TITLE PAGE

Protocol Title: Study to elucidate the association of the Renin-angiotensin system and right ventricular function in mechanically ventilated patients

Protocol Number: 205821 – Amendment 3

Short Title: Study of Renin-angiotensin system in mechanically ventilated patients

Compound Number: Non-Compound Specific

Sponsor Name and Legal Registered Address:

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SPONSOR SIGNATORY

PPD

27 Feb 2019

Aili Lazaar, MD, PPD
Discovery Medicine
Clinical Pharmacology and Experimental Medicine

Date

PPD

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Protocol Amendment 3	27-Feb-2019
Protocol Amendment 2	27-Nov-2017
Protocol Amendment 1	02-Jun-2017
Original Protocol	26-Apr-2017

The Summary of Changes tables for Protocol Amendment 1 (02-Jun-2017) and Protocol Amendment 2 (27-Nov-2017) are in Section 12.6 (Appendix 6).

Amendment 3 (27-FEB-2019)

Overall Rationale for the Amendment: The primary reason for the protocol amendment is to increase the duration that a participant may be mechanically ventilated, before they are included in the study – from ≤24 hours to ≤48 hours. This change has been made to assist enrolment of participants into the study, but the sponsor considers that it will not impact the objectives of the study. Additionally, minor clarifications have been made.

In the table below, in the column 'Description of Change', new text is shown in bold and deleted text has been striked through.

Section # and Name	Description of Change	Brief Rationale
Sponsor Signatory	~~Andy Fowler~~ ~~Discovery Medicine Physician, Respiratory Therapy Area~~ **Aili Lazaar, MD,** **Discovery Medicine** **Clinical Pharmacology and Experimental Medicine**	Sponsor signatory change due to change in study personnel.
2. Schedule of activities – *Simplified acute physiology score (SAPS II)*	SAPS II score to be taken within approximately 24 hours of admission to intensive care. Not at time of intubation.	To align the protocol with standard of care.

Section # and Name	Description of Change	Brief Rationale
2. Schedule of activities *Echocardiogram*	First echocardiogram must be completed within ~~24~~ **48** hours of starting mechanical ventilation **(but ≤24 hours, whenever possible)**	To align the protocol with the revised inclusion criteria – that a participant may be mechanically ventilated for ≤48 hours before inclusion in the study.
5. Study Design – *5.1 Overall study design*	The initial echocardiogram will take place within ~~24~~ **48** hours of mechanical ventilation **(but ≤24 hours, whenever possible).** Figure 1 updated to show up to 48 hours from mechanical ventilation to inclusion.	To align the protocol with the revised inclusion criteria – that a participant may be mechanically ventilated for ≤48 hours before inclusion in the study.
6. Study Population *6.1 Inclusion Criteria* Inclusion 2	2. Participants who are receiving invasive mechanical ventilation (duration of ventilation ~~≤24~~ **≤48** hours, **but ≤24 hours, whenever possible**).	To increase the duration that a participant may be mechanically ventilated, before they are included in the study – from ≤24 hours to ≤48 hours. This change has been made to assist enrolment of participants into the study.
6. Study Population *6.2 Exclusion Criteria*	Investigator will make every effort to ensure that the following exclusion criteria 3 to 10 are met; however, in some instances it may not be possible to assess all of these criteria within the ~~24~~ **48**-hour window.	To align the protocol with the revised inclusion criteria – that a participant may be mechanically ventilated for ≤48 hours before inclusion in the study.
9.2 Pharmacodynamics *9.2.1 Echocardiograms*	The first echocardiogram must be undertaken within ~~24~~ **48** hours of the start of mechanical ventilation (**but within 24 hours, whenever possible**)	To align the protocol with the revised inclusion criteria – that a participant may be mechanically ventilated for ≤48 hours before inclusion in the study.
9.5 Participant Status *9.5.1 SAPS II*	The SAPS II score ~~will be measured at screening only and~~ is comprised of the following components:	Removed timing of SAPS II score, because this is described more accurately in the Schedule of Activities (Section 2).

Section # and Name	Description of Change	Brief Rationale
9.7.1 Serious Adverse Events (SAE)	Although no investigational product will be given, SAEs will be recorded, **as follows: SAEs relating to study procedures; and SAEs related to GlaxoSmithKline (GSK) medication used as co-medication.** This ensures that any SAEs relating to study procedures or any GSK medication used as co-medication, are captured.	Clarification made in the SAE section, but no change in the guidance.

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1. SYNOPSIS

Protocol Title: Study to elucidate the association of the Renin-angiotensin system and right ventricular function in mechanically ventilated patients

Short Title: Study of Renin-angiotensin system in mechanically ventilated patients

Rationale:

Right ventricular (RV) dysfunction may result from increased RV afterload secondary to increased pulmonary vascular resistance in patients with severe respiratory failure, including acute respiratory distress syndrome (ARDS). RV dysfunction, particularly severe dysfunction [acute cor pulmonale (ACP)], has been associated with worse outcomes in critically ill patients, including patients with ARDS. Increased mortality has been associated with RV dilatation and increased pulmonary artery pressures [termed pulmonary circulatory dysfunction (PCD)]. The Renin-angiotensin system (RAS) is a key regulator of vascular tone in the pulmonary vasculature and Angiotensin II (Ang II) in particular drives both vasoconstriction and vascular remodelling in human diseases such as pulmonary arterial hypertension. RAS peptides such as Ang II and Ang(1-7) may play a role in the observed changes in pulmonary vasoconstriction in patients with severe respiratory failure with consequent adverse effects on the right ventricle. This study is designed to assess whether circulating Ang II and Ang(1-7) levels are associated with RV dysfunction in mechanically ventilated patients. It is also designed to further characterise the patient population, including severity of RV dysfunction.

Objectives and Endpoints:

Objectives	Endpoints
Primary	

• To evaluate the association between plasma Ang II levels and RV function in mechanically ventilated participants.	• Ang II levels
	• Echocardiographic measures:
	  • Ratio of right ventricular to left ventricular end-diastolic area
	  • Paradoxical septal motion
	  • Pulmonary arterial systolic pressure (PASP) estimated from transtricuspid pressure and right atrial pressure or inferior vena cava (IVC) diameter (whichever is available)
	Up to and including Day 3 of observation

Objectives	Endpoints
Secondary	
• To define the incidence of ACP and PCD in mechanically ventilated participants.	• Presence of PCD, ACP (severe PCD) and severe ACP Up to and including Day 3 of observation

• To evaluate the association between plasma Ang(1-7) levels, Ang II/Ang(1-7) ratio and RV function in mechanically ventilated participants.	• Ang(1-7) levels
	• Ang II/Ang(1-7) ratio
	• Echocardiographic measures:
	  • Ratio of right ventricular to left ventricular end-diastolic area
	  • Paradoxical septal motion
	  • PASP estimated from transtricuspid pressure and right atrial pressure or IVC diameter (whichever is available)
	Up to and including Day 3 of observation

Overall Design:

This study will investigate the association of RAS peptides and markers of RV function, as measured by echocardiography, in participants requiring acute mechanical ventilation. It is a low interventional study without an investigational product. Participants will be evaluated over a 3-day period using standard of care investigations, including transthoracic echocardiography (TTE) and/or transesophageal echocardiography (TOE). Three separate echocardiographs over three consecutive days will be undertaken, and at the same time point blood samples will be taken for assessment of RAS peptides to allow the association with right ventricular function to be assessed.

Number of Participants:

A total of approximately 150 participants are planned to be included.

Treatment Groups and Duration:

There are no treatment groups as no investigational product will be administered.

The total duration of this study for participants is 28 days. The study period is 3 days and health outcomes will be recorded daily for up to 28 days. Health outcomes will not be recorded beyond hospital discharge.

2. SCHEDULE OF ACTIVITIES (SOA)

Procedure	Study Period					Notes
	Screening[1]	Day 1	Day 2	Day 3	Up to day 28	
Start of mechanical ventilation	X					
Inclusion/exclusion criteria	X					Reason for intubation (underlying diagnosis e.g. sepsis, pneumonia) to be collected where possible.
Informed consent (ICF)	X					Consent process will be followed as outlined in Informed Consent Process (Section 12.3.3)
Demography	X					If it is not possible to collect at screening can be collected at any point during the study.
Height and weight	X					Collected for the purposes of evaluating BMI. Clinical estimates are acceptable if direct measurement is not possible.
Medical history	X					If possible to collect, includes medication and cardiovascular risk factors. If it is not possible to collect at screening can be collected at any point during the study.
Diagnosis of pulmonary circulatory dysfunction (PCD) and acute cor pulmonale (ACP)		X	X	X		As per echocardiographic output.
Participant status	X	X	X	X		Intubation status, proned status; diagnosis of acute respiratory distress syndrome (ARDS) including severity at time of diagnosis (if available). Post screening status to be assessed at the same time point as echocardiogram (± 1 hour).
Participant management	X	X	X	X		Management with extracorporeal membrane oxygenation (ECMO) and extracorporeal CO_2 ($ECCO_2R$) removal. Post screening status to be assessed at the same time point as echocardiogram (± 1 hour).
Simplified acute physiology score (SAPS II)	X					To be taken within approximately 24 hours of admission into the intensive care unit, as per standard of care.

Procedure	**Study Period**					**Notes**
	Screening[1]	**Day 1**	**Day 2**	**Day 3**	**Up to day 28**	
Sequential Organ Failure (SOFA) score	X	X	X	X		
Echocardiogram		X	X	X		Transthoracic echocardiogram (TTE) and/or transoesophageal echocardiogram (TOE). First echocardiogram must be completed within 48 hours of starting mechanical ventilation (but ≤24 hours, whenever possible); the following echocardiograms will be undertaken as per standard of care 24 hours apart (± 2 hours) on subsequent consecutive days (Day 2 and 3).
Vital signs	X[3]	X	X	X		Blood pressure (mean arterial, systolic and diastolic), heart rate, body temperature to match echocardiogram time points (± 30 minutes)[2].
Laboratory assessments	X	X	X	X		Clinical chemistry and haematology.
Ventilator settings	X[3]	X	X	X		Includes tidal volume, respiratory rates, static respiratory compliance, level of positive end expiratory pressure (PEEP), peak and plateau ventilator pressures, mean airway pressure and driving pressure. Measurements to be taken at intubation and at the time of each echocardiogram (± 30 mins)[2].
Lung function and blood gas measures	X[3]	X	X	X		Oxygen saturation (SaO_2) via pulse oximetry. Arterial Blood Gases (ABGs) measuring partial pressure of oxygen and carbon dioxide (PaO_2 and $PaCO_2$).[4] Oxygen requirement (FiO_2) should be documented at the same time point. pH, lactate and bicarbonate levels and base excess or deficit when available should also be documented when ABGs are assessed. Measurements to be taken at intubation and at the time of each echocardiogram (± 30 mins)[2].
Electrical Impendance Tomography	X	X	X	X		Measurements to be taken at intubation and at the time of each echocardiogram when available (±2 hours)[2].

Procedure	**Study Period**					**Notes**
	Screening[1]	**Day 1**	**Day 2**	**Day 3**	**Up to day 28**	
Serious Adverse Event (SAE) Review	<==========>					SAEs will only be collected during the 3-day observation period or until participant withdrawal, whichever is sooner.
Concomitant medication review	<==========>					Only specified prescription medication (name, start and end dates required) as described in Section 7.1. Vasopressor use to be documented for each time point of echocardiography.
Intensive Care Unit (ICU) length of stay		<==========>				Will be recorded daily up to 28 days.
Ventilator free days		<==========>				Will be recorded daily up to 28 days; however, follow up is not necessary beyond hospital discharge.
In hospital mortality		<==========>				Will be recorded daily up to 28 days; however, follow up is not necessary beyond hospital discharge.
Biomarkers						
Blood sample for renin-angiotensin system (RAS) biomarkers		X	X	X		Blood samples to be taken at the same time as echocardiography (± 30 mins)[2].
Blood sample for biomarkers		X	X	X		Blood samples to be taken at the same time as echocardiography (± 30 mins)[2].

1. Screening may be considered part of Day 1.
2. When multiple measurements are to be taken at the same time in relation to echocardiography, blood sampling for RAS peptides should be given priority. Other measurements (e.g. ventilator settings) to be prioritized as per investigator for patient care.
3. Will be recorded at the time of intubation if available.
4. Where available, a blood sample is required for arterial blood gases (ABGs) for PaO_2/FiO_2 and oxygenation index. A FiO_2 measurement should be assessed at the same time point as ABGs are taken for PaO_2.

Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files, but will not constitute a protocol amendment. The Independent Ethics Committees (IEC) will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICF.

3. INTRODUCTION

3.1. Study Rationale

Right ventricular (RV) dysfunction may result from increased RV afterload secondary to increased pulmonary vascular resistance in patients with severe respiratory failure including acute respiratory distress syndrome (ARDS). RV dysfunction, particularly severe dysfunction [acute cor pulmonale (ACP)], has been associated with worse outcomes in critically ill patients including patients with ARDS [Boissier 2013; Mekontso-Dessap 2016]. Patients with changes in RV size and pulmonary artery pressures [termed pulmonary circulatory dysfunction (PCD)] have also been observed to have an increased mortality with particularly high mortality in those patients with ACP (designated as severe PCD in this study) [Bull 2010, Boissier 2013]. The renin-angiotensin system (RAS) is a key regulator of vascular tone in the pulmonary vasculature and Angiotensin II (Ang II) in particular is thought to play a role in both vasoconstriction and vascular remodelling in human diseases such as pulmonary arterial hypertension. RAS peptides such as Ang II and Ang(1-7) may play a role in the observed changes in pulmonary vasoconstriction in patients with severe respiratory failure. This study is designed to assess whether circulating Ang II and Ang(1-7) levels are associated with RV dysfunction in mechanically ventilated patients. It is also designed to further characterise the patient population including the severity of RV dysfunction.

3.2. Background

Increased pulmonary vascular resistance (PVR) is a common finding in patients with severe respiratory failure [Zapol 1977; Bull 2010]. There is evidence of worse outcomes for patients with increased PVR. PVR has been observed to fall in survivors, whilst remaining elevated in patients who died [Zapol 1977]. PVR may often lead to pulmonary hypertension and increased RV afterload, a frequent complication of ARDS [Ware 2000]. The acutely increased after-load of the RV causes RV dilatation, impaired RV function, and reduced cardiac output. Worse outcomes, including increased mortality, have been observed in patients with ARDS and RV dysfunction [Bull 2010; Boissier 2013]. In a study of 752 patients, hospital mortality in ARDS patients with ACP was 48% (compared with 42% in non ACP patients), rising to 57% with severe ACP [Mekontso-Dessap 2016]. Patients with ACP exhibit a higher incidence of shock, increased heart rates and lower arterial pressures.

The mechanism for increased RV afterload in patients with respiratory failure is multi-factorial and may result from pulmonary vasoconstriction, micro-embolism and/or endothelial dysfunction in addition to the direct effects of mechanical ventilation. Pulmonary vasoconstriction may be mediator induced and the RAS is a key regulator of vascular tone in the pulmonary vasculature. Ang II in particular drives both acute vasoconstriction and vascular remodelling in human diseases including pulmonary arterial hypertension. Ang(1-7) appears to be a counter-regulatory peptide in the RAS, associated with vasodilation, anti-proliferative and anti-inflammatory activity, and reduced vascular leak. Specifically, the RAS regulates pulmonary vascular tone with an increase in the Ang II/Ang(1-7) ratio, driving increased PVR with subsequent deleterious effects on RV function. Therefore, whilst other effects e.g. mechanical ventilation may

affect pulmonary vascular resistance, in some patients with respiratory failure, activation of the RAS may contribute to pulmonary vascular and consequently RV dysfunction.

Attenuation of RAS-mediated vasoconstriction, without compromising the normo-adaptive pulmonary vascular response, may improve pulmonary blood flow, cardiac output, ventilation/perfusion matching and reduce afterload which should ultimately improve outcomes in mechanically ventilated patients. RAS mediated vasoconstriction might be improved through the dual action of angiotensin converting enzyme (ACE2) by simultaneously reducing Ang II and forming Ang(1-7). Thus, increasing the total amount of active enzyme may be beneficial in the treatment of RV dysfunction in patients with severe respiratory failure [Tom 2001; Idell 1987; Santos 2003; Wenz 2000].

Data exploring the RAS status and its association with acute changes in pulmonary hemodynamics and worsening RV function is limited in patients with severe respiratory failure, including in those who are mechanically ventilated. This study is designed to test the hypothesis that an association exists between RAS peptides, primarily plasma Ang II and Ang(1-7) levels, and RV function in mechanically ventilated patients. The hypothesis will be tested by assessing the plasma levels of RAS peptides and RV function as measured by echocardiography assessing RV size, sepal kinetics and degree of pulmonary hypertension as measured by pulmonary arterial systolic pressure (PASP). A secondary aim of the study is to further characterise this population to inform future interventional studies, by assessing the incidence and severity of RV dysfunction, and assessing the variability of key echocardiographic parameters including RV size and PASP as well as RAS peptide levels in mechanically ventilated patients. An exploratory aim of the study is to consider regional lung ventilation and perfusion assessed by global inhomogeneity index, which may improve understanding of venitilation/perfusion matching in patients undergoing mechanical ventilation with changes in pulmonary vascular resistance.

3.3. Benefit/Risk Assessment

3.3.1. Risk Assessment

There is no investigational product administered in this study.

This is a low interventional study without administration of an investigational product. All procedures with the exception of 3 blood draws are standard of care in the involved centres, including TTE/TOE assessments. Therefore, there is a limited extra risk to the participants beyond standard of care.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures (if applicable)		
Phlebotomy for biomarkers: Bruising, irritation or redness at the site of blood draw	Blood samples will be collected for assessment of RAS peptides and other biomarkers of relevance	Careful blood draw to minimise bruising, irritation and redness; samples may be collected from central or arterial line already in place.

3.3.2. Benefit Assessment

There is no benefit to the individual participant from being included as part of the study.

3.3.3. Overall Benefit:Risk Conclusion

Although individual participants will not benefit, the information derived from the study will inform future interventions in an area of high unmet medical need. Given the limited additional procedures beyond standard of care (3 blood draws) there is limited extra risk to the participants beyond standard of care. Consequently, given the potential future benefit to critically unwell participants, the small additional risk of the blood draws is considered justified.

4. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	

• To evaluate the association between plasma Ang II levels and RV function in mechanically ventilated participants.	• Ang II levels
	• Echocardiographic measures:
	  • Ratio of right ventricular to left ventricular end-diastolic area
	  • Paradoxical septal motion
	  • PASP estimated from transtricuspid pressure and right atrial pressure or IVC diameter (whichever is available)
	Up to and including Day 3 of observation

Secondary	
• To define the incidence of ACP and PCD in mechanically ventilated participants.	• Presence of PCD, ACP (severe PCD) and severe ACP Up to and including Day 3 of observation

• To evaluate the association between plasma Ang(1-7) levels, AngII/Ang(1-7) ratio and RV function in mechanically ventilated participants.	• Ang(1-7) levels
	• Ang II/Ang(1-7) ratio
	• Echocardiographic measures:
	  • Ratio of right ventricular to left ventricular end-diastolic area
	  • Paradoxical septal motion
	  • PASP estimated from transtricuspid pressure and right atrial pressure or IVC diameter (whichever is available)
	Up to and including Day 3 of observation

Objectives	Endpoints
Exploratory	

• To evaluate the association between plasma Ang II and Ang(1-7) levels and other measures of right ventricular function in mechanically ventilated participants.	• Ang II, Ang(1-7) levels
	• Echocardiographic measures:
	  • Paradoxical septal motion (systolic eccentricity index)
	  • Maximal velocity of s wave (tissue Doppler imaging) at the tricuspid annulus
	  • Fractional area contraction (end diastolic area minus end systolic area normalised to end diastolic area)
	  • Acceleration time of RV ejection flow
	  • Mean acceleration of RV ejection flow (maximum velocity/ acceleration time) at end-expiration and end-inspiration
	  • Inferior vena cava diameter at end-expiration
	  • Respiratory variations of RV velocity time integral (end-expiration minus end-inspiration normalised to end-expiration)
	  • Tricuspid annular plane systolic excursion (TAPSE)
	Up to and including Day 3 of observation

• To evaluate the association between plasma Ang II and Ang(1-7) levels and other measures of cardiac function and pulmonary hemodynamics in mechanically ventilated participants.	• Ang II and Ang(1-7)
	• Central venous pressure (if available)
	• Mean arterial pressure
	• Echocardiographic measures:
	  • Respiratory variations of diameter of superior vena cava
	  • Cardiac output (pulsed wave Doppler, left ventricle (LV) outflow track)
	  • LV ejection fraction
	  • LV Velocity (E') tissue doppler imaging (TDI) of annulus
	  • LV early to late ventricular filling velocities (E/A)
	Up to and including Day 3 of observation

Objectives	Endpoints
• To characterise disease severity by clinical assessment.	• Oxygen saturation (SpO_2) via pulse oximetry • pH of arterial blood gases • PaO_2/FiO_2 ratio • $PaCO_2$ • Peak and plateau ventilator pressure • PEEP • Oxygenation index • Static respiratory system compliance • Mean airway pressure • Driving pressure (plateau pressure – total PEEP) • Clinical Safety labs • Vital Signs Up to and including Day 3 of observation
• To characterise levels of organ dysfunction.	Sequential Organ Failure Assessment (SOFA) score Up to and including Day 3 of observation
• To assess the association of cardiac and inflammatory biomarkers and RV function.	• PCD and ACP status • Biomarkers: for example, cardiac biomarkers (B-type natriuretic peptide (BNP)) or inflammatory biomarkers (cytokines such as IL-6, IL-8 and sTNFR1) may be analysed as sample availability allows Up to and including Day 3 of observation
• To evaluate the association between additional markers of the RAS system (if samples permit) and primary/secondary or exploratory endpoints.	Markers could include but are not limited to: Ang(1-5) in relation to primary, secondary or exploratory endpoints if sufficient sample is available Up to and including Day 3 of observation
• To characterize health outcome measures.	• Duration of intubation • Length of hospital stay • ICU length of stay • Ventilator free days • In ICU and hospital mortality Up to 28 days after first echocardiogram
• To assess lung ventilation and perfusion distribution (in selected site only).	• Lung regional ventilation and perfusion with global inhomogeneity (GI) index Up to and including Day 3 of observation

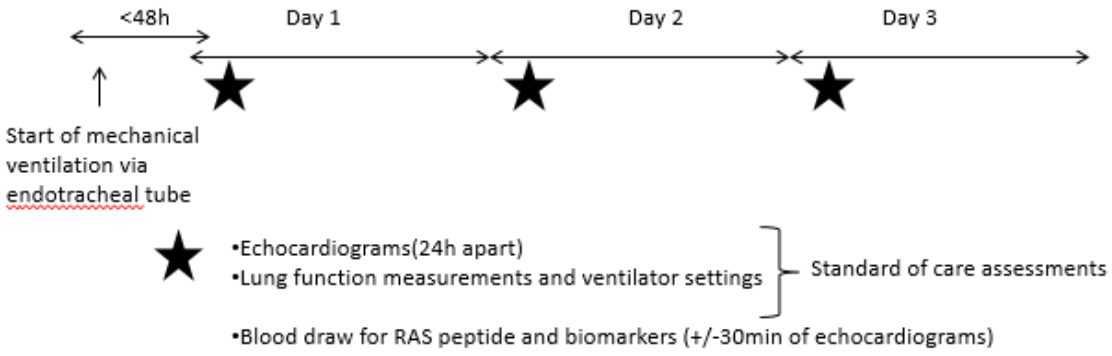
5. STUDY DESIGN

5.1. Overall Design

This study will investigate the association of RAS peptides and markers of RV function in participants requiring acute mechanical ventilation. This is a low interventional study without an investigational product. Participants will be evaluated over a 3-day period using standard of care investigations, including TTE and/or TOE echocardiography. Additional investigations will be limited to blood samples for RAS peptides and cardiac biomarkers (inflammatory markers if samples allow).

Participants will be enrolled in the study following intubation and mechanical ventilation. The initial echocardiogram will take place within 48 hours of mechanical ventilation (but ≤24 hours, whenever possible); this will be considered Day 1. Participants will be assessed every 24 hours for a further two days with daily echocardiography to monitor right and left ventricular function. Time 0 h for the study will be defined as the start of the first echocardiogram. Blood samples for RAS peptides will be collected at the time of the echocardiogram on each day. Additional parameters (including ventilator settings and arterial blood gases) will be collected at the time of intubation and daily as per standard of care. Daily assessment of mechanical ventilation and mortality will continue until hospital discharge or 28 days whichever is sooner.

Figure 1 Study Schematic



5.2. Number of Participants

Up to a maximum of 150 participants may be enrolled.

If participants prematurely discontinue the study, additional replacement participants may be recruited at the discretion of the Sponsor in consultation with the investigator.

5.3. Participant and Study Completion

A participant is considered to have completed the study if he/she has completed Day 3 assessments as shown in the SoA. If participants are extubated after the first echocardiogram and prior to the second/third echocardiogram he/she will remain in the study and be followed using echocardiography if conducted as per standard of care. In the event that further echocardiographs are undertaken, RAS peptide sampling will continue as per SoA.

Participants enrolled but not undergoing any echocardiograms will be considered withdrawn from the study.

The end of the study is defined as the date of last scheduled assessment of the last participant in the trial.

5.4. Scientific Rationale for Study Design

This study is designed to test the hypothesis that an association exists between RAS peptides, primarily plasma Ang II and Ang(1-7) levels, and RV function in mechanically ventilated participants. Therefore, this a prospective study to collect blood samples to assess RAS peptide levels and RV function as measured by echocardiography.

5.5. Dose Justification

Not applicable as there is no investigational product.

6. STUDY POPULATION

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

6.1. Inclusion Criteria

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

1. Male or female participant must be 18 to 80 years of age inclusive, at the time of enrolment.
2. Participants who are receiving invasive mechanical ventilation (duration of ventilation ≤48 hours, but ≤24 hours, whenever possible).

Weight

3. Body mass index within the range 18.0 – 38.0kg/m^2 (inclusive). Clinical estimate of height and weight is acceptable.

Informed Consent

4. Capable of giving signed informed consent as described in Section 12.3.3 (Appendix 3) which includes compliance with the requirements and restrictions listed in the ICF

and in this protocol. If participants are not capable of giving signed informed consent an emergency consent procedure will be followed as detailed in Section 12.3.3 (Appendix 3).

In France, a subject will be eligible for inclusion in this study only if either affiliated to or a beneficiary of a social security category.

6.2. Exclusion Criteria

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

1. Participants who are moribund or whose clinical condition is deteriorating rapidly or any participant for whom the investigator does not consider there is a reasonable expectation that they will be able to complete the 3 days of observation in the study.
2. Participants undergoing elective surgery.

Investigator will make every effort to ensure that the following exclusion criteria 3 to 10 are met; however, in some instances it may not be possible to assess all these criteria within the 48-hour window. In this case, a participant can be included, and investigator will obtain the information when available.

3. COPD requiring long term oxygen treatment or home mechanical ventilation.
4. Documented pre-existing chronic pulmonary hypertension.
5. Massive pulmonary embolism (defined by pulmonary embolism with systemic hypotension (defined as a systolic arterial pressure <90 mmHg or a drop in systolic arterial pressure of at least 40 mmHg for at least 15 min which is not caused by new onset arrhythmias) or shock (manifested by evidence of tissue hypoperfusion and hypoxia, including an altered level of consciousness, oliguria, or cool, clammy extremities)).
6. Pulmonary vasculitis or pulmonary haemorrhage including diffuse alveolar haemorrhage.
7. Lung transplantation within last 6 months.
8. Cardiopulmonary arrest during concurrent illness.

Concomitant Therapy

9. Any use of RAS modulators including ACE type 1 inhibitors, Renin inhibitors and Angiotensin Receptor Blockers within 4 days or 5.5 half-lives, whichever is longer.

If unable to obtain medication history at time of enrolment, participant is allowed to be included.

Other Exclusions

10. Do not resuscitate status.

6.3. Lifestyle Restrictions

No restrictions are required.

6.4. Screen Failures

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

Individuals who do not meet the criteria for participation in this study (screen failures) may not be rescreened.

7. TREATMENTS

No investigational product will be administered. Treatments will be based on local standard of care including fluid resuscitation, use of vasopressor drugs and renal support. Vasopressor use and renal replacement therapy will be documented.

Mechanical ventilation will be applied based on local practice: in the volume-assist control mode, with a target tidal volume of 6–8 ml/kg (predicted body weight) and a target plateau pressure (Pplat) of <30 cmH_2O. Respiratory rate can be increased in case of high arterial carbon dioxide partial pressure ($PaCO_2$) level, while avoiding intrinsic PEEP. Level of PEEP is left to the discretion of the frontline intensivist.

The use of prone position and use of inhaled nitric oxide is left to the discretion of the attending physician but should be documented in the Case Report Form (CRF).

7.1. Concomitant Therapy

Any prescription medication or vaccine (if known) that the participant is receiving at the time of enrolment or receives during the study must be recorded. Specifically collect any medication that may affect the RAS, cardiovascular or pulmonary homeostasis including but not limited to vasopressors, diuretics, beta blockers, steroids, induction agents such as Propofol, and inhaled nitric oxide.

The date and time of inhaled nitric oxide and vasopressor use must be recorded.

The following do not need to be documented: medication doses, administration of fluids or electrolyte infusions and nutrition.

8. DISCONTINUATION CRITERIA

8.1. Discontinuation of Study Treatment

Not applicable as there is no study treatment.

8.2. Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, request by their LAR, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance or administrative reasons.
    - If a participant withdraws from the study or the LAR requests that the participant is withdrawn after consenting, no additional blood samples will be taken. Samples already taken but not sent to central laboratory can be destroyed at patient or LAR's request. The investigator must document this in the site study records. If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- Participants who are withdrawn may be replaced.

9. STUDY ASSESSMENTS AND PROCEDURES

This section lists the parameters of each planned study assessment. Participants will be evaluated using standard of care investigations including parameters derived from ventilator readings, arterial blood gases, safety laboratory measures, and echocardiographic readings (TTE and/or TOE). Additional daily blood samples will be collected on Days 1, 2 and 3 to assess RAS peptides and other biomarkers which are not part of the standard of care. Health outcomes will also be measured including mortality, ICU status and ventilator free days up to a maximum of 28 days.

Detailed procedures for obtaining each assessment are provided in the Study Reference Manual (SRM). Whenever vital signs, lung function, ventilator settings, echocardiogram and blood draws are scheduled for the same nominal time, the assessments should occur in the following order: echocardiogram, blood draws, vital signs, ventilator settings, lung function. The timing of the assessments should allow the blood draw to occur at the exact nominal time. Time 0h will be defined as the start of the first echocardiogram.

- Study procedures and their timing are summarized in the schedule of assessments (SoA; Section 2).
- Protocol waivers or exemptions are not allowed. Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening

log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

- Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA.
- The maximum amount of blood collected from each participant over the duration of the study for the purpose of meeting the objectives will not exceed 30 mL. This does not include any standard of care assessments.
- A single repeat of a sample may be taken for technical reasons as long as they are within the timelines defined in the SoA.

9.1. Demographic/Medical History Assessments

The following demographic parameters will be captured: year of birth and gender. If it is not possible to collect at screening can be collected at any point during the study.

Medical and medication history including cardiovascular risk will be collected. If it is not possible to collect at screening can be collected at any point during the study.

Height and weight will be collected at screening. Clinical estimates are acceptable if direct measurement is not possible.

9.2. Pharmacodynamics

9.2.1. Echocardiograms

As per standard of care, TTE will be conducted on all participants with TOE measurements undertaken in those participants in whom adequate windows cannot be obtained via the transthoracic route. The first echocardiogram must be undertaken within 48 hours of the start of mechanical ventilation (but within 24 hours, whenever possible), with subsequent echocardiograms approximately 24 hours apart on Day 2 and Day 3. As previously described [Mekontso-Dessap 2016], echocardiography will be undertaken by trained operators in the field of advanced critical care echocardiography and in the case of TOE will be performed using a multiplane oesophageal probe. Images will be stored in a digital format at site and a computer assisted consensual interpretation will be performed off line by at least two trained senior investigators (see SRM for further details on echocardiography standards).

Variables that will be recorded from TTE and/or TOE, as data permit, include:

- Ratio of right ventricular to left ventricular end-diastolic area
- Paradoxical septal motion (systolic eccentricity index)
- PASP (estimated from transtricuspid pressure gradient and right atrial pressure or IVC diameter (whichever is available))
- Maximal velocity of s wave (tissue Doppler imaging) at the tricuspid annulus

- Fractional area contraction: End Diastolic Area minus End Systolic Area normalised to End Diastolic Area
- Acceleration time of RV ejection flow (Tacc)
- Mean acceleration of RV ejection flow (maximum velocity/ acceleration time) at end-expiration and end-inspiration
- Inferior vena cava diameter at end expiration
- Respiratory variations of RV velocity time integral (end-expiration minus end-inspiration / end-expiration)
- Tricuspid annular plane systolic excursion
- Respiratory variations of superior vena cava diameter
- Cardiac output (pulsed wave Doppler, LV outflow track)
- LV ejection fraction
- LV Velocity (E') tissue doppler imaging (TDI) of annulus
- LV Early to late ventricular filling velocities (E/A)

9.2.2. Electrical Impedance Tomography (EIT)

The Global Inhomogeneity (GI) index will be measured based on electrical impendence (Enlight, Timpel, Brazil) to assess lung regional and/or perfusion in centres where measurements are taken routinely. Measurements will be taken daily at time of echocardiography for up to three days. The GI index will be measured according to the methods set out by Zhao 2009.

9.3. Ventilator Settings

Measurements will include tidal volume, respiratory rates, respiratory static compliance of the respiratory system, level of total PEEP, peak and plateau ventilator pressures, mean airway pressure and driving pressure.

9.4. Lung Function

Measurements will include oxygen saturation (SpO_2) via pulse oximetry and Partial pressure of oxygen and carbon dioxide in arterial blood (PaO_2 and $PaCO_2$). The pH, lactate and bicarbonate levels of ABGs will also be documented when ABGs are assessed as well as the base excess/deficit if available. Oxygen requirement (FiO_2) will be collected and documented at the same time as ABG collection.

9.5. Participant Status

9.5.1. SAPS II

The SAPS II score is comprised of the following components:

- Age
- Heart Rate
- Systolic Blood Pressure
- Temperature
- Mechanical Ventilation or Continuous Positive Airway Pressure
- PaO_2
- FiO_2
- Urine Output
- Blood Urea Nitrogen
- Sodium
- Potassium
- Bicarbonate
- Bilirubin
- White Blood Cell
- Chronic Diseases
- Type of Admission
- Glasgow Coma Score (GCS)

If the patient is sedated at the time of assessment, an estimated GCS prior to sedation will be used instead.

More information is provided in the SRM.

9.5.2. Sequential Organ Failure Assessment (SOFA) score

The SOFA score is made up of the following components:

- PaO_2/FiO_2 (mmHg)
- Mean Arterial Pressure or administration of vasopressors required
- Bilirubin
- Platelets
- Creatinine (or urine output)

Due to the difficulties in accurately scoring Glasgow coma score in sedated patients, the neurologic system will not be included.

More information is provided in the SRM.

9.5.3. Disease diagnosis

Diagnosis of ARDS, PCD and ACP.

- ACP is defined as a dilated RV in the mid-esophagus longitudinal view or apical 4-chamber view [end-diastolic RV / left ventricle (LV) area ratio (0.6)] associated with the presence of a septal dyskinesia in the (transgastric) short-axis view of the heart.
- Severe ACP is defined as severely dilated RV (end-diastolic RV / LV area ratio ≥1) with septal dyskinesia. Septal dyskinesia will be particularly sought at end-systole, while analysing loops in slow motion.
- PCD is defined as moderate dysfunction (PASP [>40 mmHg] or a dilated RV end-diastolic RV / left ventricle (LV) area ratio [≥0.6] but without septal dyskinesia). In case of the inability of measuring transtricuspid pressure gradient, pulmonary hypertension will be defined as a Tacc below 100 ms.
- ARDS is defined as per the Berlin definition (Ranieri 2012):

Timing	Within 1 week of a known clinical insult or new or worsening respiratory symptoms
Chest imaging	Bilateral opacities—not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload Need objective assessment (eg, echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation	
Mild	200 mm Hg _< PaO2/FIO2 _ ≤ 300 mm Hg with PEEP or CPAP ≥_5 cm H2Oc
Moderate	100 mm Hg <_ PaO2/FIO2 ≤_ 200 mm Hg with PEEP _5 cm H2O
Severe	PaO2/FIO2 ≤_ 100 mm Hg with PEEP ≥_5 cm H2O

9.5.4. General management status

The following measurements of general management status will be recorded at the time of echocardiogram for each of the three study days:

- Proned status
- Intubation status
- Management with extracorporeal membrane oxygenation (ECMO)
- Management with extracorporeal CO_2 ($ECCO_2R$) removal

9.6. Additional Clinical assessments

The following data will be collected for up to a maximum of 28 days:

- Mortality
- ICU status
- Ventilator free days

Date and time of any concomitant surgery will also be recorded.

9.7. Adverse Events

Safety assessments will be conducted as part of standard of care and include serious adverse event collection, safety laboratory assessments, and vital signs. More information will be provided in the SRM.

9.7.1. Serious Adverse Events (SAE)

The definitions of an adverse event (AE) or SAE can be found in Appendix 4.

Although no investigational product will be given, SAEs will be recorded, as follows: SAEs relating to study procedures; and SAEs related to GlaxoSmithKline (GSK) medication used as co-medication.

This ensures that any SAEs relating to study procedures or any GSK medication used as co-medication are captured.

AEs (other than SAEs, as described above) will not be captured in the study.

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an SAE, as provided in this protocol. During the study when there is a safety evaluation, the investigator or site staff will be responsible for detecting, documenting and reporting SAEs, as detailed in both this section of the protocol and in the SAE section of the study reference manual.

9.7.2. Time Period and Frequency for Collecting SAE Information

- All SAEs will be collected over the 3 days of study period from the time of enrolment.
- All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study procedure or GSK medication, the investigator must promptly notify the sponsor.

- The method of recording, evaluating, and assessing causality of SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

9.7.3. Method of Detecting SAEs

SAEs will be detected by the medical team.

9.7.4. Follow-up of SAEs

After the initial SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs, will be followed until the event is resolved, stabilized, otherwise explained, or the participant is discharged from ICU or up to 28 days (whichever is sooner).

9.7.5. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of any GSK product or study procedure. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IEC, and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

9.8. Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Section 2) as per standard of care.

9.8.1. Vital Signs

- Systolic and diastolic and mean arterial blood pressure and heart rate will be assessed.
- Blood pressure and heart measurements will be assessed (supine if possible) with a completely automated device. Manual techniques will be used only if an automated device is not available.

9.8.2. Clinical Laboratory Assessments

Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA (Section 2) for the timing and frequency.

9.9. Biomarkers

The following samples are required and will be collected from all participants in this study as specified in the SoA

- The levels of Ang II and Ang(1-7) will be analysed in plasma. Additional components of the RAS [e.g. Ang(1-5)] may be evaluated from the same blood sample to better understand the correlation of the RAS to RV function if samples allow.
- Collection of samples for other biomarker research is also part of this study. For example cardiac biomarkers (eg. BNP) and other biomarkers (eg. Inflammatory markers or lung injury markers) may be evaluated if sample availability allows.
- Samples will be stored to assess future biomarkers of interest in lung injury pathophysiology as sample availability allows.

10. STATISTICAL CONSIDERATIONS

10.1. Sample Size Determination

Up to 150 participants may be enrolled in the study (as described in Section 5.2). The sample size is deemed to be sufficient to evaluate the secondary objectives of the study. In particular, the sample size of 150 participants would provide a precision of ±5 percentage points (defined as the half-width of the 95% Wald confidence interval) around a point estimate for ACP incidence (based on an estimated incidence rate of 10%).

The primary analysis (association between Ang II and RV function measured by PASP and RV size ratio) will be evaluated after ~50 participants have been enrolled in the study. If results are inconclusive, then a second analysis after ~100 participants will be carried out. Full details of the assumptions and simulations used to determine the sample size are given in Section 12.5.

In the event of a conclusion of "success" or "inconclusive" at an interim analysis, only the decision to continue with the study will be communicated with the study sites, to mitigate against the possibility of the nature of the interim conclusion influencing the data collection process and introducing bias into the later interim and final analysis results. In the event of a "futility" decision, recruitment to the study will be halted.

Simulations were carried out to evaluate the operating characteristics of the success/futility criteria (described Section 10.3.3) at the primary analysis timepoint (i.e. after 50 participants have completed their assessments), under a range of scenarios describing the correlation between Ang II and the two endpoints of PASP and RV size ratio. The main results from these simulations are as follows:

- In the case of there being no relationship between Ang II and either of the endpoints, the decision-making criteria will return a conclusion of "futility" 99.1% of the time.

- In the case of there being a strong relationship (defined as a correlation of 0.6) between Ang II and both of the endpoints, the mean difference in probabilities of RV dysfunction between the two reference values will be 33.9%, and the decision-making criteria will return a conclusion of "success" 74.7% of the time (with "inconclusive" being returned the remaining 25.3% of the time).
- When the relationship between Ang II and the endpoints is less clear, there is a greater likelihood of returning an inconclusive result and continuing the study up to the backup interim analysis at 100 participants.
- The conclusions returned by the decision-making criteria are more sensitive to Ang II's correlation with RV size ratio than to its correlation with PASP. For example, when the correlation between Ang II and RV size ratio is reduced from 0.6 to 0.3, the decision-making criteria return "success" only 12.6% of the time and the mean difference in probabilities of RV dysfunction is 22.5%. Conversely, if the correlation between Ang II and PASP is reduced from 0.6 to 0.3, the decision-making criteria will return "success" 30.1% of the time and the mean difference in probabilities of RV dysfunction is only reduced to 26.9%.

A full description of the simulation methods is given in Appendix 5 (Section 12.5) and a summary of the results from all the scenarios simulated is given in Table 1.

Table 1 Operating characteristics of decision-making criteria at the primary interim analysis (N = 50) under a range of scenarios regarding the relationship between Ang II and the two endpoints PASP and RV size ratio

a) Probabilities of returning conclusions of success, futility and inconclusive, by scenario

Correlations between Ang II and			Probability of reaching a conclusion of (%)		
PASP	**RV size ratio**		**Success**	**Futility**	**Inconclusive**
Extreme null scenario: no correlation between Ang II and either component of RV dysfunction					
0.0	0.0		0.0	99.1	0.9
Extreme positive scenario: strong correlation between Ang II and both components of RV dysfunction					
0.6	0.6		74.7	0.0	25.3
"Middling" scenario: weak correlation between Ang II and both components of RV dysfunction					
0.3	0.3		0.6	49.3	50.1
Mixture scenarios (I): Ang II correlated more strongly with PASP than with RV size ratio					
0.3	0.0		0.0	92.6	7.4
0.6	0.0		0.6	60.9	38.5
0.6	0.3		12.6	10.9	76.5
Mixture scenarios (II): Ang II correlated more strongly with RV size ratio than with PASP					
0.0	0.3		0.2	81.3	18.5
0.0	0.6		12.6	12.5	74.9
0.3	0.6		30.1	2.1	67.8
Other assumptions in the simulation: N = 50 participants; correlations between PASP and RV size = 0.5. For each scenario, results are based on 1000 simulated study datasets, each analysed using a MCMC simulation with 3 chains of 1000 iterations (after a burn-in of 1000 iterations) each.					

b) Mean difference in predicted probability of RV dysfunction, PASP and RV size ratio between estimated pre-dose and post-dose Ang II reference values, by scenario

Correlations between Ang II and			Mean difference between predicted values		
PASP	**RV size ratio**		**P (RV dysfunction) %**	**PASP (mmHg)**	**RV size ratio**
Extreme null scenario: no correlation between Ang II and either component of RV dysfunction					
0.0	0.0		0.3	0.0	0.00
Extreme positive scenario: strong correlation between Ang II and both components of RV dysfunction					

Correlations between Ang II and			Mean difference between predicted values		
PASP	**RV size ratio**		**P (RV dysfunction) %**	**PASP (mmHg)**	**RV size ratio**
0.6	0.6		33.9	5.7	0.11
"Middling" scenario: weak correlation between Ang II and both components of RV dysfunction					
0.3	0.3		14.9	2.8	0.05
Mixture scenarios (I): Ang II correlated more strongly with PASP than with RV size ratio					
0.3	0.0		6.1	2.9	0.00
0.6	0.0		13.2	5.6	0.00
0.6	0.3		22.5	5.6	0.05
Mixture scenarios (II): Ang II correlated more strongly with RV size ratio than with PASP					
0.0	0.3		9.1	0.0	0.05
0.0	0.6		22.5	0.0	0.11
0.3	0.6		26.9	2.9	0.11
Other assumptions in the simulation: N = 50 participants; correlations between PASP and RV size = 0.5. For each scenario, results are based on 1000 simulated study datasets, each analysed using a MCMC simulation with 3 chains of 1000 iterations (after a burn-in of 1000 iterations) each.					

10.2. Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Completed	All participants for whom PASP, RV size ratio, Ang II and Ang(1-7) data have been recorded for all three study days. For the purpose of evaluating ACP incidence, participants who have ACP either on entry or occurring during the study period will also be included in this population, even if they subsequently do not have three days' worth of study assessments, provided consent is not withdrawn.
Evaluable	All participants for whom PASP, RV size ratio, Ang II and Ang(1-7) data have been recorded for at least one study time point.
Safety	All participants for whom at least one echocardiograph and/or blood sample has been taken.

10.3. Statistical Analyses

10.3.1. Pharmacodynamic Analyses

Endpoint	Statistical Analysis Methods
Primary	The association of Ang II with RV function (measured by PASP and RV size ratio) will be modelled using a Bayesian repeated measures mixed effects linear regression model, where PASP and RV size ratio are joint response variables, and including subject as a random effect and log(Ang II), age and sex as fixed effects. The model will be used to make a probabilistic prediction of PASP and RV size ratio for two reference values of Ang II, and hence an estimate for the probability of RV dysfunction (defined as PASP > 40 mmHg or RV size ratio > 0.6, following Boissier 2013) for the two given Ang II levels will be obtained using mean values for the other continuous covariates and averaging over all levels of class effects for categorical covariates. The above modelling may use a modified Ang II value (modification based on the corresponding Ang(1-7) result to account for potential inhibitory interactions); details to be provided in the reporting and analysis plan (RAP). The primary analysis will use the Evaluable population.
Secondary	Association of Ang(1-7) with RV function (measured by PASP and RV size ratio) and of Ang II/Ang(1-7) with RV function (measured by PASP and RV size ratio) will both be modelled as per the primary analysis described above. These analyses will be done using the Evaluable population. A model including both Ang II and Ang(1-7) as co-predictors of RV function may also be built, to explore the relationship between RV function and the envisaged dual action of angiotensin converting enzyme (ACE)2 effecting a reduction in Ang II together with a simultaneous increase in Ang(1-7). Incidence of PCD and ACP during the three days of the study period and prevalence of pre-existing PCD/ ACP on entry to the study will be reported with 95% confidence intervals. This analysis will be done using the Completed population (which includes a clause to include participants who have an onset of PCD/ ACP but are subsequently withdrawn from the study before all three days' worth of data are collected without withdrawing consent). The distribution of PASP, RV size ratio and other measurements of RV function in mechanically ventilated participants will be assessed via a combination of descriptive summary tables and outputs from the statistical modelling.
Exploratory	Will be described in the reporting and analysis plan

Given that the hypothesis to this study is that RAS-mediated pulmonary vasoconstriction may be reduced through the dual action of ACE2 by simultaneously reducing Ang II and forming Ang(1-7) (see Section 3.2), an important secondary analysis will be to explore the joint effect of both Ang II and Ang(1-7) as related to RV dysfunction. That is to say, in addition to exploring the individual hypotheses that Ang II is deleterious to RV function and that Ang(1-7) is beneficial, a model will be developed that integrates the combined effects of these two mediators, since the expected effect of treatment with ACE2 is that both will change. Several models may be analysed in order to explore the exact nature of the relationship between the dual action of changes in Ang II and Ang(1-7) and the probability of RV dysfunction as measured by PASP and RV size ratio.

10.3.2. Safety Analyses

All safety analyses will be performed on the Safety Population. Safety data will be presented in tabular and/or graphical format and summarised descriptively according to GSK's Integrated Data Standards Library (IDSL) standards.

10.3.3. Interim Analyses

Two interim analyses are planned during the course of this study, both of which will provide an opportunity to stop the study for futility (close study to future recruitment but allow existing participants to complete the study as planned). A third interim analysis may optionally be performed if the second is inconclusive. The primary analysis of the association between Ang II and RV function (measured by PASP and RV size ratio) will be performed at each interim analysis, and will be used for the purposes of decision-making. The results of these analyses are to be considered "rules of thumb" and may be overridden by other considerations such as an observed association between the secondary RAS biomarkers and measurements of RV (dys)function that is strong enough to warrant further investigation. The interim analysis may be postponed if there is insufficient coverage in the first 25 participants of Ang II values to make an interpolative prediction of RV (dys)function for the two pre-specified reference values (see below).

RV function will be measured using PASP and RV size ratio. The general principle underlying all the analyses of RV function and RAS peptide biomarkers will be:

- Model the joint distribution of PASP and RV size ratio as a function of the RAS peptide(s) in question (together with confounding variables also included as covariates)
- Choose reference values of the RAS peptide(s) in question and estimate a posterior distribution of PASP and RV size ratio for those given values, and
- Use the posterior distribution to determine the probability of RV dysfunction (for a future individual participant), defined (following the definition of moderate/severe pulmonary circulatory disease given in Figure 2 of Boissier 2013) as:
    - PASP > 40 mmHg
    - RV size ratio > 0.6

The advantage of this method is that all the information on the continuous scales of PASP and RV size ratio is maintained throughout the modelling process, and that dichotomising participants into binary categories takes place at the end after the modelling has been done.

In order to evaluate the estimated effect of administering rhACE2 on RV outcomes (via its effect on the RAS peptides), predictions for the probability of RV dysfunction will be calculated for specific, pre-determined values of RAS peptide measurements that represent reasonable estimates for each RAS peptide both before and after dosing with rhACE2 in this patient population. These values will be hereafter referred to as *reference values*. Reference values for the RAS peptides will be defined based on available observed data from an investigator sponsored study (NCT01884051) (where RAS peptides were observed both before and after dosing with rhACE2) and will be calculated using the geometric mean of the observed pre-dose values (for the first reference value, hereafter referred to as the *pre-dose reference*) and the geometric mean of the observed 2 hours post-dose values (for the second reference value, hereafter referred to as the *post-dose reference*). (Note: the terminology "pre-dose" and "post-dose" should not be taken to imply that investigational product is used in this study; these values should be taken as being borrowed from that interventional study. Furthermore, the term "reference values" in this context should not be interpreted as upper or lower limits to the normal range. They represent the best guess available for a patient's typical experience before and after rhACE2 administration that may reasonably relate to a change in pulmonary haemodynamics.)

In the case that an assessment of inconclusive or futile conclusions based on Ang II at an interim analysis Ang 1-7 and the ratio of Ang II:Ang1-7 (and/or other peptides of the RAS as possible) will be evaluated and may lead to continuation of the study. In all the results from the simulation exercises described in this section, Ang II was taken to be the analyte in question for the purpose of evaluating the operating characteristics of the decision-making criteria, had they been strictly applied, with no fallback on the analyses of other RAS peptides. Additionally, for the sake of simplicity, only one measurement per participant was assumed (rather than three repeated measurements). This represents the worst case scenario (in terms of independence of samples) where all measurements within the same participant are perfectly correlated with each other.

At the time of writing, the reference values for Ang II were as follows (based on the two available observations of sufficient quality from the aforementioned investigator sponsored study (NCT01884051).

- Pre-dose reference value: 55.4 pg/ml
- Post-dose reference value 7.1 pg/ml

For the purposes of evaluating sample size considerations, these were the reference values for Ang II that were used in the assumptions underlying the simulations. An initial set of reference values for all RAS peptides measured in this study will be agreed before the first subject enters the study, and will be detailed in the RAP.

The first interim analysis will take place after approximately 25 evaluable participants have completed the 3 day observational part of the study. The primary analysis of the association between Ang II and RV function will be performed and a probabilistic prediction for the difference in probability of RV dysfunction associated with a change in Ang II from the pre-dose reference value to the post-dose reference value will be obtained. If the median estimate of this difference is positive (i.e. probability of RV dysfunction at the pre-dose reference value is lower than that at the post-dose reference value) then additional follow-on analyses of the association of the other RAS biomarkers with RV (dys)function will be conducted. If similar conclusions are drawn from these analyses, then the study may be stopped for futility. If an association between another RAS biomarker and RV (dys)function is observed in the absence of an association between Ang II and RV (dys)function, then this biomarker may be substituted in place of Ang II as the primary endpoint for subsequent analyses.

As part of the sample size simulations (see Section 10.1), the operating characteristics of the first interim analysis were assessed to evaluate the probability of falsely declaring futility after approximately 25 participants when the study would have gone on to show success after 150 participants. None of the simulations, in any of the scenarios returned a futility result after 25 participants that would have gone on to be a success after 150 participants. Full results are given in Table 2.

Table 2 Proportion of simulated studies returning conclusion of futility at N under a range of scenarios regarding the relationship between Ang II and the two endpoints PASP and RV size ratio

Correlations between Ang II and			Probability (%) of futility at N=25	Probability (%) of futility at N=25 followed by success at N=150	Probability (%) of futility at N=25 followed by inconclusive at N=150
PASP	**RV size ratio**				
Extreme null scenario: no correlation between Ang II and either component of RV dysfunction					
0.0	0.0		49.7	0.0	0.0
Extreme positive scenario: strong correlation between Ang II and both components of RV dysfunction					
0.6	0.6		0.0	0.0	0.0
"Middling" scenario: weak correlation between Ang II and both components of RV dysfunction					
0.3	0.3		4.6	0.0	1.4
Mixture scenarios (I): Ang II correlated more strongly with PASP than with RV size ratio					
0.3	0.0		23.3	0.0	0.1
0.6	0.0		8.3	0.0	1.0
0.6	0.3		0.4	0.0	0.4
Mixture scenarios (II): Ang II correlated more strongly with RV size ratio than with PASP					
0.0	0.3		13.4	0.0	0.0
0.0	0.6		1.2	0.0	1.2
0.3	0.6		0.1	0.0	0.1
Other assumptions in the simulation: N = 50 participants; correlations between PASP and RV size = 0.5. For each scenario, results are based on 1000 simulated study datasets, each analysed using a MCMC simulation with 3 chains of 1000 iterations (after a burn-in of 1000 iterations) each.					

The second interim analysis will take place after approximately 50 evaluable participants have completed the 3-day observational part of the. This interim analysis will be used as the main go/no-go decision-making point as to whether to continue gathering data on biomarkers and other background information and to start internal GSK preparations for future interventional studies. The primary analysis of the association between RV (dys)function and Ang II will be performed and a probabilistic prediction for the difference in the probability of RV dysfunction associated with a change in Ang II from the pre-dose reference value to the post-dose reference value will be obtained (as at the first interim analysis). The absolute difference between these probabilities will then be calculated as and conclusions made as follows:

- If the absolute difference between P(RV dysfunction at the pre-dose reference value and P(RV dysfunction at the post-dose reference value) ≤ 15%, then the study may be stopped for futility.
- If the absolute difference between P(RV dysfunction at the pre-dose reference value and P(RV dysfunction at the post-dose reference value) ≥ 30%, then the study may continue, collecting biomarker data up to a maximum of 150 participants, and GSK may begin enabling activities to prepare for a future interventional study.
- If the absolute difference lies somewhere between these values (i.e. between 15 and 30%), then a decision will be made by the Sponsor in conjunction with the investigator as to whether or not to continue based on observed associations between the secondary RAS biomarkers and RV (dys)function in addition to the primary analysis. A further interim analysis after 100 participants may additionally be performed in this case.

Recruitment will continue whilst the interim analyses are being performed.

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

11. REFERENCES

Boissier F, Katsahian S, Razazi K, Thille AW, Roche-Campo F, Leon R, et al. Prevalence and prognosis of cor pulmonale during protective ventilation for acute respiratory distress syndrome. *Intensive Care Med*. 2013 Oct;39(10):1725-1733.

Bull TM, Clark B, McFann K, Moss M; National Institutes of Health/National Heart, Lung, and Blood Institute ARDS Network. Pulmonary vascular dysfunction is associated with poor outcomes in patients with acute lung injury. *Am J Respir Crit Care Med*. 2010 Nov 1;182(9):1123-1128

Idell S, Kueppers, F, Lippmann, M, Rosen, H, Niederman, M, Fein, A. ACE elevated in ARDS BAL. Angiotensin converting enzyme in bronchoalveolar lavage in ARDS. *Chest*, 1987; 91:52-56.

Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, McAnulty RJ, Humphries SE, Hill MR, Laurent GJ. Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am J Respir Crit Care Med*. 2002 Sep 1; 166(5):646-650.

Mekontso-Dessap A, Boissier F, Charron C, Bégot E, Repessé X, Legras A, Brun-Buisson C, Vignon P, Vieillard-Baron A. Acute cor pulmonale during protective ventilation for acute respiratory distress syndrome: prevalence, predictors, and clinical impact. *Intensive Care Med*. 2016 May; 42(5):862-870.

Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS (ARDS Definition Task Force). Acute respiratory distress syndrome: the Berlin Definition. *JAMA*. 2012; 307(23):2526-33.

Santos RAS, Simoes e Silva, AC, Maric, C, Silva, DMR, machado, RP, de Buhr, I,Heringer-Walther, S, Pinheiro, SVB, Lopes, MT, Bader, M, Mendes, EP, Soares Lemos,V, Campagnole-Santos, MJ, Schultheiss, H-P, Speth, R and Walther, T. ANG (1-7) as the agonist of mas. 'Angiotensin (1-7) is an endogenous ligand for the G-protein coupled receptor Mas' *Proc. Natl. Acad. Sci*. USA. 2003; 100:8258-8263.

Tom B, de Vries R, Saxena PR, Danser AH. Bradykinin potentiation by angiotensin-(1-7) and ACE inhibitors correlates with ACE C- and N-domain blockade. *Hypertension*. 2001 Jul; 38(1):95-99.

Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med*. 2000 May 4; 342(18):1334-49.

Wenz M, Hoffmann, B, Bohlender, J, Kaczmarczyk, G. ANG II elevated in ARDS.Anfgiotensin II formation and endothelin clearance in ARDS participants in supine and prone positions. *Intensive Care Med*. 2000; 26:292-298.

Zapol WM, Snider MT: Pulmonary hypertension in severe acute respiratory failure. *N Engl J Med* 1977, 296: 476–480.

Zhao Z1, Möller K, Steinmann D, Frerichs I, Guttmann J. Evaluation of an electrical impedance tomography-based Global Inhomogeneity Index for pulmonary ventilation distribution.Intensive Care Med. 2009 Nov;35(11):1900-6

12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

ABG	Arterial Blood Gases
ACP	Acute Cor Pulmonale
ACE	Angiotensin Converting Enzyme
rhACE2	Recombinant Human Angiotensin Converting Enzyme 2
AE	Adverse Event
Ang	Angiotensin
ARDS	Acute Respiratory Distress Syndrome
CRF	Case Report Form
CO_2	Carbon Dioxide
CONSORT	Consolidated Standards of Reporting Trials
cmH_2O	Centimeter of Water
E'	LV Velocity
FiO_2	Oxygen Requirement
GCS	Glasgow Coma Score
GI	Global Inhomogeneity
GSK	GlaxoSmithKline
h	Hour
ICF	Informed Consent Form
ICU	Intensive Care Unit
IDSL	Integrated Data Standards Library
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IVC	Inferior Vena Cava
Kg/m^2	Kilogram/Square Meter
LAR	Legally Acceptable Representative
LV	Left Ventricle
Mins	Minutes
mL	Milliliter
mmHg	Millimeter of Mercury
ms	Millisecond
$PaCO_2$	Partial Pressure of Carbon Dioxide
PaO_2	Partial Pressure of Oxygen
PASP	Pulmonary Arterial Systolic Pressure
PCD	Pulmonary Circulatory Dysfunction
PEEP	Positive End Expiratory Pressure
Pg/ml	Picograms per millilitre
Pplat	Target Plateau Pressure
PI	Principle Investigator
PVR	Pulmonary Vascular Resistance
RAP	Reporting and Analysis Plan
RAS	Renin Angiotensin System

RV	Right Ventriculum
SAE	Serious Adverse Event
SAPS	Simplified Acute Physiology Score
SaO_2	Oxygen Saturation
SoA	Schedule of Assessments
SOFA	Sequential Organ Failure Assessment
SRM	Study Reference Manual
SUSAR	Suspected Unexpected Serious Adverse Reactions
TAPSE	Tricuspid Annular Plane Systolic Excursion
TDI	Tissue Doppler Imaging
TOE	Transesophageal echocardiography
TTE	Transthoracic echocardiography
SpO_2	Oxygen Saturation

Trademark Information

Trademarks of the GlaxoSmithKline group of companies	**Trademarks not owned by the GlaxoSmithKline group of companies**
NONE	None

12.2. Appendix 2: Clinical Laboratory Tests

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

Table 3 Protocol-Required Safety Laboratory Assessments

Collected as per standard of care

Laboratory Assessments	**Parameters**		
Haematology	Platelet Count	WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
	RBC Count		
	WBC (absolute)		
	Haemoglobin		
	Haematocrit		
Clinical Chemistry	BUN	Potassium	Total bilirubin
	Creatinine	Sodium	Troponin I or T
	Chloride	Total CO_2	

12.3. Appendix 3: Study Governance Considerations

12.3.1. Ethical Considerations

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 current version of the Declaration of Helsinki

- This study will be conducted in accordance with the protocol and with:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
  - Applicable ICH Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) must be submitted to an IEC by the investigator and reviewed and approved by the IEC before the study is initiated.
- Any amendments to the protocol will require IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IEC
  - Notifying the IEC of SAE or other significant safety findings as required by IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

12.3.2. Financial Disclosure

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

12.3.3. Informed Consent Process

Given participants may be mechanically ventilated upon enrolment, obtaining informed consent directly from the participants may not always be feasible. Therefore, participants may be enrolled in three ways:

1. Participant is enrolled by signing the informed consent

If the participant is capable of understanding and signing the informed consent prior to enrolment, the below points will apply:

- The investigator or his/her representative will explain the nature of the study to the participant and answer all questions regarding the study.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

2. Participant is enrolled by his/her LAR

If the participant is unable to provide informed consent, best efforts will be undertaken by the investigator or delegate to obtain consent from a LAR prior to enrolment. If consent is obtained from a LAR, the following points will apply:

- Best efforts will be undertaken to obtain written consent from a LAR; however, if only oral LAR consent is feasible, participants can be enrolled on LAR's oral consent and a written LAR consent will be sought as soon as possible thereafter.
- The investigator has responsibility for applying local laws in the matter of who has the capacity to consent and who qualifies as a LAR of a potential subject.
- A LAR is defined as an individual or juridical or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical trial.
- LAR can be a member of the participant's family, next-of-kin or trusted person previously designated by the participant.
- As soon as is practically possible following a participant regaining capacity, participants will be asked to provide informed consent to remain in the study. If they decline, then they will be withdrawn from the study. The subjects will decide if they want to allow samples already collected to be used or request for them to be discarded if not yet analysed.

3. Participant is enrolled upon emergency consent process

If the participant in unable to provide informed consent and the LAR cannot be reached when the study selection criteria are fulfilled, the investigator will be able to enrol the participant according to the emergency consent process, and the following points will apply.

- The emergency consent process is indicated in last resort (article L1122-1-2 of French Public Health code) as per the following arguments: the urgency and gravity of the situation has mandated invasive mechanical ventilation and intensive care admission; the use of echocardiography for hemodynamic monitoring is routine in the participating centres in this situation.

- As soon as it is practically possible following a participant regaining capacity, participants will be asked to provide informed consent to remain in the study. If they decline, then they will be withdrawn from the study. The participants will decide if they want to allow samples already collected to be used or request for them to be discarded if not yet analysed.

Documentation of Informed consent process

The management of the informed consent process will be documented in the medical source document. This will include which process was used to obtain consent.

The following should be noted in regard to the informed consent process:

- If consent is provided by LAR, name, time and date of LAR and name of the personnel at site that collected this information

If consent is not obtained the time and date of attempt(s) must be collected along with the name(s) of LAR and personnel at site that attempted contact. Participants/LAR must be informed that their participation is voluntary. Participants and/or LAR will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IEC or study centre.

A copy of the signed ICF(s) must be provided to the participant or the participant's LAR.

Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.

Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicentre studies only in their entirety and not as

individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Dissemination of Clinical Study Data

- Study information from this protocol will be posted on publicly available clinical trial registers before enrolment of subjects begins.
- 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 subjects, as appropriate.
- The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.
- A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The investigator must permit study-related monitoring, audits, IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years after study completion

unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Study and Site Closure

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

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

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator

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

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of a study treatment*, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study treatment. *Although there is no "study treatment" in this study, AE/SAE definitions still apply to reports of serious adverse events explicitly attributed to GSK products or to study procedures.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of concomitant GSK product. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the

Events NOT Meeting the AE Definition
participant's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE

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

A SAE is defined as any untoward medical occurrence that:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d. Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

A SAE is defined as any untoward medical occurrence that:
e. Is a congenital anomaly/birth defect
f. Other situations: • Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording SAE

SAE Recording
• When a SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event. • The investigator will then record all relevant SAE information in the CRF. • It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page. • There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to GSK. • The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the SAE.

Assessment of Causality
• The investigator is obligated to assess the relationship between study procedure, GSK product and each occurrence of each SAE. • A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. • The investigator will use clinical judgment to determine the relationship.

Assessment of Causality
• Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated. • The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment. • For each SAE, the investigator **must** document in the medical notes that he/she has reviewed the SAE and has provided an assessment of causality. • There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to GSK.** • The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment. • The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of SAE
• The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK to elucidate the nature and/or causality of the SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology. New or updated information will be recorded in the originally completed CRF. • The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool
• The primary mechanism for reporting SAE to GSK will be the electronic data collection tool. • If the electronic system is unavailable for more than 24 hours, then the site will use the paper SAE data collection tool (see next Section).

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

SAE Reporting to GSK via Paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the medical monitor or the SAE coordinator. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames. • Contacts for SAE reporting can be found in the SRM.

12.5. Appendix 5: Assumptions for sample size calculations

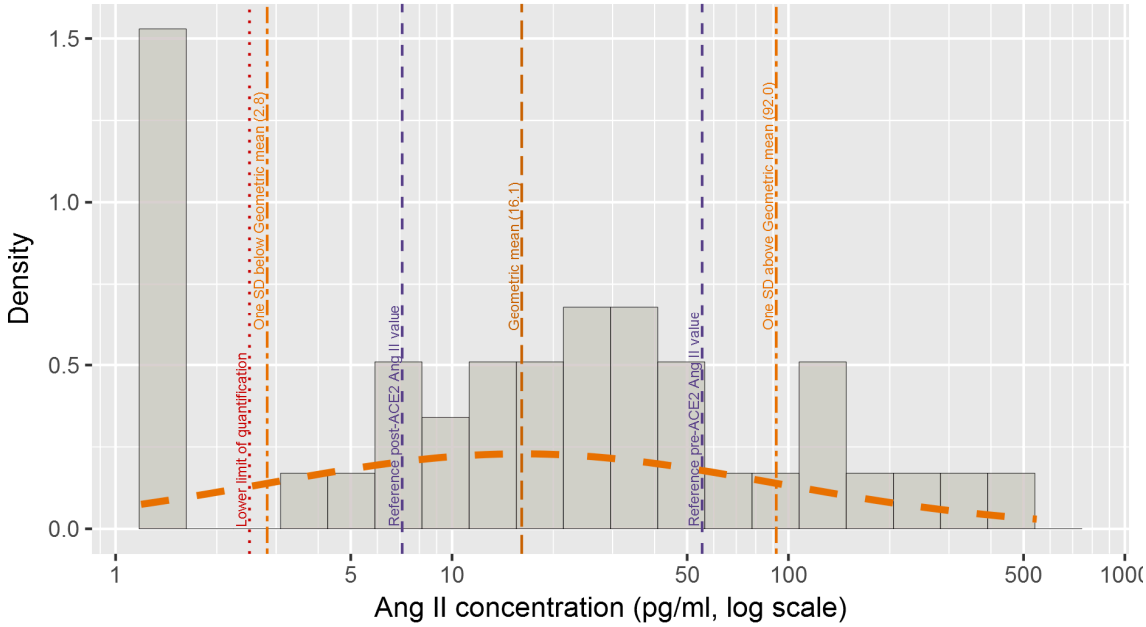
12.5.1. Distribution of Ang II

For the purposes of these sample size calculations, Ang II was chosen as the primary explanatory variable. This choice is based on the *a priori* belief that lowering Ang II is associated with improvements in RV outcomes. Similar modelling approaches can be applied to other RAS biomarkers, in particular Ang(1-7) (the other component of the dual action of Angiotensin Converting Enzyme (ACE)2); indeed these will be applied in the final analysis. However, for the purposes of defining sensible decision criteria, and assessing whether the proposed sample size of 50 participants (for the primary interim analysis) is suitable for purpose, only Ang II is considered in these simulations.

The assumptions underlying the distribution of Ang II in the participant population are as follows:

- Ang II follows log-normal distribution with mean and standard deviation as observed in 42 participants of the previous ARDS study (ACE114622; baseline measurements)
    - Geometric mean of 16.1 pg/ml.
    - Standard deviation of log(Ang II) is 1.74; equivalent to 5.7-fold change in Ang II.
    - Implies that ~⅔ of population will be in the range (2.8, 92.1) (one SD below mean to one SD above mean)

Figure 2 Distribution of observed Ang II in study ACE116422 with super-imposed normal distribution curve



12.5.2. Definition of outcome: an objective measure of right ventricular (RV) dysfunction

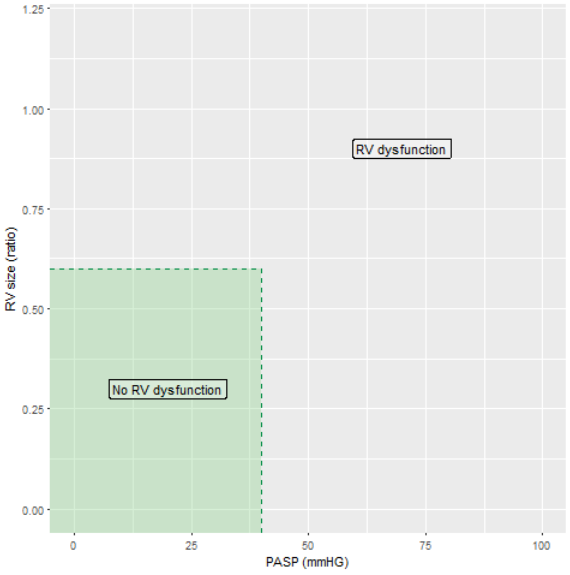
Following the definition of moderate pulmonary circulatory dysfunction (PCD) presented by Boissier 2013, quantitative criteria for an objective measurement of right ventricular (RV) dysfunction were set out as follows:

- A participant is defined as having right ventricular (RV) dysfunction if one of the following two criteria is true:
    - pulmonary arterial systolic pressure (PASP) > 40 mmHg
    - ratio of the right ventricular to the left ventricular end-diastolic areas (hereafter referred to as RV size ratio) > 0.6

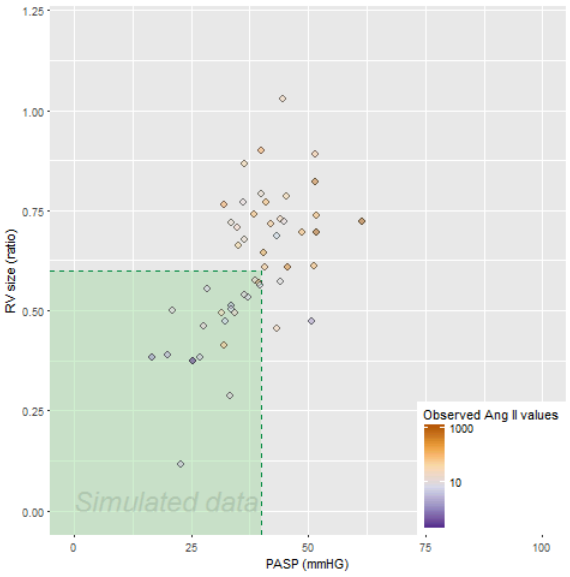
These two criteria can be represented on a 2-dimensional plane divided into two regions representing "no RV dysfunction" and "RV dysfunction" (***Figure*** 3a).

However, rather than dichotomising participants into those with and those without RV dysfunction based on individual measurements (***Figure*** 3b), the two component variables, PASP and RV size ratio, will be modelled as bivariate continuous outcomes, with Angiotensin II (Ang-II) level as a continuous predictor variable. The joint posterior distribution of PASP and RV size ratio for given reference levels of Ang-II will be calculated based on the observed data, and a posterior probability of the mean PASP and mean RV jointly lying in the "RV dysfunction" region of the plane calculated from these posterior distributions (***Figure*** 3c).

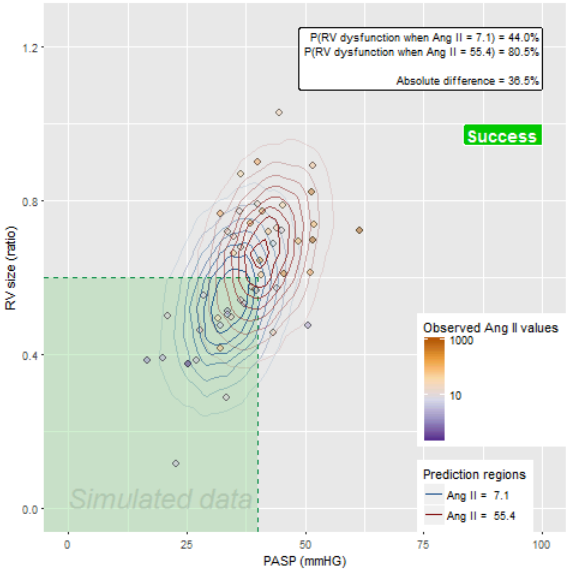
Figure 3 Illustration of the process of modelling the joint distribution of PASP and RV size ratio and obtaining a predicted probability of RV dysfunction for given Ang II levels



a) The criteria used for defining RV dysfunction are represented as a plane divided into two regions. Points (representing participant data) lying in the green region are categorised as not having RV dysfunction ("normal"), points lying in the grey region are categorised as having RV dysfunction ("dysfunctional").



b) Illustrative mock data showing simulated values of the two component variables RV dysfunction (PASP and RV size ratio, represented on the *x* and *y* axes) and their relationship to Ang II values (represented by colour). It is possible to dichotomise participants into those with RV dysfunction and those with normal RV function at this stage; this is not the approach proposed here.



c) Illustration of posterior prediction regions for the two component variables of RV dysfunction for two reference values of Ang II. Instead of modelling the association of Ang II with the dichotomised RV dysfunction response variable (and losing the information from the continuous variables), a Bayesian linear regression model is fitted with PASP and RV size ratio as bivariate outcome variables and Ang II as the explanatory variable. Prediction regions and the probability of RV dysfunction (defined as the weighted proportion of the prediction region being within the grey region of the plane) are calculated for two reference Ang II values. The absolute difference between these two probabilities is then calculated. If this difference is ≥30%, then the relationship between Ang II and RV dysfunction is considered strong enough for there to be potential for the action of ACE2 on Ang II to be of clinical benefit and further study to be conducted ("success"). If the difference is ≤15%, then the relationship is considered too weak to warrant further inquiry ("futile"). The reference values are derived as the geometric mean of the observed Ang II values at pre-dose and at 2 hours post rhACE2 dose in the investigator sponsored study (NCT01884051).

12.5.3. "Success" and "futility" criteria

Criteria for "success" (that is to say, the strength of association between Ang II and RV dysfunction required to confirm potential for the effect of rhACE2 on Ang II to yield clinical benefits, warranting further study) and "futility" (an association too weak to justify continuing development) are based on posterior predictive regions for PASP and RV size ratio for two given Ang II values. These reference values are the geometric mean of observed pre-dose and 2 hours' post-dose Ang II values from an investigator-sponsored study (NCT01884051), and represent the best estimate for a typical "before rhACE2" and "after rhACE2" measurement. The reference values used in this simulation are based on the two participants in that study for which, at the time of writing, data of sufficient reliability were available, and were derived as follows:

- Pre-dose Ang II: 55.4 pg/ml (geometric mean of 121.3 and 25.3 pg/ml)
- Post-dose Ang II: 7.1 pg/ml (geometric mean of 16.3 and 3.1 pg/ml)

"Success" and "futility" criteria are derived using the predicted probabilities of RV dysfunction for a hypothetical future participant with the reference values listed above. The predicted probability of RV dysfunction for a given Ang II value is defined as the proportion of the posterior joint predicted region of PASP and RV size ratio (weighted by density) that satisfies the criteria for RV dysfunction (i.e. what portion of the prediction region lies in the grey zone in ***Figure 3***). The absolute difference between the two

probabilities is then calculated and compared with the following criteria for success or futility (for N = 50, 100 and 150):

- "Success": P(RV dysfunction when Ang II = 55.4) - P(RV dysfunction when Ang II = 7.1) ≥ 30%
- "Futile": P(RV dysfunction when Ang II = 55.4) - P(RV dysfunction when Ang II = 7.1) ≤ 15%

The results are deemed "inconclusive" otherwise.

For N = 25, a conclusion of futile was returned if the difference between P (RV dysfunction when Ang II = 55.4) and P (RV dysfunction when Ang II = 7.1) was negative. A result of inconclusive was returned otherwise.

12.5.4. Assumptions for simulated datasets

A simulation was carried out to assess the operating characteristics of the above success and futility criteria, given reasonable assumptions for the distributions of PASP, RV size and Ang II, and the three pairwise correlations between them.

The assumptions for the distributions of these variables are as follows:

- Ang II is log-normally distributed, with mean(log(Ang II)) = 2.78 and SD(log(Ang II)) = 1.74 (as described above)
- PASP is normally distributed, with mean(PASP) = 38 mmHg and SD(PASP) = 8 mmHg
- RV size ratio is normally distributed, with mean(RV size ratio) = 0.61 and SD(RV size ratio) = 0.15

The assumed pairwise correlations between these three variables were as follows:

- The correlation between PASP and RV size ratio (the two outcome variables) was assumed to be 0.5
- Three different scenarios each for the correlation between PASP and log(Ang II), and between RV size ratio and log(Ang II) were considered (for a total of nine scenarios in all): zero, 0.3 and 0.6. This was to test the operating characteristics for a wide variety of scenarios ranging from "no association whatsoever" (both correlations zero) to "Ang II strongly correlated with both components of RV dysfunction" (both correlations 0.6).

A single simulated study dataset consisted of 50 values for Ang II, PASP and RV size, drawn from a multi-variate normal distribution with the above characteristics.

For each of the nine scenarios, a total of 1000 study datasets were generated and analysed, which was the maximum that was feasible given computational constraints.

12.5.5. Analysis of simulated data

For each individual study dataset that was generated, the relationship between Ang II (as the explanatory variable) and the joint bivariate distribution of PASP and RV size ratio (the outcome variables) was analysed using a Bayesian multivariate linear regression model. Estimates for the posterior distributions of coefficients in the model were obtained using an MCMC simulation (using Gibbs sampling) running three chains of 2000 iterations each. The first 1000 iterations of each chain were discarded (the "burn-in") and the posterior distributions estimated from the 3000 remaining iterations (1000 from each chain).

The multivariate normal model was specified as follows:

$$\begin{pmatrix} y_{i1} \\ y_{i2} \end{pmatrix} \sim MVNormal\left(\begin{pmatrix} \mu_{i1} \\ \mu_{i2} \end{pmatrix}, \Sigma\right)$$

where y_{ij} represents the observed value for the *i*th subject and the *j*th outcome (i.e. *j=1* representing PASP and *j=2* representing RV size ratio), μ_{ij} represents the expected value for the *i*th subject and the *j*th outcome (*j=1* and *j=2* as above), and Σ represents a 2×2 variance-covariance matrix for the two outcome variables. The expected values, μ_{ij}, are modelled as follows:

$$\mu_{ij} = \alpha_j + \beta_j x_i$$

where α_j is the intercept term for the *j*th outcome (i.e. PASP or RV size), β_j represents the slope coefficient for log(Ang II) in the model, and x_i represents the observed log(Ang II) value for the *i*th subject. For simplicity the simulations have no additional covariates (e.g. age, sex), and do not take into account multiple observations in the same participant (i.e. they represent the extreme worst case scenario where there is perfect within-participant correlation of all variables).

Uninformative prior distributions are assigned for α, β, and σ as follows:

- α is assigned a bivariate normal distribution with mean zero (for both outcomes), large variances (for both outcomes) and zero covariance (between the two outcomes),
- β is assigned a bivariate normal distribution with the same specifications as for α,
- T, defined as the inverse of Σ, is assigned an uninformative Wishart prior distribution: T ~ Wishart(R, df) where:

$$R = \begin{pmatrix} 0.001 & 0 \\ 0 & 0.001 \end{pmatrix}$$ and df=3.

12.5.6. Metrics for operating characteristics

For each scenario, 1000 simulated datasets of 150 participants each were generated and analysed according to the above modelling process, and the following metrics were calculated for the first 25, 50, 100 and the whole 150-participant dataset using the 1000 results so generated:

- the proportion of studies returning "success" results
- the proportion of studies returning "futile" results
- the proportion of studies returning “inconclusive” results
- the mean absolute difference in predicted P(RV dysfunction) between the two reference Ang II values
- the mean difference in predicted PASP between the two reference Ang II values
- the mean difference in predicted RV size ratio between the two reference Ang II values

Results are summarised for the nine scenarios and presented in the main body of the protocol (Section 10.1 and Section 10.3.3).

12.6. Appendix 6: Protocol Amendment History

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

Protocol Amendment 2 (27-Nov-2017)

Overall Rationale for the Amendment: In this amendment, an exploratory outcome and emergency consent have been added. In addition, clarification was added throughout the protocol.

Section # and Name	Description of Change	Brief Rationale
2. Schedule of Assessments	Added Sequential Organ Failure (SOFA) score	Clarification that SOFA will be calculated on a daily basis. All components needed to calculate the score were already being collected.
2. Schedule of Assessments	The following language was added in Footnote 2 of Schedule of Assessments: *Other measurements (e.g. ventilator settings) to be prioritized as per investigator for patient care.*	The timing of exploratory outcomes can be prioritized by investigators in the best interest of patients as part of standard of care.
2. Schedule of Assessments	A note was added that SAPS II will only be measured at screening.	The timing of the assessment was clarified.
2. Schedule of Assessments 4. Objectives and Endpoints 9.5.5 General management status	Added Participant Management which includes use of ECMO and ECCO2R.	Sites have recently included use of ECMO and ECCO2R as part of patient's standard management. The use needs to be documented in case of interaction with outcomes.
2. Schedule of Assessments 4. Objectives and Endpoints 9.3 Ventilator settings	Static compliance was added as a measurement that will be collected with other ventilator settings.	It had previously been missed; static compliance is a standard measure read from the ventilator.
2. Schedule of assessments 4. Objectives and Endpoints 9.4 Lung Function	pH and base excess were added as measurements that will be collected with other lung function measurements.	These measurements had previously been missed. • pH is a standard measure automatically calculated from blood gas analysis. • Base excess is a standard blood gas analysis. Depending on the base status of the patient a base

Section # and Name	Description of Change	Brief Rationale
		excess result may be collected rather than base deficit.
2. Schedule of Assessments 4. Objectives and Endpoints 9.9 Biomarkers 12.2 Appendix 2: Clinical Laboratory Tests	Troponin I or T was removed from the schedule of assessments, objectives and endpoints and biomarker sections and added to Table 3. *Protocol required safety laboratory assessments.*	Troponin I or T was removed as a test performed by GSK and will be performed as a safety lab by sites when taken as standard of care.
4. Objectives and Endpoints	Systolic eccentricity index was moved from Primary to exploratory endpoint.	The primary analysis will use the binary outcome of paradoxical septal motion. Systolic eccentricity index is a continuous variable and was included in the primary endpoint in error. This will now be analysed separately as an exploratory outcome.
4. Objectives and Endpoints 9.2.2 Electrical Impedance Tomography (EIT)	New exploratory objective was added.	EIT assesses lung ventilation and perfusion. Understanding regional ventilation/perfusion may be of importance in understanding the role of the RAS in ventilation/perfusion matching in severe respiratory failure.
4. Objectives and Endpoints 9.2.1 Echocardiograms	It was added that the inferior vena cava diameter will be taken at end-expiration. The formula for calculating Respiratory variations of RV velocity time integral was added for completeness. The formula is (end-expiration minus end-inspiration / end-expiration).	Values that will be recorded from the echocardiograms were further defined for clarity.
9.1 Demographic/Medical History Assessments	Clarification was added that *demographic and medical history assessments* could be collected at any point in the study if it is not possible at screening.	Whilst important to collect this data where possible, sometimes it is extremely challenging to collect in the acute care setting.

Section # and Name	Description of Change	Brief Rationale
9.5.2 SAPS II	It was clarified that an estimated GCS can be used if a patient is sedated at the time of assessment	When a patient is sedated the GCS cannot be calculated, therefore an estimate prior to sedation must be used (where available).
9.5.3 Disease Diagnosis	ARDS Definition was added.	The definition was missing from previous version.
9.5.3 Disease Diagnosis	PCD definition clarified	Definition was previously missing > and ≥ for PASP and RV size respectively.
9.6 Additional clinical assessments	It was defined that date and time of only *concomitant surgery* will be recorded.	To clarify that only *concomitant surgery* needs to be recorded.
9.9 Biomarkers	Troponin I or T was removed as a test performed by GSK and will be performed by sites when taken as standard of care. Clarified that samples will be stored, and other biomarkers may be assessed eg. BNP and eg. Inflammatory markers/lung injury markers.	Troponin I or T cannot be measured at GSK. To clarify other biomarkers may be tested and samples will be stored.
10.3.1 Pharmacodynamics Analyses	Statistical analysis (PD) wording updated to clarify the rationale for additional RAS peptide analyses.	The biological effects of the RAS are complex and effects of peptides beyond Ang II (peptide to be assessed in primary analysis) may be observed e.g. Ang 1-7.
12.2 Appendix 2: Clinical Laboratory Tests	It was clarified that the safety assessments in Table 3 will be as per standard of care.	To ensure assessments are only completed as per standard of care
12.3.3 Informed consent process	The option to enrol a participant with emergency consent was added.	To ensure the consenting process is appropriate for the participant population.
Throughout	Deleted mention of Institutional Review Board (IRB) throughout.	This protocol does not require submission to an IRB due to lack of investigational product

Protocol Amendment 1 (02-Jun-2017)

Overall Rationale for the Amendment: Protocol was amended to remove the option for participants to be included without informed consent and to change errors in the previous version.

Section # and Name	Description of Change	Brief Rationale
2. Schedule of Assessments And 9.1 Demography and Medical History Assessments	Height & Weight assessment were added as they were missed from the prior version.	Prior version mentioned these parameters only under the inclusion criteria, but missed them in Section 2 and Section 9.1.
2. Schedule of Assessments	Assessments of Diagnosis of PCD & ACP were removed from Participant status and added as a separate line.	To provide more clarity on the time points of these assessments.
9.5.1 SAPS II	Glasgow Coma scale was removed from the components defined for the SAPS II score.	It was incorrectly included in the prior version.
12.3.3 Informed consent process and 6.1 inclusion criteria	Removed the option for participants to be included without informed consent.	It was decided participants must have a signed informed consent by their legally acceptable representative prior to enrolment.