample if there is a liver event. This had been omitted in error.

Appendix 1: Liver Safety Process

ADDED TEXT

- **<u>Blood sample for pharmacokinetic (PK) analysis, obtained within 72 hours from last dose. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are included in the SPM.</u>**

CHANGE 8

Update of suppliers.

Section 12.6 Appendix 6 Preparation of Active and Placebo Drug for Dosing

PREVIOUS TEXT

- Pari eFlow Filter Valve Set (041G0500) (supplied by Fisher Scientific)
- Pari disposable filters (for use with Filter Valve Set) (041B0522/3) (supplied by Fisher Scientific)

REVISED TEXT

- Pari eFlow Filter Valve Set (041G0500) (supplied by ~~Fisher Scientific~~ **<u>Ancillare</u>**)
- Pari disposable filters (for use with Filter Valve Set) (041B0522/3) (supplied by ~~Fisher Scientific~~ **<u>Ancillare</u>**)

Amendment 3

Amendment 3 was produced after discussions concerning logistical set-up of the study. Changes include: widening the window for dosing study treatment; incorporating a process for instances of inoperable cases (so called 'open-and-shut' cases); and allowing randomisation to be conducted up-to 72 hours prior to day of surgery.

Section 3.3.1

Previous Text:

Patients will be randomized via a central allocation system to receive either GSK2862277 or placebo as an inhaled (via nebulisation) dose. A single inhaled dose of GSK2862277 or placebo to match will be administered approximately 1-3 hours prior to the subjects scheduled surgery, before the initiation of pre-operative procedures.

Revised Text:

Patients will be randomized via a central allocation system to receive either GSK2862277 or placebo as an inhaled (via nebulisation) dose. A single inhaled dose of GSK2862277 or placebo to match will be administered approximately 1-**5** hours prior to the subjects **start of (knife to skin)** surgery, before the initiation of pre-operative procedures.

Section 4.3

Additional text:

A screen failure is defined as any subject who has been assigned a subject identifier, but does not continue in the study beyond Visit 1 (screening) or any subject who completes Visit 1 but is subsequently found to be ineligible for the study based on findings from laboratory or any other screening test conducted at Visit 1.

Additionally, if a subject completes written informed consent and experiences a serious adverse event (SAE) in the time period between completing written informed consent and the planned Visit 1 date, the subject will be assigned a subject identifier and classified as a screen failure.

The interactive voice response system (IVRS) used to track study enrolment will be notified and at a minimum the following information will be collected in the eCRF for screen failures:

- Date of screening visit
- Subject identifier
- Demographic information, including race, age and gender
- Inclusion/exclusion criteria

- Reason subject failed screening

Serious Adverse Events information, if applicable, for any SAE that occurred in the time period between completing written informed consent and planned Visit 1 date

Section 4.4

Additional Text:

In some cases, a subject may be randomised to the study and surgery initiated, but subsequently halted due to a decision being made that the case is in fact inoperable. In the event of an open and shut case subjects will be withdrawn from the study by the investigator. As the subjects will have received a dose of study dose prior to surgery, every effort should be made to complete as many of the Day 28 follow-up assessments and procedures as possible, prior to hospital discharge.

Section 5.6

Additional Text:

A description of the methods and materials required for preparation of GSK2862277 and reconstitution will be detailed in Section 12.6 and the Study Procedures Manual (SPM).

Additional Text:

Blood sample for PK**/Urea/Total Protein**.

Section 6.1 Additional rows in Time & Events Tables

	Pre-dose	0[1] h	1 h	Immediately prior or at start of surgery	On completion of surgery
Randomisation	X[2]				
Brief Physical Examination	X				
Concomitant Medication	X				
IVRS	X[2]				
Vital Signs	X		X		
12-lead ECG	X		X		
Adverse Events	X				
Serious Adverse Events	X				
Clinical Labs including Haematology and Chemistry	X				
Blood sample for immunogenicity	X				
Blood sample for PK	X		X		X
Blood sample for urea and total protein					X
Blood samples for translational sub-study[3]	X				X
Sample for Urinalysis	X				
Blood sample for serum biomarkers	X				X
Nebulised (IH) Study Treatment		X			
Transpulmonary thermodilution				X	X
P/F Ratio: • Arterial blood sample • Record FiO_2				X	X
Oxygenation Index				X	X
BAL Sampling					X

1. Dosing may occur 1-5 hours prior to surgery
2. Randomisation & associated IVRS activities may be conducted up-to 72 hours prior to administration of study treatment.
3. **Only applicable at designated sites**

NB: Time points (except pre-dose) refer to post-start of drug administration on Day 1.
Pre-dose is defined as the time from admission to hospital up to the administration of study treatment.

Procedures	Day 2	Day 3	Day 4	Daily until discharge	Day 8[1]	Day of discharge	Day 28 (FU) ±3 days	1. If discharged before Day 8 take blood and urine samples on day of discharge 2. PVPI will be measured via single-indicator transpulmonary thermodilution as long as the subject remains in the ICU with a patent indwelling PiCCO catheter, up to Day 4. 3. Sequential Organ Failure Assessment (SOFA) Score will only be calculated up to Day 4. 4. Sample to be taken 24 to 26 hours post-dose of study treatment. 5. Sample to be taken 46 to 50 hours post-dose of study treatment. 6. **Only applicable at designated sites**
Concomitant Medication	X	X	X	X		X	X	
IVRS						X	X	
Transpulmonary thermodilution[2]	X	X	X					
P/F Ratio (as available) • Arterial blood sample • Record FiO_2	X	X	X					
SOFA Components[3] • PaO2/FiO2 (mmHg) (as available) • Glasgow Coma Score (GCS) • Mean Arterial Pressure or administration of vasopressors required • Bilirubin • Platelets • Creatinine (or urine output)	X	X	X					
SpO_2/FiO_2 ratio: • Pulse Oximetry • Record FiO_2	X	X	X					
Vital Signs			X			X	X	
12-lead ECG	X		X			X		
Adverse Events	X	X	X	X	X	X	X	
Serious Adverse Events	X	X	X	X	X	X	X	
Clinical Labs including Haematology and Chemistry	X	X	X		X			
Blood sample for serum biomarkers	X	X	X		X			
Blood samples for translational sub-study[6]		X						
Blood sample for Immunogenicity					X		X	
Blood sample for PK	X[4]	X[5]						
Sample for Urinalysis					X			
Organ Failure Free Days							X	
ARDS diagnosis status						X		
Ventilator Free Days							X	

Procedures	Day 2	Day 3	Day 4	Daily until discharge	Day 8[1]	Day of discharge	Day 28 (FU) ±3 days	
ICU & Hospital Length of Stay						X		
Survival							X	

Section 6.3.5. Clinical Laboratory Assessments

Clinical Chemistry Table: Total Protein Removed

Section 6.5.3

Previous Text

Blood (serum and plasma) and BAL sample(s)...

Revised Text

Blood ~~(serum and plasma)~~ and BAL sample(s)...

Previous Text

Candidate biomarkers and subsequently discovered biomarkers of the biological response associated with the development of ARDS or medically related conditions and/or the action of GSK2862277 may be identified by application of:

- Detection of protein endpoints in serum and BAL by immunoassay and/or equivalent alternative technologies (these may include but are not limited to vWF and sRAGE).
- Total protein determination in plasma for protein permeability analysis

Revised Text

Candidate biomarkers and subsequently discovered biomarkers of the biological response associated with the development of ARDS or medically related conditions and/or the action of GSK2862277 may be identified by application of:

- Detection of protein endpoints in serum and BAL by immunoassay and/or equivalent alternative technologies (these may include but are not limited to vWF and sRAGE).
- Total protein determination in plasma for protein permeability analysis
- **Cellular assays and RNA analysis**

Amendment 2

Amendment 2 was made to reduce the upper threshold for AST & ALT liver enzyme values for inclusion in the study, as per instruction from the UK competent authority (Medicines and Healthcare Regulatory Authority [MHRA]). A change to the QTc inclusion criteria was also made to correct an error not previously identified.

Previous text:

5. Liver parameters according to the thresholds below: AST and ALT < 5xULN; alkaline phosphatase and bilirubin ≤ 1.5xULN (isolated bilirubin > 1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
6. QTcB or QTcF ≦ 480 msec at screening

Either QTcB or QTcF, machine or manual over-read can be used. This applies to both males and females. The QT correction formula used to determine inclusion and discontinuation for an individual subject should be the same throughout the study.

Revised text:

5. Liver parameters according to the thresholds below: AST and ALT < **3xULN**; alkaline phosphatase and bilirubin ≤ 1.5xULN (isolated bilirubin > 1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
6. QTcB or QTcF ≦ **450** msec at screening

Either QTcB or QTcF, machine or manual over-read can be used. This applies to both males and females. The QT correction formula used to determine inclusion and discontinuation for an individual subject should be the same throughout the study.

Amendment 1

Amendment 1 was made to correct an error in the protocol title:

Previous text:

A Placebo Controlled, Double-blind, Multi-centre, Repeat Dose, Parallel Group, Randomised Clinical Trial of GSK2862277 in Patients undergoing Oesophagectomy Surgery

Revised text:

A Placebo Controlled, Double-blind, Multi-centre, Single Dose, Parallel Group, Randomised Clinical Trial of GSK2862277 in Patients undergoing Oesophagectomy Surgery

A revision to the exclusion criteria around alcohol consumption was also made:

Previous text:

7. 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.

Revised text:

3. History of regular alcohol consumption within 6 months of the study, defined as:
    - an average weekly intake of >28 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.

12.6. Appendix 6 Preparation of Active and Placebo Drug for Dosing

Subjects will be administered either a single 26mg dose of GSK2862277 or placebo via IH.

For IH administration, compatibility has been established with the following components:

Material Use	Component Composition
Syringes	Polypropylene
Needles	18G to 23G Stainless Steel

The following materials will be required (if catalogue numbers change then you will be notified):

- GSK2862277, 40mg vials (supplied by GSK)
- Placebo/Diluent to Match IH GSK2862277 (supplied by GSK)
- Reconstitution Fluid for IH GSK2862277 (supplied by GSK)
- Nebuliser System (Controller + Handset) (PARI eFlow nebuliser with 4 mL cup and size 30 mesh, article number 678G2003) (supplied by GSK)
- Additional Nebuliser Handsets (PARI eFlow nebuliser with 4 mL cup and size 30 mesh, article number 678G8203) (supplied by GSK).
- Pari eFlow Filter Valve Set (041G0500) (supplied by Ancillare)
- Pari disposable filters (for use with Filter Valve Set) (041B0522/3) (supplied byAncillare)

12.6.1. Dose Preparation for 26mg GSK2862277

1. For each subject, remove 1 vial of GSK2862277, 40mg/vial from 2°C to 8°C storage.
2. Reconstitute GSK2862277, 40mg/vial lyophilized DP to 20mg/mL solution of GSK2862277 by injecting 2.3mL Reconstitution Fluid for IH GSK2862277 directly into the vial using a fresh 3mL syringe and needle assembly (Use a fresh syringe and needle assembly to reconstitute each lyophilized DP vial). Following addition of Reconstitution Fluid for IH GSK2862277, gently swirl the vial to dissolve the lyophilized cake. Avoid vigorous shaking or agitation of the reconstituted 20mg/mL solution of GSK2862277.
3. ***Prior to withdrawal into syringe,*** invert the vial 5 times to ensure complete mixing. Then, using a fresh needle and a 3mL PP syringe, slowly withdraw 1.3mL reconstituted 20mg/mL solution of GSK2862277 and add to nebuliser cup.

This reconstituted solution in the syringe must be transferred to the nebulizer cup as soon as possible. The reconstituted 20mg/mL solution of GSK2862277 is stable for 12 hours at 25°C and for 24 hours when stored at 5°C in the original vial.

Dosing must occur within 24hrs of reconstitution of the GSK2862277 lyophilised DP.

12.6.1.1. Preparation of placebo dose

1. Use 1 vial of GSK2862277 Placebo/Diluent to Match IH GSK2862277 for each subject.
2. Using a fresh needle and a 3mL PP syringe, slowly withdraw 1.3mL and add to nebuliser cup.

This solution can be stored for 12 hours at 25°C and for 24 hours at 5°C prior to use.

12.6.2. Instructions for Transportation

If the reconstituted 20mg/mL solution of GSK2862277 needs to be transported from a pharmacy to a clinical site, the solution must be stored at 2°C to 8°C in the glass vial (primary container) prior to transportation. The transportation must take no more than 4 hours at 2°C to 8°C while the reconstituted solution is in the glass vial.

Dosing must occur within 24hrs of reconstitution of the GSK2862277 lyophilised DP while stored at 2°C to 8°C.