

TITLE PAGE

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Title:	An evaluation of DCE-MRI measures of pulmonary oedema and vascular permeability in healthy subjects and in patients with cardiac failure: A methods validation study for evaluation of novel treatments limiting pulmonary oedema in cardiac failure
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2013N186309_01	2014-APR-24	Amendment No.: 01
<p>This protocol amendment clarifies the exercise protocol to be conducted, details measures for endpoints, defines the biomarker to be measured (NT-pro BNP), reduces total blood volume to be collected, updates study schematic, adds an interim review of the data, removes collection of AEs as there is no IP included in the study, alters physical exams to brief physical exams, and adds a dyspnea scale and the Borg Rating of Perceived Exertion.</p>		
2013N186309_02	2015-FEB-16	Amendment No.: 02
<p>This protocol amendment changes modifies inclusion criteria, clarifies the exercise protocol, and corrects the footnote numbering in the time and events table.</p>		
2013N186309_03	2016-APR-08	Amendment No. 3
<p>Amendment No. 3 adds an evaluation of an additional group of subjects who have been hospitalized for acute decompensated heart failure to determine whether DCE-MRI can detect changes in measures of pulmonary oedema with standard of care treatment.</p>		

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INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol 201137

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

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

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

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Investigator Signature	Date

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LIST OF ABBREVIATIONS

3D SPGR	3D Spoiled Gradient Echo
ADHF	Acute Decompensated Heart Failure
AE	Adverse Event
AIF	Arterial Input Function
ALT	Alanine Transaminase
AST	Aspartate Transaminase
BMI	Body Mass Index
BNP	B-Type Natriuretic Peptide
BP	Blood Pressure
CXR	Chest X-Ray
CHF	Chronic Heart Failure
DCE	Dynamic Contrast Enhanced
DLCO	Diffusing Capacity of the Lung for Carbon Monoxide
DLNO	Diffusing Capacity of the Lung for Nitrous Oxide
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
FDA	Food and Drug Administration
FENa	Fractional excretion of sodium
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
Gd	Gadolinium
GSK	GlaxoSmithKline
HF	Heart Failure
HV	Healthy Volunteers
IB	Investigator Brochure
ICH	International Conference on Harmonisation
IDSL	Integrated Data Standards Library
IEC	Institutional Ethics Committee
IRB	Institutional Review Board
Kg	Kilogram
Ktrans	Exchange Rate
LSLV	Last Subject's Last Visit
LUS	Lung Ultrasound
MD	Medical Doctor
MedDRA	Medical Dictionary for Regulatory Activities
mL	Milliliter
MRI	Magnetic Resonance Imaging
mSv	Milli-sievert
NHS	National Health Service
NO	Nitric Oxide
NSF/NFD	Nephrogenic Systemic Fibrosis/Nephrogenic Fibrosing Dermopathy
NT-proBNP	N-terminal of pro-Brain-type Natriuretic Peptide
NYHA	New York Heart Association

PACS	Picture Archiving and Communication System
PE	Pulmonary Oedema
PIMS	Phase I Information Management System
RBC	Red Blood Cells
RER	Respiratory Exchange Ratio
RPE	Rating of Perceived Exertion
RIS	Radiology Information System
SAE	Serious Adverse Event
SaO ₂	Saturation of Oxygen
SPM	Study Procedures Manual
Sv	Sievert
TE	Constant Echo Time
TR	Repetition Time
TRPV4	Transient Receptor Potential Vanilloid 4
UK	United Kingdom
ULN	Upper Limit of Normal
VCO ₂	Carbon Dioxide Production [Volume of Carbon Dioxide]
Ve	Interstitial Volume
VE	Ventilation
VO ₂	Oxygen Consumption [Volume of Oxygen]
Vp	Plasma Volume
WBC	White Blood Cells

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NONE	The Borg CR10 scale

1. INTRODUCTION

1.1. Study Rationale

In cardiogenic pulmonary oedema, increased venous pressure causes fluid extravasation from the vascular to the pulmonary interstitial and alveolar spaces. Reduced exercise tolerance, orthopnoea and paroxysmal nocturnal dyspnoea are all thought to be associated symptoms related to interference with gas exchange or up-regulation of baroreceptors activating a centrally mediated response (Redfield, 2003). The therapeutic arsenal is limited, and many patients are found refractory to current therapies. This is a substantial clinical problem, waiting for a novel therapeutic option such as a transient receptor potential vanilloid 4 (TRPV4) channel blocker.

TRPV4 channels are expressed at the pulmonary alveolar septal barrier, limiting the movement of intravascular fluid into the interstitial and alveolar air spaces. TRPV4 is activated by increases in pulmonary vascular pressure, resulting in endothelial cell contraction and breakdown of the septal barrier. The breakdown of the barrier results in the development of pulmonary oedema.

TRPV4 channel blockade may be promising in the treatment of pulmonary oedema and dyspnoea in heart failure patients by re-establishing the alveolar septal barrier. Although these drugs are effective in animal models, pharmacological studies in humans are likely to be restricted by difficulty in accurately quantifying pulmonary oedema.

There is interest in the potential role of magnetic resonance imaging (MRI) in quantifying pulmonary oedema. MRI has demonstrated increased lung water content in people with heart failure (Chow, 2009); however quantification remains problematic, and it is not possible to distinguish between intravascular and extravascular water using existing techniques.

Dynamic contrast enhanced (DCE) MRI is an established technique for assessing changes in vascular permeability and interstitial water volume. DCE-MRI measures the exchange of an extracellular MRI contrast agent as it travels between the intravascular space and the interstitial space. DCE-MRI enables quantification of the plasma volume (v_p), interstitial volume (v_e), and exchange rate (k^{trans}), a parameter strongly dependent on vascular permeability. DCE-MRI is routinely used within oncology studies (O'Connor, 2007), and is used increasingly in other indications such as renal disease (Buckley, 2006) and rheumatoid arthritis (Hodgson, 2007). DCE-MRI has been used to detect alterations in vascular permeability and interstitial water volume in the lungs of smokers relative to healthy controls (Naish, 2008). However, DCE-MRI has not yet been used in the context of heart failure (HF).

The primary objective of this study is to characterize the capability of MRI to detect increased interstitial lung fluid or altered vascular permeability. First, the DCE-MRI markers of vascular permeability and pulmonary oedema will be measured in healthy volunteers (HV) and subjects with HF at rest to determine whether there is a difference between the two populations. Next, exercise-induced changes (relative to rest) in interstitial volume and exchange rate will be evaluated in both HV and subjects with HF.

In patients with chronic HF, an abnormal alveolar-vascular interface leading to increased fluid flux at high pulmonary arterial pressures, as well as a tendency toward greater exercise-induced pulmonary vascular pressure, increases the chance for excess fluid under these conditions compared to what may be observed in normal subjects.

The secondary outcome measure will be the intrasubject variability in DCE-MRI measures of pulmonary physiology, determined by a same-subject comparison of two imaging sessions.

To correlate the potential clinical significance of MRI findings, exploratory endpoints will include comparison of DCE-MRI measures with a dyspnoea scale validated in acute pulmonary oedema (Mebazaa, 2010), disease severity, and pulmonary function (e.g., diffusing capacity of the lung for carbon monoxide (DL_{CO})).

Additionally, the capability of DCE-MRI to detect changes in interstitial lung fluid in patients with acute decompensated heart failure (ADHF) will be investigated. DCE-MRI markers of pulmonary oedema will be assessed when patients are initially hospitalized with ADHF and subsequently after receiving standard of care treatment to determine whether differences can be detected by this methodology. Lung ultrasound (LUS) may also be explored in patients with ADHF as a potential tool for assessment of extra-vascular lung water through measurement of B-lines.

1.2. Brief Background

Specific TRPV4 channel blockers limit the development of pulmonary oedema in animal models. Target validation and dose-ranging studies in humans are likely to be restricted by a lack of sensitive and specific endpoints. MRI can provide quantitative measures of vascular permeability and pulmonary oedema. The aim of this study is to establish the potential utility of MRI as a novel endpoint for future dose-ranging and proof-of-mechanism studies with TRPV4 blockers.

2. OBJECTIVE(S) AND ENDPOINT(S)

The objectives and endpoints for the study are summarised in the table below:

Objectives	Endpoints
Primary	
<ul style="list-style-type: none"> Establish whether DCE-MRI can detect differences in measures of pulmonary oedema or vascular permeability between HF and HV groups. Explore the effect of exercise on DCE-MRI measures of pulmonary oedema and vascular permeability in HF and HV groups. 	<ul style="list-style-type: none"> Interstitial volume (v_e) and exchange rate (k^{trans}) measured using DCE-MRI in HF patients and HVs at baseline. Change in interstitial volume (v_e) and exchange rate (k^{trans}) measured using DCE-MRI in HF patients and HVs before and following exercise.

Objectives	Endpoints
<ul style="list-style-type: none"> Explore the effect of standard of care treatment on DCE-MRI measures of pulmonary oedema and vascular permeability in patients with ADHF. 	<ul style="list-style-type: none"> Change in interstitial volume (v_e) and exchange rate (k^{trans}) measured using DCE-MRI in patients with ADHF during hospitalization and following the resolution of pulmonary oedema.
Secondary	
<ul style="list-style-type: none"> Estimate the intrasubject variability of DCE-MRI measures of pulmonary oedema and vascular permeability. 	<ul style="list-style-type: none"> Estimation of the variability in the interstitial volume (v_e) and exchange rate (k^{trans}) between 2 MRI visits approximately 1 week apart.
Exploratory	
<ul style="list-style-type: none"> Explore the relationship between DCE-MRI measures of pulmonary physiology and disease severity, symptoms, pulmonary function, volume status and renal function Estimate differences in plasma volume and MRI physical properties influenced by the tissue microenvironment relaxation rate, and proton density between HF and HV groups as measured using DCE-MRI Explore the distribution of DCE-MRI measures of pulmonary physiology in HF and HV groups both before and after exercise Explore the effect of standard of care treatment on plasma volume and MRI physical properties influenced by the tissue microenvironment relaxation rate, and proton density in patients 	<ul style="list-style-type: none"> Correlation of MRI measures with clinical and biochemical measures: dyspnoea score, NYHA grade, respiratory rate, NT-proBNP, exercise capacity (including Borg RPE, heart rate, blood pressure, and gas exchange endpoints such as peak VO_2, VE/VCO_2 slope), DL_{CO}, DL_{NO}, body weight, urine specific gravity, fractional excretion of sodium (FENa), blood urea to creatinine ratio. Plasma volume (v_p), T_1 relaxation rate, and proton density measured using MRI in HF patients and HVs. Interstitial volume (v_e) and exchange rate (k^{trans}), and other clinical and biochemical measures as listed above if data permit. Change in Plasma volume (v_p), T_1 relaxation rate, and proton density measured using MRI in patients with ADHF during hospitalization and following the resolution of

Objectives	Endpoints
<p>with ADHF.</p> <ul style="list-style-type: none"> Explore the effect of standard of care treatment on ultrasound measures of pulmonary oedema in patients with ADHF. Explore the distribution of DCE-MRI measures of pulmonary physiology in ADHF patients both before and after standard of care treatment 	<p>pulmonary oedema</p> <ul style="list-style-type: none"> Change in B-lines measured using ultrasound in patients with ADHF during hospitalization and following the resolution of pulmonary oedema Interstitial volume (v_e) and exchange rate (k^{trans}), and other clinical and biochemical measures as listed above if data permit

3. STUDY DESIGN

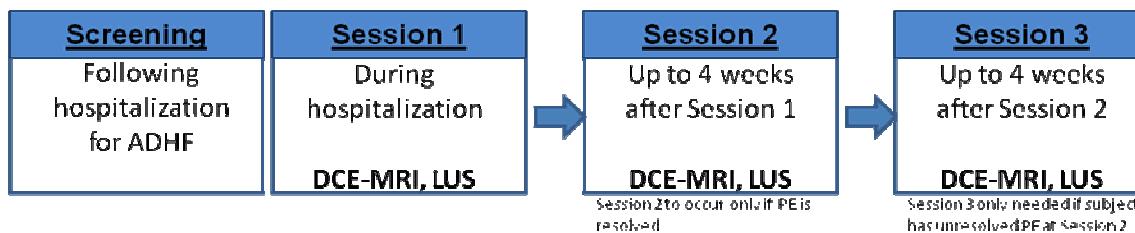
3.1. Study Schematic

Protocol waivers or exemptions are not allowed. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential.

For healthy volunteers and subjects with HF (Groups 1 and 2, respectively), the study design schematic is shown below:



For subjects with ADHF (Group 3), the study design is shown below:



3.2. Study Design Detail

For Groups 1 and 2, the study will consist of four sessions. The first visit will be a Screening Visit during which an appropriately trained study team member will describe the study, answer any questions the potential participant may have about the study seek

informed, written consent, and assess eligibility to enrol in the study. Within 35 days, subjects will return for the first scanning session, where subjects will undergo the baseline procedure. The second imaging session will occur approximately one week later to measure within-subject variability. A third imaging session (which will be conducted in 2 visits) will incorporate a bicycle exercise challenge prior to the MRI scan, and this third scan will be performed approximately one to three days after the second imaging session.

For subjects with ADHF (Group 3), Screening will occur during hospitalization to assess eligibility and enrolment in the study. Session 1 will be conducted while the subject is still hospitalized with evidence of pulmonary oedema and receiving standard of care treatment. Session 2 will be conducted within 4 weeks of the first scan, when the signs of pulmonary oedema are considered to be resolved as judged by the Investigator. If a subject's pulmonary oedema has not resolved at Session 2, as judged by the Investigator using lung auscultation and/or chest x-ray, then the subject will be not be scanned by MRI at Session 2 and will be brought back for Session 3 up to 4 weeks after Session 2 for their second MRI.

3.3. Risk Management

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

All inclusion criteria will be satisfied before patients undergo any procedures.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
Risk of exacerbation of dyspnoea in heart failure subjects when lying supine.	This is expected to be rapidly reversible on sitting upright, as the orthopnoea effect is dependent upon venous pressure	To minimize the risk, a trial of the supine position will be performed during the screening visit.	All procedures will be medically supervised. There is continuous monitoring of peripheral oxygen saturations and heart rate in the scanner control room, as well as direct vision down the magnet bore from the control room. The subject has a squeeze-bulb call bell to interrupt scanning. MRI compatible trolleys, wheel chairs and oxygen cylinders allow for removal from the scanner room into the foyer of the scanning suite where a full resuscitation trolley is available. All scanning staff members are trained in the emergency removal of subjects from the scanners. In the event of significant dyspnoea, treatments available in the study area will include sitting upright and oxygen if required. If further therapies are required, best practice clinical management including transfer to hospital for further management will be

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
			undertaken.
Use of Gd in subjects with abnormal renal function may result in Nephrogenic Systemic Fibrosis/Nephrogenic Fibrosing Dermopathy (NSF/NFD).	<p>Gadobutrol (Gd) (Gadovist; Bayer) at a dose less than or equal to 0.1mmol/kg will be used in the MRI protocol. It is excreted in the urine with approximately 50% and 90% of the dose excreted by 2 and 12 hours, respectively, post-injection.</p> <p>Approximately 200 million patients have had contrast enhanced Gd MRI examinations since such scans were first introduced 20 years ago [Thomsen, 2006]. The contrast agents are widely used off label for MRI angiography and cardiac MRI due to their low side effect profile and low risk of nephrotoxicity. From June 2006 to 12 April 2007, more than 215 individuals with NSF/NFD had been reported to the FDA [Cowper, ICNSFR Website, 2007]. All these individuals were exposed to Gd prior to developing NSF and all had moderate to end-stage renal disease prior to exposure to Gd: approximately 90% of the cases were dialysis dependent whilst the remaining 10% of subjects had chronic kidney disease or acute kidney injury [Perazella, 2007]. The incidence of NSF in dialysis and end-stage renal disease patients when exposed to Gd is in the range 3-5% [Marckmann, 2006; Kuo, 2008; Perazella, 2007; Deo, 2007; Chewning, 2007; Thomsen, 2006]. It is concluded that subjects dependent on dialysis and those with estimated glomerular filtration rate (eGFR) <30mL/min (chronic kidney disease stage 4/5) are at risk of developing NSF on exposure to Gd [Perazella, 2007].</p>	<p>As a result of this recent evidence, subjects with creatinine clearance levels < 60mL/min will be excluded from this study (Groups 1 and 2) and < 40mL/min in Group 3.</p>	<p>Gd contains unpaired electrons that interact with neighbouring water molecules and thereby generate MRI contrast. Free unbound Gd is extremely toxic, but when chelated to a ligand, its safety profile is dramatically improved, assisted by a 500-fold increase in renal excretion [Kramer, 2007].</p> <p>The elimination half-time in patients with normal renal function is approximately 1.5 hours, but in patients with severely reduced kidney function (GFRs between 2-10), it is as long as 34 hours. The decreased elimination time may allow Gd to dissociate from its chelating agent. Free, unbound Gd may then deposit in tissue beds and incite a fibrotic process, perhaps via circulating fibrocytes. Patients with normal renal function appear to have negligible risk of developing NSF/NFD [Kramer, 2007].</p>
Side effects from bicycle testing	Side effects of bicycle testing can include shortness of breath, light-headedness, drop in blood pressure, and abnormal heart rhythm. In rare cases, these side effects can be serious or life-threatening. Also possible are direct injuries such as	Inclusion criteria. Medical history and PI discretion as to patient's ability to participate in the exercise test.	Medical oversight during the test.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
	bruises, sprains, and strains and indirect problems such worsening of pain from arthritis.		
Subject may not be able to enter the MRI machine	Certain interventions, prostheses, or foreign bodies might be incompatible with the MRI scanner.	Exclusion criteria and local MRI scanning facility criteria	All participants will be screened according to local hospital criteria and trial inclusion/exclusion before entering the MRI room to ensure they are able to have the MRI conducted.
Subject may not be able to complete MRI once started	MRI can be associated with claustrophobia. The radio fields used in MRI can cause mild nerve stimulation and tissue heating, both of which are strictly regulated by both software and hardware controls implemented by the scanner manufacturer that cannot be bypassed.	Exclusion criteria and medical history.	All scans will be conducted by experienced radiographers and radiologists in the nominated scanning sites. If a subject becomes claustrophobic, the subject will be withdrawn from the study if they are unable to tolerate it again.
Within group 3, exposure to ionizing radiation from chest x-ray.	The dose from a single chest X-ray should not exceed 20 micro-sievert (Sv) and this corresponds to a lifetime risk of a fatal malignancy of about 1 in 1 million (ICRP 103). The study will minimize exposure by using x-rays acquired as part of clinical care whenever possible. It is expected that all subjects will require a single chest x-ray, but may have a maximum of 3 chest x-rays (up to maximum 60 micro-Sv within this study, lifetime risk of fatal malignancy of approximately 1 in 333,333).	Females of reproductive potential and all subjects under 50 years of age will be excluded from participation in group 3.	All procedures will be performed by a qualified technician.

4. STUDY POPULATION

4.1. Number of Subjects

With respect to Groups 1 and 2, this study plans to enrol a sufficient number of subjects to have at least 24 subjects (12 HV and 12 subjects with HF) with evaluable MRI data. For each subject, the MRI data must be of sufficient quality to enable DCE-MRI modelling from both Session 1 and Session 2. Additionally, at least 6 HV and 6 subjects with HF are needed to complete the third scanning session with evaluable MRI data following exercise testing.

After 12 subjects (6 HV and 6 subjects with HF) have completed Sessions 1 and 2, an interim review will be performed. Following the interim review, the study team will decide whether to (a) terminate the study if it is determined that the image quality is poor (e.g., due to excessive movement during scanning or related to specifics around lung tissue) or the procedures are not well tolerated in the HF group (e.g., inability to lie flat

for the duration of the imaging session), or (b) continue the study to enrol a sufficient number of additional subjects to meet the designated numbers required (see above).

The interim decision will be based on the expert opinion of the Investigator in consultation with the GSK Study Team.

For Group 3, it is planned that a sufficient number of subjects with ADHF will be enrolled so that at least 5 subjects complete two scanning Sessions (1 and 2 or 1 and 3).

4.2. Eligibility Criteria

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

4.2.1. Inclusion Criteria

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

4.2.1.1. Inclusion Criteria for Heart Failure Group (Group 2)

1. Established diagnosis of mild to moderate heart failure of any aetiology with symptoms defined as corresponding to the New York Heart Association (NYHA) class II or III

4.2.1.2. Inclusion Criteria for Healthy Volunteer Group (Group 1)

1. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, brief physical examination, clinical laboratory tests, and ECG

4.2.1.3. Inclusion Criteria for Both the Healthy Volunteer and Heart Failure Groups (Group 1 and 2)

1. Males or females over 18 years of age at the time of signing the informed consent
2. Body weight \geq 50kg and BMI within the range 18-40 kg/m² (inclusive)
3. Able to understand and comply with protocol requirements, instructions and protocol-stated restrictions and is willing to take part in the imaging sessions
4. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form

4.2.1.4. Inclusion Criteria for Subjects with ADHF (Group 3)

1. Male subjects OR female subjects of non reproductive potential as defined as pre-menopausal females with a documented tubal ligation or hysterectomy, or

post-menopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) > 40 MIU/mL and oestradiol < 40 pg/mL (< 140 pmol/L) is confirmatory.

2. 50 years of age or over at the time of signing the informed consent
3. Hospitalized for the management of acute decompensated HF
4. Presence of dyspnoea at rest or with minimal activity
5. Presence of at least one of the following signs:
 - Tachypnea with respiratory rate ≥ 20 breaths/min
 - Rales or crackles audible on auscultation
6. Chest x-ray with evidence of pulmonary congestion/oedema performed approximately within the last 48 hours (if not available – an additional research CXR may be requested)
7. Have received at least one treatment with an intravenous diuretic prior to the first MRI scan
8. Body weight ≥ 50 kg and BMI within the range 18-40 kg/m² (inclusive)
9. Able to understand and comply with protocol requirements, instructions and protocol-stated restrictions and is willing to take part in the imaging sessions
10. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form

4.2.2. Exclusion Criteria

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

4.2.2.1. Exclusion Criteria for Heart Failure Group (Group 2)

1. History of known primary pulmonary disease requiring current medication or other therapy
2. Orthopnoea of sufficient severity to preclude supine scanning as determined at screening
3. Unstable angina within the past 3 months
4. Uncontrolled hypertension (resting systolic BP > 160 mmHg or resting diastolic BP > 100 mmHg)
5. Resting hypoxia while breathing room air (SaO₂ $< 88\%$)

4.2.2.2. Exclusion Criteria for Both the Healthy Volunteer and Heart Failure Groups (Group 1 and 2)

1. Current smoker, defined as having smoked in the preceding 6 months

2. Contraindication for MRI scanning (as assessed by local MRI safety questionnaire), which includes but is not limited to:
 - a. Intracranial aneurysm clips (except Sugita) or other metallic objects
 - b. Intra- orbital metal fragments that have not been removed
 - c. Pacemakers or other implanted cardiac rhythm management devices and non-MRI compatible heart valves
 - d. Inner ear implants
 - e. History of claustrophobia
3. Pregnant females as determined by positive urine HCG test at screening or prior to any scanning session
4. Positive test for drugs of abuse, not due to current prescription drugs as determined by the GSK Medical Monitor and PI, and alcohol screen
5. Estimated creatinine clearance (Cockcroft-Gault) <60mL/minute

4.2.2.3. Exclusion Criteria for Subjects with ADHF (Group 3)

1. End-stage heart failure defined as requiring left ventricular assist devices, intra-aortic balloon pump or any type of mechanical support
2. Chronic or intermittent renal support therapy (hemodialysis, ultrafiltration, or peritoneal dialysis)
3. Ongoing or planned intravenous diuretic treatment within 1 hour of MRI scan appointment
4. History of known primary pulmonary disease requiring current medication or other therapy
5. Orthopnoea of sufficient severity to preclude supine scanning (as determined by a 15-minute test of lying supine with or without the use of oxygen)
6. Contraindication for MRI scanning (as assessed by local MRI safety questionnaire), which includes but is not limited to:
 - a. Intracranial aneurysm clips (except Sugita) or other metallic objects
 - b. Intra-orbital metal fragments that have not been removed
 - c. Pacemakers or other implanted cardiac rhythm management devices and non-MRI compatible heart valves
 - d. Inner ear implants
 - e. History of claustrophobia
7. Estimated creatinine clearance (Cockcroft-Gault) <40mL/minute
8. Contraindication to MRI contrast agents
9. Previous inclusion in a research and/or medical protocol involving nuclear medicine, PET or radiological investigations with significant radiation burden (a significant radiation burden being defined as 10 mSv in addition to natural

background radiation, in the previous 3 years including the dose from this study).

4.3. Lifestyle and/or Dietary Restrictions

4.3.1. Contraception Requirements

4.3.1.1. Female Subjects

For subjects in Groups 1 and 2, no specific forms of contraception are required for female subjects of childbearing potential, though they will be informed that they will be withdrawn from the study if they become pregnant. Female subjects under the age of 50 years or of childbearing potential are not eligible for Group 3.

4.3.1.2. Male Subjects

Male subjects with female partners of child-bearing potential do not require contraception in this study.

4.3.2. Exercise

In Groups 1 and 2, participants will be told to avoid strenuous exercise for 24 hours prior to each scanning session.

4.3.3. Caffeine and Alcohol (Groups 1 and 2)

For 24 hours prior to screening and each scanning session, subjects will abstain from alcohol consumption. Subjects with a positive alcohol breath test at any session will be excluded from the study.

On the day of each scanning session, subjects will refrain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate).

4.4. Withdrawal Criteria and Procedures

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

For Groups 1 and 2, subjects who withdraw or do not have evaluable DCE-MRI data from Sessions 1 and 2 will be replaced in order to meet the designated number of subjects (see Section 4.1). For subjects in group 3, who withdraw or do not have evaluable DCE-MRI scans from either Session 1 and 2 or Session 1 and 3 will be replaced in order to meet the designated number of subjects.

Should a subject fail to attend the clinic for a required study visit, the site should attempt to contact the subject and re-schedule the missed visit as soon as possible. The site should also counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study based on previous non-compliance. In cases where the subject does not return for the rescheduled visit or cannot be reached to reschedule the missed visit, the site should

make every effort to regain contact with the subject so that they can be withdrawn appropriately from the study.

4.5. Subject Completion

A completed subject is defined as one who has completed all sessions of the study.

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

5. STUDY TREATMENT

No treatment (investigational product) will be given in this study.

5.1. Concomitant Medications and Non-Drug Therapies

Group 1: Subjects in the healthy group may take all regularly prescribed and over-the-counter medication as planned throughout this study.

Group 2: Subjects in the heart failure group will take all regularly prescribed and over the counter medication as planned - except for loop diuretics, aldosterone antagonists, and thiazide diuretics on the day of imaging. These subjects will take their dose of these medicines after imaging has completed on that day.

Group 3: Subjects who have been hospitalized for ADHF will receive standard of care treatment both during the hospitalization period and following discharge. Within 1 hour of a planned MRI scan appointment, subjects should not have any ongoing or planned intravenous diuretic treatment (i.e., any treatment should be given after the planned scan appointment). Current diuretic dose will be recorded at each session.

6. STUDY ASSESSMENTS AND PROCEDURES

This section lists the procedures and parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Tables, Section [6.1](#).

For Groups 1 and 2, the procedures and assessments related to exercise testing in Session 3, will be conducted on two separate visits as detailed in the Time and Events Table, Section [6.1.1](#). The visits do not need to be on consecutive days.

Whenever vital signs, 12-lead ECGs and blood draws are scheduled for the same nominal time, the assessments should occur in the following order: 12-lead ECG, vital signs, blood draw.

The change in timing or addition of time points for any planned study assessments must be approved and documented by GSK, but this will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme. No more than 50mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

6.1. Time and Events Tables

6.1.1. Time and Event Table for Healthy Volunteers and Subjects with HF (Groups 1 and 2)

Day:	Screening	Session 1	Session 2	Session 3 ⁴	
Visit Window	-35 to -1 days	Day 1	7±2 days after Session 1	Scan up to 3 days after Scan in Session 2	
				Visit 1	Visit 2
Informed Consent	X				
Demography/Medical History/Concomitant Medications ¹	X				
Brief Physical Examination	X	X	X	X	
Orthopnoea Assessment ²	X				
Urinalysis, Pregnancy test ³ , Drugs of abuse screen, alcohol breath test	X	X	X	X	
Electrocardiograph (ECG)	X				
Vital Signs	X	X	X	X	X
Respiratory Rate ⁶		X	X	X	X
Clinical Laboratory Assessments	X	X ²	X ²	X ²	
NT- pro BNP ²	X			X ⁵	X ⁵
Dyspnoea Scoring ⁶		X	X	X	X
Exercise Testing ⁷				X	X
Magnetic Resonance Imaging with pulse oximetry monitoring		X	X		X
DL _{CO} and DL _{NO} ⁸		X	X		X
Record any SAEs, and any AEs related to study procedures ⁹	X	X	X	X	X

1. For subjects with HF, additional history assessments are detailed in Section 6.2.
2. HF subjects only, details in the SPM.
3. Women of childbearing potential only.
4. Session 3 will be conducted over two visits. The two visits do not need to be on consecutive days.
5. In Session 3, a blood sample for NT-proBNP will be collected before and after the maximal exercise test, before the constant workload exercise test, and after the MRI scan.

6. The dyspnoea score will be obtained before and after the maximal exercise test, before the constant workload exercise test, and after the MRI scan. Respiratory rate will be measured at these same timepoints (and as indicated during the exercise testing).
7. The exercise testing (maximal exercise to exhaustion and constant workload tests) may be conducted over a two-day (consecutive or non-consecutive) period. The maximal exercise test will be conducted before the constant workload test.
8. DLco and DLno measurements will be made after each MRI scan has been completed and prior to the constant workload exercise test in Session 3 (Visit 2).
9. See Section [6.12.2](#) and Section [6.12.3](#).

6.1.2. Time and Event Table for Subjects with ADHF (Group 3)

PROCEDURE	SCREENING	SESSION 1	SESSION 2 ⁴	SESSION 3 ⁵
	Following Hospital Admission For ADHF	During Hospitalization For ADHF	Up To 4 Weeks After Session 1	Up To 4 Weeks After Session 2
Informed Consent	X			
Demography/Medical History/Concomitant Medications ¹	X	X ⁸	X ⁸	X ⁸
Vital Signs including weight	X	X ²	X	X
Lung Auscultation	X	X ²	X	X
Orthopnoea Assessment	X			
Chest X-Ray ³		X ²	X	X
Oximetry	X	X	X	X
Lung Ultrasound ⁶		X ⁷	X	X
Magnetic Resonance Imaging (DCE-MRI)		X	X	X
Dyspnoea Score (5-Point Likert Scale)	X	X	X	X
Respiratory Rate	X	X	X	X
Creatinine	X	X	X	X
Haematocrit ⁹	X	X	X	X
FSH and Estradiol ¹⁰	X			
NT proBNP		X	X	X
Record Any SAEs and any AEs Related To Study Procedures		X	X	X

1. Information will be reviewed based on available hospital records
2. Assessment does not need to be repeated if Session 1 is conducted within 48 hours of Screening
3. At any designated visit, a chest X-ray need not be repeated if one has already been conducted as part of standard care
4. Session 2 will only be conducted when the pulmonary oedema is considered resolved. If pulmonary oedema is considered unresolved, only CXR and LUS may be collected.
5. Session 3 only in subjects deemed to not have pulmonary oedema resolved at session 2 by the Investigator.
6. Where feasible, Ultrasound may be performed to assess pulmonary oedema in addition to lung auscultation and / or CXR. The LUS may be completed at a separate visit (\pm 3 Days). Additional anatomic structures may be assessed during any ultrasound.
7. Multiple ultrasounds may be performed during hospitalization period of Session 1 up to one daily with a maximum of 3.
8. Sessions 1, 2 and 3 will include recording current diuretic dosage
9. Haematocrit values that are no more than 1 week old may be used
10. In women of non child bearing potential only

6.2. Demographic/Medical History Assessments

The following demographic parameters will be recorded:

- Year of birth
- Gender
- Race and ethnicity

For subjects with HF only, the following items pertaining to medical and medication history will be recorded/assessed at Screening (See eligibility criteria listed in Section 4.2):

- Confirm heart failure class
- Onset and type of symptoms
- Years since diagnosis of HF
- Degree of exercise intolerance: distance/ stairs/time prior to breathlessness
- Presence of orthopnoea and/or paroxysmal nocturnal dyspnoea
- Peripheral oedema: level above ankle, non-dependent limb
- Significant past medical history including onset, aetiology, and results of any recent relevant investigations of heart failure, as applicable
- Medication history

6.3. Safety

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

6.3.1. Brief Physical Examinations

A brief physical examination will be performed at each session for both groups of subjects (Groups 1 and 2). In subjects with HF (Group 2), peripheral oedema (level above ankle, non-dependent limb) should be monitored at all sessions. Height will be measured only on the first visit (Screening), and weight will be measured at every session. Lung auscultation will be performed on patients with ADHF in Group 3 at Screening, Sessions 1, 2, and 3 by investigator or medically trained designee.

6.3.2. Vital Signs

- Vital sign measurements will include systolic and diastolic blood pressure and heart rate.
- Vital signs will be collected with the subject semi-supine and after they have been resting for 5 minutes.

For Groups 1 and 2, additional measurements of heart rate and blood pressure to be made during the exercise testing will be detailed in the SPM.

Group 3 will include a weight measurement at Sessions 1, 2 and 3 (if needed).

6.3.3. **Electrocardiogram (ECG)**

- For Groups 1 and 2, 12-lead ECGs will be obtained during the study using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.
- ECGs will be obtained in the semi-supine position after the subject has been resting for at least 5 minutes.

6.3.4. **Clinical Laboratory Assessments and Urinalysis**

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed below for Groups 1 and 2. Details for the preparation and shipment of samples will be provided by the local laboratory.

Haematology

Platelet Count	<i>Automated WBC Differential:</i>
RBC Count	Neutrophils
WBC Count (absolute)	Lymphocytes
Hemoglobin	Monocytes
Hematocrit	Eosinophils
	Basophils

Clinical Chemistry

Urea	Calcium	Total and direct bilirubin
Creatinine	Troponin	CrCl (estimated using Cockcroft-Gault)
Glucose	ALT (SGPT)	
Sodium	AST (SGOT)	
Potassium		

Routine Urinalysis

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

Other Screening Tests

FSH and estradiol (as needed in women of non-child bearing potential only)
Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines)

Biomarkers

N-terminal pro-Brain-type Natriuretic Peptide (NT- pro BNP)

If additional non-protocol specified laboratory assessments are performed at the site's local laboratory, which result in a change in subject management or are considered clinical significant by the Investigator (for example, SAE), the results must be captured and sent to GSK along with other study data as defined in [Appendix 1](#).

6.3.4.1. Pregnancy Testing

For Groups 1 and 2, a standard urine pregnancy test will be performed at Screening and at every Session (first day of Session 3) in women of childbearing potential. If the test is positive, the subject will be withdrawn from the study. For Group 3, FSH and estradiol will be testing if needed to confirm non child bearing potential status.

6.4. N-terminal pro-Brain-type Natriuretic Peptide (NT-pro-BNP)

For subjects in Group 2, blood samples for NT-proBNP determinations will be drawn at screening and in Session 3 before and after the maximal exercise test, before the constant workload exercise test and after MRI scanning only in subjects with HF. For subjects participating in Group 3, blood samples for NT proBNP levels will be drawn according to the time and events table. Record the date and time that each sample is collected. Details of the processing, storage, and shipping of samples are located in the SPM.

6.5. Dyspnoea Scale Scoring

After the MRI scan in Sessions 1 and 2, a standardised, validated dyspnoea 5-point Likert scale ([Mebazaa, 2010](#)) will be completed for Groups 1 and 2. In Session 3 only, the score will be completed before and after the maximal exercise test, and before and after the constant workload exercise test (before scanning) and after MRI scanning.

For Group 3, the dyspnoea 5-point Likert scale will be completed at Screening and before and after the MRI scan in Sessions 1, 2, and 3 (if needed).

Details of the scale and administration are located in [Appendix 2](#) (Section 11.2) and the SPM.

6.6. Respiratory Rate

Respiratory rate will be recorded over a 60-second period.

For Groups 1 and 2, respiratory rate will be measured after the MRI scan in Sessions 1 and 2. In Session 3 only, respiratory rate will be measured immediately before and after the maximal exercise test, and before and after the constant workload exercise test (before scanning), and after MRI scanning. Any additional measurements to be taken during the exercise testing procedure will be detailed in the SPM.

For Group 3, the respiratory rate will be measured at Screening and before the MRI scan in Sessions 1, 2 and 3 (if needed).

6.7. Chest X-rays

For subjects with ADHF in Group 3, a clinically indicated chest X-ray (CXR) will be performed to assess cardiogenic pulmonary oedema. CXRs used as part of standard clinical care may be used when a CXR is acquired within 48 hours of a scheduled CXR per T&E Table Section 6.1.2. A maximum of 3 CXR scans will be performed as part of this study, each with a maximum of 20 microsievert. Additional details regarding the chest X-rays and the designation of resolution of pulmonary oedema may be found in the SPM.

6.8. Lung Ultrasound

For subjects with ADHF in Group 3, lung ultrasound may be used to assess pulmonary oedema, where feasible, specifically through measurement of the number of B lines. Additional anatomic structures may be assessed during any ultrasound. Additional details regarding the ultrasounds will be included within the SPM.

6.9. Magnetic Resonance Imaging

6.9.1. Subject Scanning Procedures

Subjects will undergo MRI scanning at Addenbrookes Radiology Department using a 1.5 T system. On attendance at the Radiology Department, subjects will be placed supine in the scanner and will be prepared for intravenous contrast agent administration.

The scanning protocol will include routine localisers followed by T1 measurement sequences and a dynamic time series. The T1 measurement sequences will consist of 3D spoiled gradient echo (3D-SPGR) with constant echo time (TE) and repetition time (TR) and varying flip angles. The dynamic series will also consist of 3D-SPGR acquisitions with constant TE, TR and constant flip angle. Contrast agent will be administered intravenously as a bolus using a power injector during the dynamic series. Scanner gains will be controlled manually between all of the 3D-SPGR acquisitions to allow subsequent DCE-MRI parameter quantification.

Subjects within Group 3 may receive medical oxygen during the exam provided they receive oxygen during all MRI sessions and delivery rate is kept constant during a scanning session and matched for both scanning sessions. Further details of scanning site training procedures and scanning protocols will be provided in a dedicated Imaging Manual.

All MRI scans will be reported (non-anonymised) for clinical abnormalities as per normal NHS Clinical Governance requirements for subject safety purposes.

6.9.2. Phantom Scanning

In order to maximise image quality and data consistency throughout the study, regular scanning of a Eurospin TO5 phantom will be required. The same scanning protocol as used in study subjects will be repeated using the phantom at intervals of no less than once every 6 weeks and immediately after any software or hardware upgrade or maintenance.

6.9.3. Data Transfer

All MRI data will be anonymised at site and transferred to Bioxydyn Limited. Details for image transfer will be provided in the Imaging Manual.

6.9.4. DCE-MRI Data Analysis

All DCE-MRI data analysis will take place at Bioxydyn Limited. DCE-MRI data will be corrected for breathing motion using registration and the lungs will be segmented from the image volume. An arterial input function (AIF) will be extracted from the pulmonary artery or right ventricle; if not available a population AIF will be substituted. The 'extended Tofts' tracer kinetic model will be applied to the data to provide measurements of k^{trans} (capillary transfer coefficient of contrast agent - /min), v_e (leakage space - fraction) and v_p (plasma volume - fraction). Median values of k^{trans} and v_e will be provided from each lung (total lung, apical and basal) for subsequent statistical analysis. All values will be corrected for the individual's haematocrit value. If not available, a typical haematocrit value will be assumed.

6.10. Exercise Test

In Session 3, subjects in Groups 1 and 2 will perform two exercise tests.

Maximal Exercise Test Limited by Dyspnoea or Fatigue

Subjects will first be asked to perform a maximal exercise test limited by dyspnoea or fatigue on a cycle ergometer. Baseline measurements (heart rate, blood pressure, respiratory rate, and breath-by-breath gas exchange measurements: oxygen consumption (VO_2), ventilation/carbon dioxide production (VE/VCO_2) slope, respiratory exchange ratio (RER), and VO_2 and time at ventilator threshold) will be performed with subjects seated on the cycle ergometer for approximately 3 minutes. Subjects will then be asked to commence cycling. The workloads will be increased in a stepwise fashion until volitional fatigue. Breath-by-breath gas measurements and heart rate will be recorded throughout exercise. At the end of each workload, Borg Rating of Perceived Exertion (RPE) (See Section 11.3 [Appendix 3](#)) and blood pressure will be obtained. At peak exercise, heart rate, blood pressure, Borg RPE, and breath-by-breath gas exchange measurements including peak VO_2 will be recorded. Respiratory rate at the end of exercise will also be recorded.

Constant Workload Exercise Test

Subjects will be asked to perform cycle exercise for 10 minutes at a workload between 75% to 80% of the peak work rate achieved during the maximal exercise to exhaustion test described above. Breath-by-breath gas measurements (VO_2 , RER, exercise time) and heart rate will be measured throughout the exercise period. Blood pressure and respiratory rate will also be measured.

Full details of the exercise test protocols are included in the SPM.

6.11. DLco and DLno

Pulmonary diffusion is impaired at rest in patients with chronic heart failure (HF) and has been implicated in the generation of symptoms and exercise intolerance. It is hypothesized that exercise increases pulmonary vascular pressures, thereby mediating a further lessening of the integrity of the vascular: alveolar membrane. This results in enhanced interstitial fluid, thereby altering membrane gas exchange as measured by DLco. This is based on the alveolar: vascular membrane conductance as well as the affinity for hemoglobin. To better characterize the membrane conductance alone, nitrogen oxide (NO) absorption will also be tested, as it is mostly dependent on the membrane itself. This is due to its profound affinity for hemoglobin well beyond that of CO. A correlation between DLco and DLno and the change in MRI imaging will be evaluated.

For Groups 1 and 2, DLco and DLno measurements will be made after each MRI scan has been completed and prior to the constant workload exercise test in Session 3, Visit 2.

Details of the pulmonary testing methodology including diffusing capacity and measured components are included in the SPM.

6.12. Serious Adverse Events (SAEs)

The Investigator (or designated site staff) is responsible for detecting, documenting and reporting events that meet the definition of an SAE.

6.12.1. Time Period for Collecting SAE Information

Any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) will be recorded from the time a subject consents to participate in the study up to and including the last visit. All SAEs will be recorded and reported to GSK within 24 hours, as indicated in [Appendix 1](#).

Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the Investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to study participation, the Investigator would promptly notify GSK.

NOTE: The method of, recording, evaluating and follow-up of SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in [Appendix 1](#).

6.12.2. Definition of Adverse Events

An AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.

Since no investigational product will be administered in this study, information regarding the occurrence of adverse events will not be routinely collected. Medical occurrences (non-serious events) and non-serious events related to study procedures that begin during the study will be recorded in the medical notes.

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

- Any abnormal laboratory test results (haematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the Investigator.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study treatment administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae.).

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

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

6.12.3. Definition of Serious Adverse Events

If an event is not an AE per Section [6.12.2](#), then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease, etc).

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

- a. Results in death

b. Is life-threatening

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

c. Requires hospitalization or prolongation of existing hospitalization.

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

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

d. Results in disability/incapacity, or

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

e. Is a congenital anomaly/birth defect.

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

6.12.4. Death Events

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

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

6.12.5. Prompt Reporting of SAEs to GSK

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

If the Investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before notifying GSK of the event and completing the appropriate data collection tool. The Investigator will always provide an assessment of causality at the time of the initial report as described in [Appendix 1](#).

6.12.6. Regulatory Reporting Requirements for SAEs

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

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

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

6.13. Pregnancy

If a woman in the study becomes pregnant, she will be withdrawn from the study.

6.13.1. Time Period for Collecting Pregnancy information

Any pregnancies occurring after the first session and up to and including the last visit should be reported.

6.13.2. Action to be Taken if Pregnancy Occurs

The Investigator will collect pregnancy information on any female subject, who becomes pregnant while participating in this study. The Investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of a subject's pregnancy. The subject will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE.

A spontaneous abortion is always considered to be an SAE and will be reported as such. Furthermore, any SAE occurring as a result of a post-study pregnancy will be reported to

GSK as described in Section [6.12.5](#). While the Investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

7. DATA MANAGEMENT

The MRI data will be transferred to a separate analysis company in an anonymised fashion. Instructions on MRI data transfer will provided to the scanning site.

For this study, subject data will be entered into an electronic Phase I Information Management System (PIMS), transmitted electronically to GSK, and combined with data provided from other sources in a validated data system.

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

8. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

8.1. Hypotheses Comparisons

Estimation approach will be used for the comparison of interests. No formal hypothesis testing will be performed. There are two comparisons of interest in this study to be performed between Group 1 and Group 2. The first is to estimate the feasibility of whether DCE-MRI can detect differences in DCE-MRI measures of pulmonary oedema or vascular permeability between HF and HV groups. The second comparison is to determine whether a burst of exercise activity can enhance the interstitial lung fluid (and/or exchange rate) from baseline.

8.2. Sample Size Considerations

Sample size is based on feasibility. A similarly sized study using DCE-MRI was able to detect statistically significant differences in the lungs of smokers compared to HVs ([Naish, 2008](#)).

8.3. Data Analysis Considerations

Anonymised data may be transferred within GSK or to external sites. In some cases, we may use pooled or anonymised individual image data to support scientific reports.

All imaging data will be anonymised and stored on a Picture Archiving and Communication System (PACS) and RIS.

8.3.1. Interim Analysis

Interim analysis will be conducted as described in Section 3.1

8.3.2. Final Analyses

The formal statistical analysis described below will be performed for Groups 1 and 2. For the primary endpoints, contrast agent interstitial volume (v_e) and exchange rate (k^{trans}) data will be fitted separately using a mixed effect model with subject treated as a random effect, patient population (HF or HV) and scanning session as a fixed effect. Point estimates and associated 95% confidence intervals (CI) will be constructed to provide a plausible range of values for the true comparisons of interest such as the mean difference DCE-MRI measures of interests between HF and HV groups for the different scanning sessions of interest. If model assumptions of normality appear grossly violated, alternative methods (e.g., use of raw data with log transformation or non-parametric methods) will be considered.

For the secondary endpoint, the estimation of the within subject variability of DCE-MRI measures of pulmonary oedema and vascular permeability between study visits will be calculated based on the mixed model above using data from baseline scans by group.

For the exploratory analysis on the differences in plasma volume, relaxation rate, and proton density between HF and HV groups as measured using DCE-MRI, the similar analysis will be provided as above if data permit. The correlations of MRI measures with clinical and biochemical measures: dyspnoea score, NYHA grade, respiratory rate, NT-proBNP, exercise capacity, DLco, DLno, body weight, urine specific gravity, fractional excretion of sodium (FENa), blood urea to creatinine ratio and others will also be explored as data permit.

The distribution of DCE-MRI measures of pulmonary physiology (interstitial volume (v_e), exchange rate (k^{trans}) and others) in HF and HV groups both before and after exercise will be explored using empirical cdf/pdf and/or scatter plots for each subgroup of interest to help visualize the univariate distribution and the change.

For Group 3, DCE-MRI measures, lung ultrasound (B Line count), NT proBNP, dyspnoea score, and respiratory rate will be summarized by visit/session. Individual line plots will be provided for each endpoint of interest to visualize the change in these measurements during hospitalization and after standard treatment. For subjects in Group 3 who withdrew after Session 1, the DCE-MRI measures from this scan may be included in the analyses.

8.3.2.1. Safety Analyses

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

9. STUDY GOVERNANCE CONSIDERATIONS

9.1. Posting of Information on Publicly Available Clinical Trial Registers

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

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

Prior to initiation of a study site, GSK will obtain favourable opinion/approval from the appropriate regulatory agency to conduct the study in accordance with ICH Good Clinical Practice (GCP) and applicable country-specific regulatory requirements.

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

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

- IRB/IEC review and favourable opinion/approval to conduct the study and of any subsequent relevant amended documents
- Written informed consent (and any amendments) to be obtained for each subject before participation in the study
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)

Written informed consent must be obtained from each subject prior to participation in the study.

9.2.1. Urgent Safety Measures

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

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

9.3. Quality Control (Study Monitoring)

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

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

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

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

9.4. Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance assessment and/or audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study. In the event of an assessment, audit or inspection, the Investigator (and institution) must agree to grant the advisor(s), auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss the conduct of the study, any findings/relevant issues and to implement any corrective and/or preventative actions to address any findings/issues identified.

9.5. Study and Site Closure

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

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

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

9.6. Records Retention

Following closure of the study, the Investigator or the head of the medical institution (where applicable) must maintain all site study records, except for those required by local regulations to be maintained by someone else, in a safe and secure location. The records

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

GSK will inform the Investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, or GSK standards/procedures; otherwise, the retention period will default to 15 years.

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

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

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The Investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK will also provide the Investigator with the full summary of the study results. The Investigator is encouraged to share the summary results with the study subjects, as appropriate.

The results summary will be posted to the Clinical Study Register no later than eight months after the final primary completion date, the date that the final subject was examined or received an intervention for the purposes of final collection of data for the primary outcome. In addition, a manuscript will be submitted to a peer reviewed journal for publication no later than 18 months after the last subject's last visit (LSLV). When manuscript publication in a peer reviewed journal is not feasible, a statement will be added to the public register to explain the reason for not publishing.

A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

10. REFERENCES

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11. APPENDICES

11.1. Appendix 1: Procedures for Detection, Evaluation, Follow-Up and Reporting of SAEs

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

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

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

Evaluating SAEs

Assessment of Intensity

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

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

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

Severe: An event that prevents normal everyday activities.

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

Assessment of Causality

The Investigator is obligated to assess the relationship between study treatment and the occurrence of each SAE. A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. The Investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated. The Investigator will also consult

the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of 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 when an SAE has occurred and the Investigator has minimal information to include in the initial report to GSK. However, it is very important that the Investigator always make an assessment of causality for every event prior to the initial transmission of the SAE data to GSK. The Investigator may change his/her opinion of causality in light of follow-up information, amending the SAE data collection tool accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of SAEs

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

The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the SAE. The Investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals. If a subject dies during participation in the study or during a recognized follow-up period, the Investigator will provide GSK with a copy of any post-mortem findings, including histopathology.

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

Reporting of SAEs to GSK

Facsimile transmission of the SAE data collection tool is the preferred method to transmit this information to the project contact for SAE receipt. 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. Initial notification via the telephone does not replace the need for the Investigator to complete and sign the SAE data collection tool within the designated reporting time frames.

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

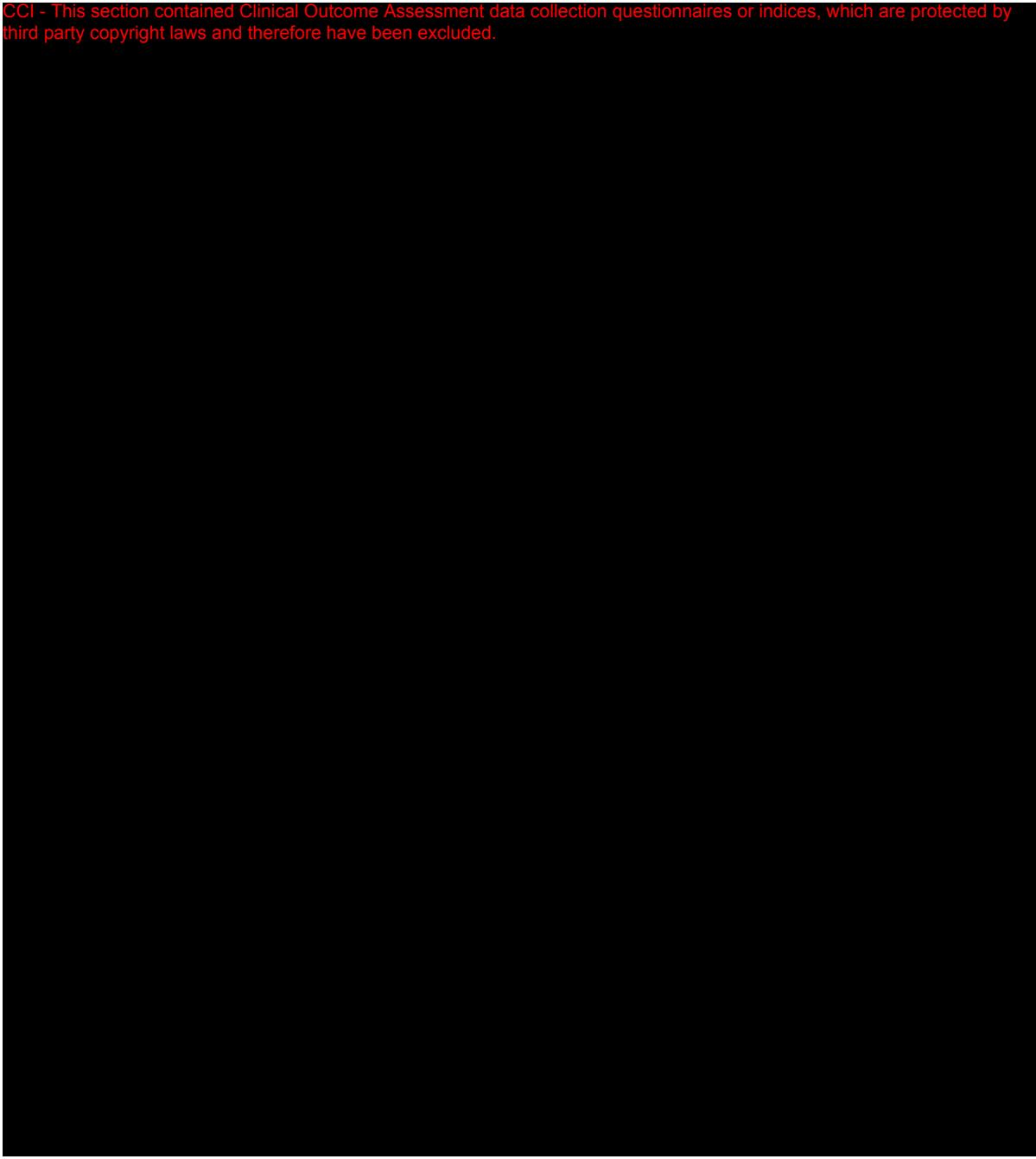
11.2. Appendix 2: Dyspnoea Scale

CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.



11.3. Appendix 3: Borg Rating of Perceived Exertion (RPE) Scale

CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.



11.4. Appendix 4: Protocol Amendment Changes

AMENDMENT 1

Where the Amendment Applies

This amendment applies to all sites (single site study).

Summary of Amendment Changes with Rationale

This protocol amendment clarifies the exercise protocol to be conducted, details measures for endpoints, defines the biomarker to be measured (NT-pro BNP), reduces total blood volume to be collected, updates study schematic, adds an interim review of the data, removes collection of AEs as there is no IP included in the study, alters physical exams to brief physical exams, and adds a dyspnea scale and the Borg Rating of Perceived Exertion.

List of Specific Changes

Trademark Information

REVISED TEXT

Trademarks of the GlaxoSmithKline group of companies	Trademarks not owned by the GlaxoSmithKline group of companies
NONE	The Borg CR10 scale NONE

Section 1.1 Study Rationale

PREVIOUS TEXT

The primary objective of this study is to characterize the capability of MRI to detect increased interstitial lung fluid or altered vascular permeability. First, the DCE-MRI markers of vascular permeability and pulmonary oedema will be measured in healthy volunteers (HV) and HF patients at rest to determine whether there is a difference between the two populations. Next, exercise-induced changes relative to rest in interstitial volume and exchange rate will be evaluated in both HV and HF patients.

REVISED TEXT

The primary objective of this study is to characterize the capability of MRI to detect increased interstitial lung fluid or altered vascular permeability. First, the DCE-MRI markers of vascular permeability and pulmonary oedema will be measured in healthy volunteers (HV) and **HF subjects patients with HF** at rest to determine whether there is a difference between the two populations. Next, exercise-induced changes (relative to rest) in interstitial volume and exchange rate will be evaluated in both HV and **subjects with HF patients. In patients with chronic HF, an abnormal alveolar-vascular**

interface leading to increased fluid flux at high pulmonary arterial pressures, as well as a tendency toward greater exercise-induced pulmonary vascular pressure, increases the chance for excess fluid under these conditions compared to what may be observed in normal subjects.

~~Exercise increases pulmonary pressure, which may result in increased vascular permeability and pulmonary oedema in HF patients compared to HVs.~~

Section 2 Objectives and Endpoints

DELETED TEXT

Primary Endpoint: Explore the effect of exercise on DCE-MRI measures of pulmonary oedema and vascular permeability in ~~a subset of the~~ HF and HV groups.

DELETED TEXT

Secondary Endpoints

<ul style="list-style-type: none"> Estimate the intrasubject variability of DCE-MRI measures of pulmonary oedema and vascular permeability between study visits. 	<ul style="list-style-type: none"> Estimation of the variability in the interstitial volume (v_e) and exchange rate (k^{trans}) within HF patients between 2 MRI visits approximately 1 week apart.
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PREVIOUS TEXT

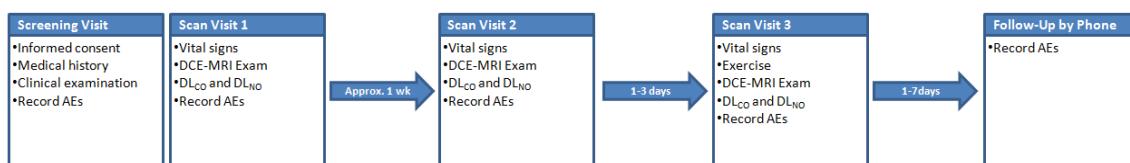
Correlation of MRI measures with clinical and biochemical measures: dyspnoea score, NYHA grade, respiratory rate, BNP, exercise capacity DL_{CO} , DL_{NO} , body weight, urine specific gravity, fractional excretion of sodium (FENa), blood urea to creatinine ratio

REVISED TEXT

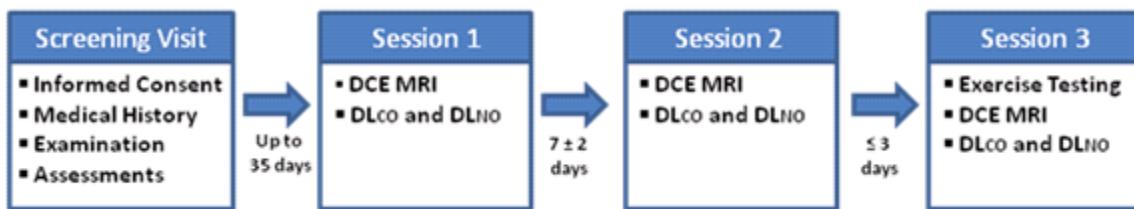
Correlation of MRI measures with clinical and biochemical measures: dyspnoea score, NYHA grade, respiratory rate, **NT-proBNP**, exercise capacity (**including Borg RER, heart rate, blood pressure, and gas exchange endpoints such as peak VO_2 , VE/VCO_2 slope**), DL_{CO} , DL_{NO} , body weight, urine specific gravity, fractional excretion of sodium (FENa), blood urea to creatinine ratio.

Section 3.1 Study Schematic

PREVIOUS TEXT



REVISED TEXT

**Section 3.2 Study Design Detail**

PREVIOUS TEXT

The study consists of five visits. The first visit will be a Screening Visit during which an appropriately trained study team member will describe the study, answer any questions the potential participant may have about the study and seek informed, written consent to enrol in the study. Subjects will be assessed for their tolerance of lying flat. Within 35 days, subjects will return for the first scanning session, where subjects will undergo the baseline procedure. The second imaging session will occur approximately one week later to measure within-subject variability. A third imaging visit will incorporate a bicycle exercise challenge prior to imaging and will occur approximately one to three days after the second imaging session. The final contact will be a follow-up phone call, which will take place up to 1 week after the last scanning session.

REVISED TEXT

The study consists of **four sessions five visits**. The first visit will be a Screening Visit during which an appropriately trained study team member will describe the study, answer any questions the potential participant may have about the study **and** seek informed, written consent, **and assess eligibility** to enrol in the study. **Subjects will be assessed for their tolerance of lying flat.** Within 35 days, subjects will return for the first scanning session, where subjects will undergo the baseline procedure. The second imaging session will occur approximately one week later to measure within-subject variability. A third imaging **session visit (which will be conducted in 2 visits)** will incorporate a bicycle exercise challenge prior to **the MRI scan, imaging and this third scan will be performed occur** approximately one to three days after the second imaging session. **The final contact will be a follow-up phone call, which will take place up to 1 week after the last scanning session.**

Section 4.1. Number of Subjects

PREVIOUS TEXT

This study will enrol a sufficient number of subjects to have up to 24 evaluable subjects (up to 12 HV and up to 12 HF patients). An evaluable subject is defined as a subject that has MRI data of sufficient quality to enable DCE-MRI modelling both Scan Visit 1 and Scan Visit 2.

REVISED TEXT

This study **plans to** **will** enrol a sufficient number of subjects to have **at least up to 24 evaluable** subjects (**up to 12 HV and up to 12 subjects with HF patients**) with evaluable MRI data. **An evaluable subject is defined** For each subject, the **as a subject that has** MRI data must be of sufficient quality to enable DCE-MRI modelling from both Session 1 and Session ~~and Visit~~ 2. Additionally, at least 6 HV and 6 subjects with HF are needed to complete the third scanning session with evaluable MRI data following exercise testing.

After 12 subjects (6 HV and 6 subjects with HF) have completed Sessions 1 and 2, an interim review will be performed. Following the interim review, the study team will decide whether to (a) terminate the study if it is determined that the image quality is poor (e.g., due to excessive movement during scanning or related to specifics around lung tissue) or the procedures are not well tolerated in the HF group (e.g., inability to lie flat for the duration of the imaging session), or (b) continue the study to enrol a sufficient number of additional subjects to meet the designated numbers required (see above).

The interim decision will be based on the expert opinion of the Investigator in consultation with the GSK Study Team.

Section 4.2.1.2 Inclusion Criteria for Healthy Volunteer Group

ADDED TEXT

1. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, **brief** physical examination, **clinical laboratory tests**, and ECG

Section 4.2.1.3 Inclusion Criteria for Both the Healthy Volunteer and Heart Failure Groups

ADDED TEXT

2. Body weight \geq 50kg and BMI within the range 18.5-32.0 kg/m² (inclusive)

Section 4.2.2 Exclusion Criteria

PREVIOUS TEXT

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

REVISED TEXT

A subject will not be eligible for inclusion in this study only if any ~~H~~ of the following criteria apply within each of the subject categories below:

Section 4.2.2.2 Exclusion Criteria for Both the Healthy Volunteer and Heart Failure Groups

DELETED TEXT

2. Contraindication for MRI scanning (as assessed by local MRI safety questionnaire), which includes but is not limited to:
 - a. Intracranial aneurysm clips (except Sugita) or other metallic objects
 - b. ~~I History of intra- orbital metal fragments that have not been removed by an MD~~

Section 4.3.2 Exercise [ADDED SECTION]

Participants will be told to avoid strenuous exercise for 24 hours prior to each scanning session.

Section 4.3.3 Caffeine and Alcohol

PREVIOUS TEXT

- Prior to each No alcohol or. People who have smoked within the previous 6 months will be excluded in accordance with study inclusion and exclusion criteria.

Subjects with a positive alcohol breath test at any session will be excluded from the study. Subjects will abstain from alcohol consumption during each scanning session.

During each scanning session, subjects will refrain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate) on the day of scan.

REVISED TEXT

For 24 hours prior to screening and each scanning session, subjects will abstain from ~~No~~ alcohol consumption. ~~People who have smoked within the previous 6 months will be excluded in accordance with study inclusion and exclusion criteria~~

Subjects with a positive alcohol breath test at any session will be excluded from the study. ~~Subjects will abstain from alcohol consumption during each scanning session.~~

On the day of ~~During~~ each scanning session, subjects will refrain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate) ~~on the day of scan~~.

Section 4.4 Withdrawal Criteria and Procedures

PREVIOUS TEXT

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

Subjects who withdraw or do not have evaluable DCE-MRI data from Scan Visit 1 and Scan Visit 2 will be replaced (see Section 4.4).

REVISED TEXT

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

Subjects who withdraw or do not have evaluable DCE-MRI data from ~~Sessions~~ ~~Scan Visit~~ 1 and ~~Scan Visit~~ 2 will be replaced in **order to meet the designated number of subjects** (see Section 4.1).

Section 5 Study Treatment

ADDED TEXT

No treatment (**investigational product**) will be given in this study.

Section 6 Study Assessments and Procedures

ADDED TEXT

In Session 3, the procedures and assessments related to exercise testing be conducted on two separate visits as detailed in the Time and Events Table. The visits do not need to be on consecutive days.

DELETED TEXT

The change in timing or addition of time points for any planned study assessments must be approved and documented by GSK, but this will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme. No more than 500mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

Section 6.1. Time and Events Table

PREVIOUS TEXT

Day:	Screening	Scan VisSessionit 1	Scan Session 2	Scan Session 3
Visit Window	-35 to -1 days	Day 1	7±2 days after Session 1	Up to 3 days after Session 2
Informed Consent	X			
Medical History / con Meds	X			
Physical examination	X			
Urinalysis, pregnancy test ¹ , drugs of abuse screen	X	X	X	X
Electrocardiograph (ECG)	X			
Vital signs and respiratory rate	X	X	X	X
Clinical Laboratory Assessments	X	X ²	X ²	X ²
B-Type Natriuretic Peptide (NT- pro BNP) ²	X	X	X	X
Dyspnoea Scoring		X	X	X
Exercise Test ⁴				X
Magnetic resonance imaging with pulse oximetry monitoring		X	X	X
DL _{CO} and DL _{NO} ⁵³		X		X
Adverse Events	X	X	X	X

1. Women of childbearing potential only
2. HF subjects only
3. The maximal exercise test will be conducted before the constant workload test.
4. DL_{CO} and DL_{NO} will be measured after the MRI scan

REVISED TEXT

Day:	Screening	Scan Vis Session 1	Scan Session 2	Scan Session 3 ⁴	
Visit Window	-35 to -1 days	Day 1	7±2 days after Session 1	Scan up to 3 days after Scan in Session 2	Visit 1 Visit 2
Informed Consent	X				
Demography/Medical History/Concomitant Medications¹	X				
Brief Physical Examination	X	X	X	X	
Orthopnoea Assessment²	X				
Urinalysis, Pregnancy test ³ , Drugs of abuse screen, alcohol breath test	X	X	X	X	
Electrocardiograph (ECG)	X				
Vital Signs and respiratory rate	X	X	X	X	X
Respiratory Rate⁶		X	X	X	X
Clinical Laboratory Assessments	X	X ²	X ²	X ²	
B-Type Natriuretic Peptide NT- pro BNP²	X	X	X	X ⁵	X ⁵
Dyspnoea Scoring ⁶		X	X	X	X
Exercise Testing ⁷				X	X
Magnetic Resonance Imaging with pulse oximetry monitoring		X	X		X
DL _{CO} and DL _{NO} ⁸		X	X		X
Record any SAEs, and any AEs related to study procedures⁹	X	X	X	X	X

1. For subjects with HF, additional history assessments are detailed in Section 6.2.

2. ~~Women of childbearing potential only~~

2. HF subjects only, details in the SPM.

3. Women of childbearing potential only

4. Session 3 will be conducted over two visits. The two visits do not need to be on consecutive days. In Session 3, a blood sample for NT-proBNP will be collected before and after the maximal exercise test, before the constant workload exercise test, and after the MRI scan.
5. The dyspnoea score will be obtained before and after the maximal exercise test, before the constant workload exercise test, and after the MRI scan. Respiratory rate will be measured at these same timepoints (and as indicated during the exercise testing).
6. The exercise testing (maximal exercise to exhaustion and constant workload tests) may be conducted over a two-day (consecutive or non-consecutive) period. The maximal exercise test will be conducted before the constant workload test.
7. DLco and DLno measurements will be made after each MRI scan has been completed and prior to the constant workload exercise test in Session 3 (Visit 2).
8. See Section 6.10.2 and Section 6.10.3.

Section 6.2 Demographic/Medical History Assessments

PREVIOUS TEXT

Medical and medication history will be assessed as related to the eligibility criteria listed in Section 4.2. Specific areas covered will include:

- Confirm heart failure class
- Onset of symptoms—when and type

REVISED TEXT

For subjects with HF only, the following items pertaining to medical and medication history will be recorded/assessed at Screening as related (See eligibility criteria listed in Section 4.2): Specific areas covered will include:

- Confirm heart failure class
- Onset and type of symptoms

Section 6.3.1. Brief Physical Examinations [RENAMED SECTION]

PREVIOUS TEXT

A physical examination will be performed by a study physician at each study visit in both groups and will include an assessment of the skin, lungs, cardiovascular system, abdomen, and legs with particular focus on fluid balance in addition to the medical history (Section 6.2). Height will be measured only on the first visit, and weight will be measured at every visit.

REVISED TEXT

A brief physical examination will be performed by a study physician at each session in both groups of subjects. In subjects with HF, peripheral oedema (level above ankle, non-dependent limb) should be monitored at all sessions. and will include an assessment of the skin, lungs, cardiovascular system, abdomen, and legs with particular focus on fluid balance in addition to the medical history (Section 6.2). Height will be measured only on the first visit (Screening), and weight will be measured at every session.

Section 6.3.2 Vital Signs Respiratory Rate [RENAMED SECTION]

PREVIOUS TEXT

- Vital sign measurements will include systolic and diastolic blood pressure and heart rate.
- Vital signs will be collected with the subject semi-supine and after they have been resting for 5 minutes.

- Respiratory rate should be recorded as a 60-second average at all time points along with vital signs. In addition immediately before exercise (Scan Visit 3 only), before scanning, and after scanning.

REVISED TEXT

- Vital sign measurements will include systolic and diastolic blood pressure and heart rate.
- Vital signs will be collected with the subject semi-supine and after they have been resting for 5 minutes.

Additional measurements of heart rate and blood pressure to be made during the exercise testing will be detailed in the SPM.

- ~~Respiratory rate should be recorded as a 60-second average at all time points along with vital signs. In addition immediately before exercise (Scan Visit 3 only), before scanning, and after scanning.~~

Section 5.3.4 Clinical Laboratory Assessments and Urinalysis

PREVIOUS TEXT

Platelet Count	<i>Automated WBC Differential:</i>
	Neutrophils
WBC Count (absolute)	Lymphocytes
Hemoglobin	Monocytes
Hematocrit	Eosinophils
	Basophils

Urea	Calcium	Bilirubin (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%)
Creatinine	Troponin	eGFR (estimated Glomerular Filtration Rate)
Glucose, fasting	ALT (SGPT)	
Sodium	AST (SGOT)	
Potassium CPK		

Biomarkers

(NT- pro BNP

REVISED TEXT

Haematology

Platelet Count	<i>Automated WBC Differential:</i>
RBC Count	Neutrophils
WBC Count (absolute)	Lymphocytes
Hemoglobin	Monocytes
Hematocrit	Eosinophils
	Basophils

Clinical Chemistry

Urea	Calcium	Total and direct bilirubin (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%)
Creatinine	Troponin	eGFR (estimated Glomerular Filtration Rate)
Glucose fasting	ALT (SGPT)	
Sodium	AST (SGOT)	
Potassium CPK		

Routine Urinalysis

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

Biomarkers

N-terminal pro-Brain-type Natriuretic Peptide (NT- pro BNP)
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**Section 6.4 N-terminal pro-Brain-type Natriuretic Peptide (NT-pro-BNP)
[RENAMED SECTION]**

PREVIOUS TEXT

Blood samples for NT-Pro BNP will be drawn at the timepoints in \ Collect each serial whole blood biomarker sample as close as possible to the planned time relative to dosing. Collect whole blood into a properly labeled 2.5mL SST blood collection tube (supplied by GSK). Record the date and exact time that each sample is collected.

Immediately after collection, gently invert (DO NOT SHAKE) the evacuated blood collection tube 5 times to mix and place upright at room temperature for 30 – 120 minutes (or at 2 – 8 °C for 12 -16 hours) to clot. Centrifuge the tube (1200 – 1400 x g for 10 minutes at room temperature) and collect 3 x 500ul of supernatant into correctly labeled vials. Transfer serum to storage in a -80 °C freezer within 30 minutes of collection.

REVISED TEXT

At Screening and in Session 3 only, blood samples for NT-Pro BNP determinations will be drawn at the timepoints in Section 6.1 before and after the maximal exercise test, before the constant workload exercise test and after MRI scanning only in subjects with HF. Collect each serial whole blood biomarker sample as close as possible to the planned time relative to dosing. Collect whole blood into a properly labeled 2.5mL SST blood collection tube (supplied by GSK). Record the date and exact time that each sample is collected. Details of the processing, storage, and shipping of samples are located in the SPM.

Immediately after collection, gently invert (DO NOT SHAKE) the evacuated blood collection tube 5 times to mix and place upright at room temperature for 30–120 minutes (or at 2–8 °C for 12–16 hours) to clot. Centrifuge the tube (1200–1400 × g for 10 minutes at room temperature) and collect 3 × 500µl of supernatant into correctly labeled vials. Transfer serum to storage in a –80 °C freezer within 30 minutes of collection.

Section 6.5. Dyspnoea Scale Scoring

PREVIOUS TEXT

A standardised, validated dyspnoea 5-point Likert scale and a visual analog scale (Mebazaa 2010) will be used immediately before exercise (Session 3 only), before scanning, and after scanning to provide a clinical correlate of changes of MRI measures of pulmonary physiology as measured by DCE-MRI. The data will be correlated to respiratory rate measured in the same time points.

Details for the scales are located in the SPM (Section 11.2).

REVISED TEXT

After the MRI scan in Sessions 1 and 2, a standardised, validated dyspnoea 5-point Likert scale and a visual analog scale (Mebazaa 2010) will be completed. In Session 3 only, the score will be completed used immediately before and after the maximal exercise test, and before and after the constant workload exercise (Session 3 only), test (before scanning) and after MRI scanning before scanning, and after scanning to provide a clinical correlate of changes of MR measures of pulmonary physiology as measured by DCE-MRI. The data will be correlated to respiratory rate measured in the same time points.

Details of the scale and administration are located in Appendix 2 (Section 11.2) and the SPM.

Section 6.6 Respiratory Rate [ADDED SECTION]

Respiratory rate will be recorded over a 60-second period. Respiratory rate will be measured after the MRI scan in Sessions 1 and 2. In Session 3 only, respiratory rate will be measured immediately before and after the maximal exercise test, and before

and after the constant workload exercise test (before scanning), and after MRI scanning. Any additional measurements to be taken during the exercise testing procedure will be detailed in the SPM.

Section 6.7.1 Subject Scanning Procedures

DELETED TEXT

~~All subjects will be scanned using this scanner.~~

Section 6.7.4 DCE-MRI Data Analysis

ADDED TEXT

...Median values of k^{trans} and v_e will be provided from each lung (**total lung, apical and basal**) for subsequent statistical analysis. All values will be corrected for the individual's haematocrit value. If not available, a typical haematocrit value will be assumed.

Section 6.8 Exercise Test

PREVIOUS TEXT

On Scan Visit 3, subjects will perform a maximal exercise test limited by dyspnoea or fatigue on a cycle ergometer. After a rest period, the workloads will be increased in a step wise fashion by 25 watts every 3 minutes. Breath by breath gas exchange and heart rate will be measured throughout exercise. During each 3 minute workload, Borg RPE and blood pressure will be obtained including same at peak exercise. Gas exchange endpoints included peak VO_2 , Ve/VCO_2 slope, RER, exercise time, and VO_2 and time at ventilator threshold will also be recorded. Details of the exercise test protocol are located in the SPM.

REVISED TEXT

In Session 3, subjects will perform **two exercise tests**.

Maximal Exercise Test Limited by Dyspnoea or Fatigue

Subjects will first be asked to perform a maximal exercise test limited by dyspnoea or fatigue on a cycle ergometer. **Baseline measurements (heart rate, blood pressure, respiratory rate, and breath-by-breath gas exchange measurements: oxygen consumption (VO_2), ventilation/carbon dioxide production (VE/VCO_2) slope, respiratory exchange ratio (RER), and VO_2 and time at ventilator threshold) will be performed with subjects seated on the cycle ergometer for approximately 3 minutes.** Subjects will then be asked to commence cycling at 20 watts for 3 minutes. The workloads will be increased in a stepwise fashion by 20 watts every 3 minutes **until volitional fatigue**. Breath-by-breath gas **measurements** and heart rate will be recorded throughout exercise. **At the end of each 3-minute workload, Borg Rating of Perceived Exertion (RPE) (See Section 11.3 Appendix 3) and blood pressure will be obtained.** At peak exercise, **heart rate, blood pressure, Borg RPE, and breath-by-breath gas exchange measurements including peak VO_2 will be recorded.** Respiratory rate at the end of exercise will also be recorded.

Constant Workload Exercise Test

Subjects will be asked to perform cycle exercise for 10 minutes at a workload between 75% to 80% of the peak work rate achieved during the maximal exercise to exhaustion test described above. Breath-by-breath gas measurements (VO₂, RER, exercise time) and heart rate will be measured throughout the exercise period. Blood pressure and respiratory rate will also be measured.

Full details of the exercise test protocols are included in the SPM.

Section 6.9 DL_{CO} and DL_{NO}

PREVIOUS TEXT

Details of test are located in the SPM.

REVISED TEXT

DL_{CO} and DL_{NO} measurements will be made after each MRI scan has been completed and prior to the constant workload exercise test in Session 3, Visit 2.

Details of the pulmonary testing methodology including diffusing capacity and measured components are included in the SPM. Details of test are located in the SPM.

**Section 6.10 Adverse Events (AE) and Serious Adverse Events (SAEs)
[RENAMED SECTION]**

DELETED TEXT

The Investigator (or designated site staff) is responsible for detecting, documenting and reporting events that meet the definition of an ~~AE or SAE~~.

Section 6.10.1 Time Period for Collecting AE and SAE Information

PREVIOUS TEXT

AEs will be collected from the start of the study (Visit 1) and until the discharge from the last visit. Medical occurrences that begin prior to the start of study (Visit 1) but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions eCRF.

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

NOTE: The method of recording, evaluating and follow-up of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Appendix 1.

REVISED TEXT

~~AEs will be collected from the start of the study (Visit 1) and until the discharge from the last visit. Medical occurrences that begin prior to the start of study (Visit 1) but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions eCRF.~~

~~SAEs will be collected over the same time period as stated above for AEs. However, Any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) will be recorded from the time a subject consents to participate in the study up to and including the last visit any follow-up contact...~~

NOTE: The method of, recording, evaluating and follow-up of ~~AEs and~~ SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Appendix 1.

~~Section 6.10.2 Method of Detecting AEs and SAEs [DELETED SECTION]~~

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

- ~~How are you feeling?"~~
- ~~"Have you had any (other) medical problems since your last visit/contact?"~~
- ~~"Have you taken any new medicines, since your last visit/contact?"~~

Section 6.10.2 Definition of Adverse Events

ADDED TEXT

Since no investigational product will be administered in this study, information regarding the occurrence of adverse events will not be routinely collected. Medical occurrences (non-serious events) that begin during the study may be recorded under Medical History/Current Medical Conditions. Non-serious events related to study procedures may also be recorded.

Section 6.10.4 Death Events

PREVIOUS TEXT

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

REVISED TEXT

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

Section 6.10.6 Regulatory Reporting Requirements for SAEs

DELETED TEXT

~~GSK has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation.~~ GSK will comply with country specific regulatory requirements relating to safety reporting to regulatory authorities, IRBs/IECs and investigators.

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

Section 7. Data Management

PREVIOUS TEXT

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

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

For this study subject data will be collected using GSK defined case report forms and combined with data provided from other sources in a validated data system.

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

REVISED TEXT

For this study, subject data will be entered into ~~an GSK-defined~~ electronic **Phase I Information Management System** ~~case report forms~~ (PIMS eCRFs), transmitted electronically to GSK, and combined with data provided from other sources in a validated data system.

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

~~For this study subject data will be collected using GSK defined case report forms and combined with data provided from other sources in a validated data system.~~

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

Section 8.1 Hypotheses and Treatment Comparisons [RENAMED SECTION]

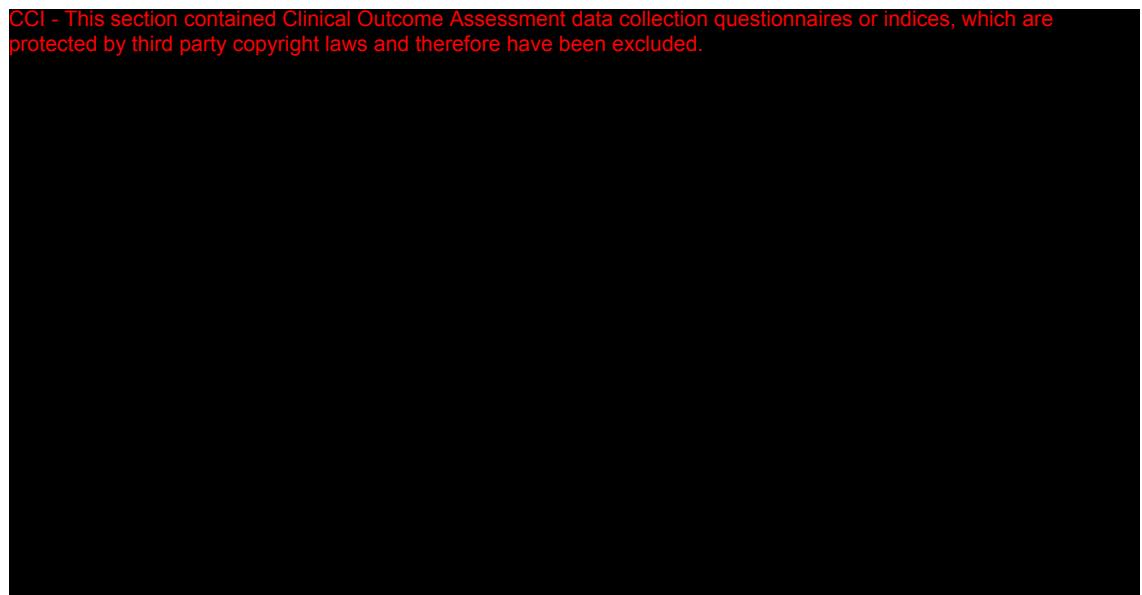
Section 8.4.2 Final Analyses

ADDED TEXT

The correlations of MRI measures with clinical and biochemical measures: dyspnoea score, **NYHA grade, respiratory rate, NT-proBNP, exercise capacity, DLco, DLno**, body weight, urine specific gravity, fractional excretion of sodium (FENa), blood urea to creatinine ratio and others will also be explored as data permit.

Section 11.3 Appendix 2: Dyspnoea Scale [ADDED APPENDIX]

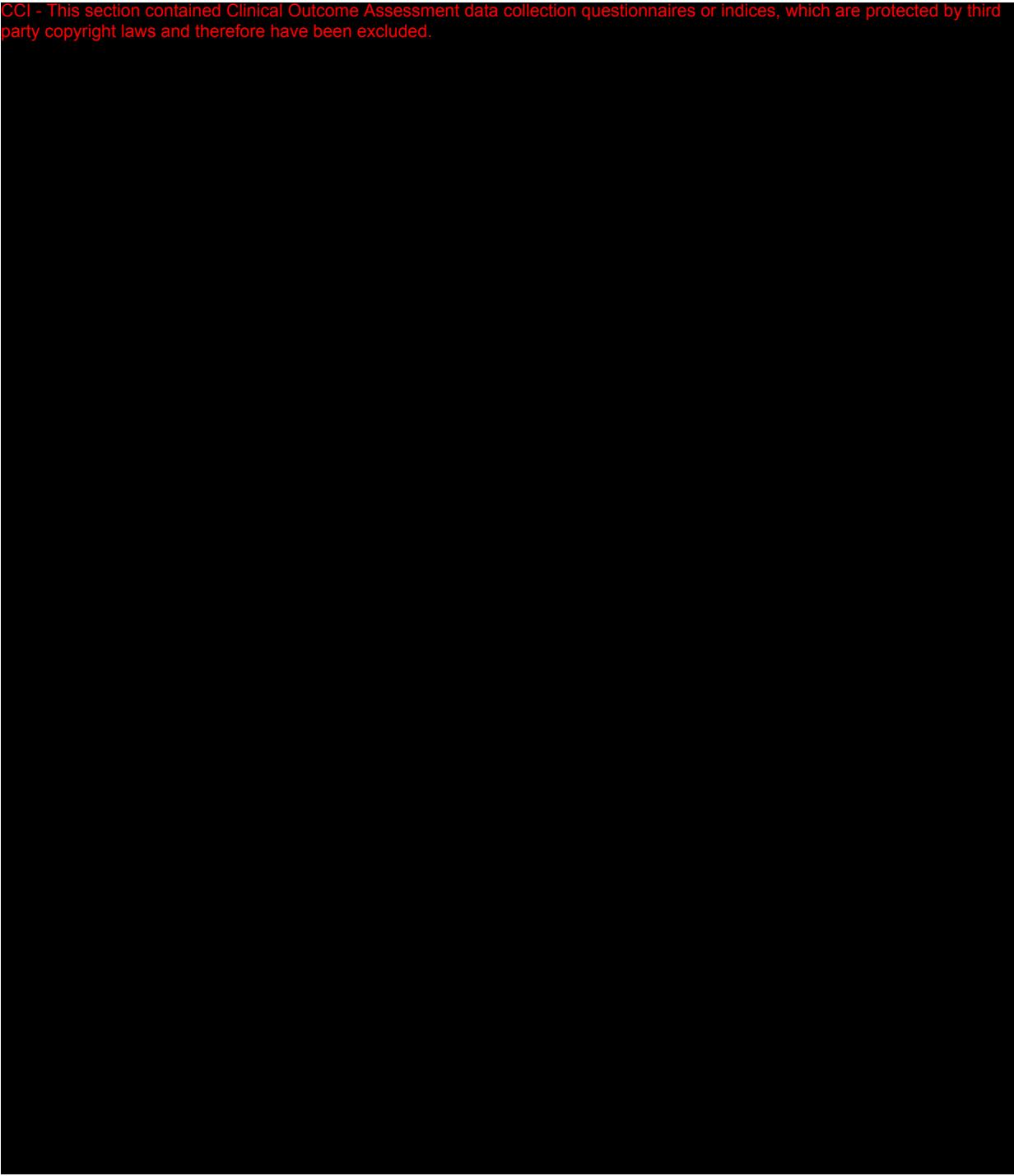
CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.



Source: Mebazaa A et al. The impact of early standard therapy on dyspnoea in patients with acute heart failure: the URGENT-dyspnea study. Eur. Heart J 2010; 31:832-841.

**Section 11.5 Appendix 3: Borg Rating of Perceived Exertion (RPE) Scale
[ADDED APPENDIX]**

CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.



AMENDMENT 2

Where the Amendment Applies

This amendment applies to all sites (single site study).

Summary of Amendment Changes with Rationale

This protocol amendment changes modifies inclusion criteria, clarifies the exercise protocol, and corrects the footnote numbering in the time and events table.

List of Specific Changes

Section 4.2.1 Inclusion Criteria

PREVIOUS TEXT

4.2.1.1 Inclusion Criteria for Heart Failure Group

1. Established diagnosis of mild to moderate heart failure of any aetiology with symptoms defined as corresponding to the New York Heart Association (NYHA) class II or III
2. Capable in participating in the exercise assessments

4.2.1.2 Inclusion Criteria for Healthy Volunteer Group

1. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, brief physical examination, clinical laboratory tests, and ECG

4.2.1.3 Inclusion Criteria for Both the Healthy Volunteer and Heart Failure Groups

1. Male or females over 18 years of age at the time of signing the informed consent
2. Body weight $\geq 50\text{kg}$ and BMI within the range $18.5\text{-}32.0 \text{ kg/m}^2$ (inclusive)
3. Able to understand and comply with protocol requirements, instructions and protocol-stated restrictions and is willing to take part in the imaging sessions
4. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form

REVISED TEXT

4.2.1.1 Inclusion Criteria for Heart Failure Group

1. Established diagnosis of mild to moderate heart failure of any aetiology with symptoms defined as corresponding to the New York Heart Association (NYHA) class II or III

2. Capable of participating in the exercise assessments

4.2.1.2 Inclusion Criteria for Healthy Volunteer Group

1. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, brief physical examination, clinical laboratory tests, and ECG

4.2.1.3 Inclusion Criteria for Both the Healthy Volunteer and Heart Failure Groups

1. Males or females over 18 years of age at the time of signing the informed consent
2. Body weight \geq 50kg and BMI within the range 18.5-32.040 kg/m² (inclusive)
3. Able to understand and comply with protocol requirements, instructions and protocol-stated restrictions and is willing to take part in the imaging sessions
4. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form

Section 6.1 Time and Events Table

PREVIOUS TEXT

Day:	Screening	Session 1	Session 2	Session 3 ⁴	
Visit Window	-35 to -1 days	Day 1	7±2 days after Session 1	Scan up to 3 days after Scan in Session 2	
				Visit 1	Visit 2
Informed Consent	X				
Demography/Medical History/Concomitant Medications ¹	X				
Brief Physical Examination	X	X	X	X	
Orthopnoea Assessment ²	X				
Urinalysis, Pregnancy test ³ , Drugs of abuse screen, alcohol breath test	X	X	X	X	
Electrocardiograph (ECG)	X				
Vital Signs	X	X	X	X	X
Respiratory Rate ⁶		X	X	X	X
Clinical Laboratory Assessments	X	X ²	X ²	X ²	
NT- pro BNP ²	X			X ⁵	X ⁵
Dyspnoea Scoring ⁶		X	X	X	X
Exercise Testing ⁷				X	X
Magnetic Resonance Imaging with pulse oximetry monitoring		X	X		X
DL _{CO} and DL _{NO} ⁸		X	X		X
Record any SAEs, and any AEs related to study procedures ⁹	X	X	X	X	X

1. For subjects with HF, additional history assessments are detailed in Section 6.2.
2. HF subjects only, details in the SPM.
3. Women of childbearing potential only.
4. Session 3 will be conducted over two visits. The two visits do not need to be on consecutive days. In Session 3, a blood sample for NT-proBNP will be collected before and after the maximal exercise test, before the constant workload exercise test, and after the MRI scan.

5. The dyspnoea score will be obtained before and after the maximal exercise test, before the constant workload exercise test, and after the MRI scan. Respiratory rate will be measured at these same timepoints (and as indicated during the exercise testing).
6. The exercise testing (maximal exercise to exhaustion and constant workload tests) may be conducted over a two-day (consecutive or non-consecutive) period. The maximal exercise test will be conducted before the constant workload test.
7. DL_{CO} and DL_{NO} measurements will be made after each MRI scan has been completed and prior to the constant workload exercise test in Session 3 (Visit 2).
8. See Section 6.10.2 and Section 6.10.3.

REVISED TEXT

6.1 Time and Events Table

Day:	Screening	Session 1	Session 2	Session 3 ⁴	
Visit Window	-35 to -1 days	Day 1	7±2 days after Session 1	Scan up to 3 days after Scan in Session 2	
				Visit 1	Visit 2
Informed Consent	X				
Demography/Medical History/Concomitant Medications ¹	X				
Brief Physical Examination	X	X	X	X	
Orthopnoea Assessment ²	X				
Urinalysis, Pregnancy test ³ , Drugs of abuse screen, alcohol breath test	X	X	X	X	
Electrocardiograph (ECG)	X				
Vital Signs	X	X	X	X	X
Respiratory Rate ⁶		X	X	X	X
Clinical Laboratory Assessments	X	X ²	X ²	X ²	
NT- pro BNP ²	X			X ⁵	X ⁵
Dyspnoea Scoring ⁶		X	X	X	X
Exercise Testing ⁷				X	X
Magnetic Resonance Imaging with pulse oximetry monitoring		X	X		X
DL _{CO} and DL _{NO} ⁸		X	X		X

Day:	Screening	Session 1	Session 2	Session 3 ⁴	
Record any SAEs, and any AEs related to study procedures ⁹	X	X	X	X	X

1. For subjects with HF, additional history assessments are detailed in Section 6.2.
2. HF subjects only, details in the SPM.
3. Women of childbearing potential only.
4. Session 3 will be conducted over two visits. The two visits do not need to be on consecutive days.
5. In Session 3, a blood sample for NT-proBNP will be collected before and after the maximal exercise test, before the constant workload exercise test, and after the MRI scan.
6. The dyspnoea score will be obtained before and after the maximal exercise test, before the constant workload exercise test, and after the MRI scan. Respiratory rate will be measured at these same timepoints (and as indicated during the exercise testing).
7. The exercise testing (maximal exercise to exhaustion and constant workload tests) may be conducted over a two-day (consecutive or non-consecutive) period. The maximal exercise test will be conducted before the constant workload test.
8. DLco and DLno measurements will be made after each MRI scan has been completed and prior to the constant workload exercise test in Session 3 (Visit 2).
9. See Section 6.10.2 and Section 6.10.3.

Section 6.8 Exercise

PREVIOUS TEXT

In Session 3, subjects will perform two exercise tests.

Maximal Exercise Test Limited by Dyspnoea or Fatigue

Subjects will first be asked to perform a maximal exercise test limited by dyspnoea or fatigue on a cycle ergometer. Baseline measurements (heart rate, blood pressure, respiratory rate, and breath-by-breath gas exchange measurements: oxygen consumption (VO_2), ventilation/carbon dioxide production (VE/VCO_2) slope, respiratory exchange ratio (RER), and VO_2 and time at ventilator threshold) will be performed with subjects seated on the cycle ergometer for approximately 3 minutes. Subjects will then be asked to commence cycling at 20 watts for 3 minutes. The workloads will be increased in a stepwise fashion by 20 watts every 3 minutes until volitional fatigue. Breath-by-breath gas measurements and heart rate will be recorded throughout exercise. At the end of each 3-minute workload, Borg Rating of Perceived Exertion (RPE) (See Section 11.3 Appendix 3) and blood pressure will be obtained. At peak exercise, heart rate, blood pressure, Borg RPE, and breath-by-breath gas exchange measurements including peak VO_2 will be recorded. Respiratory rate at the end of exercise will also be recorded.

DELETED TEXT

In Session 3, subjects will perform two exercise tests.

Maximal Exercise Test Limited by Dyspnoea or Fatigue

Subjects will first be asked to perform a maximal exercise test limited by dyspnoea or fatigue on a cycle ergometer. Baseline measurements (heart rate, blood pressure, respiratory rate, and breath-by-breath gas exchange measurements: oxygen consumption (VO_2), ventilation/carbon dioxide production (VE/VCO_2) slope, respiratory exchange ratio (RER), and VO_2 and time at ventilator threshold) will be performed with subjects seated on the cycle ergometer for approximately 3 minutes. Subjects will then be asked to commence cycling ~~at 20 watts for 3 minutes~~. The workloads will be increased in a stepwise fashion ~~by 20 watts every 3 minutes~~ until volitional fatigue. Breath-by-breath gas measurements and heart rate will be recorded throughout exercise. At the end of each ~~3-minute~~ workload, Borg Rating of Perceived Exertion (RPE) (See Section 11.3 Appendix 3) and blood pressure will be obtained. At peak exercise, heart rate, blood pressure, Borg RPE, and breath-by-breath gas exchange measurements including peak VO_2 will be recorded. Respiratory rate at the end of exercise will also be recorded.

AMENDMENT 3

WHERE THIS AMENDMENT APPLIES

This amendment applies to all sites (single site study).Amendment 3

SUMMARY OF CHANGES WITH RATIONALE

Amendment No. 3 adds an evaluation of an additional group of subjects who have been hospitalized for acute decompensated heart failure to determine whether DCE-MRI can detect changes in measures of pulmonary oedema with standard of care treatment.

List of Changes

LIST OF ABBREVIATIONS

ADDED TEXT

ADHF	Acute Decompensated Heart Failure
CXR	Chest X-Ray
LUS	Lung Ultrasound
mSv	Milli-Sievert
PE	Pulmonary Oedema
Sv	Sievert

Section 1.1 Study Rationale

ADDED TEXT

Additionally, the capability of DCE-MRI to detect changes in interstitial lung fluid in patients with acute decompensated heart failure (ADHF) will be investigated. DCE-MRI markers of pulmonary oedema will be assessed when patients are initially hospitalized with ADHF and subsequently after receiving standard of care treatment to determine whether differences can be detected by this methodology. Lung ultrasound (LUS) may also be explored in patients with ADHF as a potential tool for assessment of extra-vascular lung water through measurement of B-lines.

Section 2 Objectives and Endpoints

ADDED TEXT

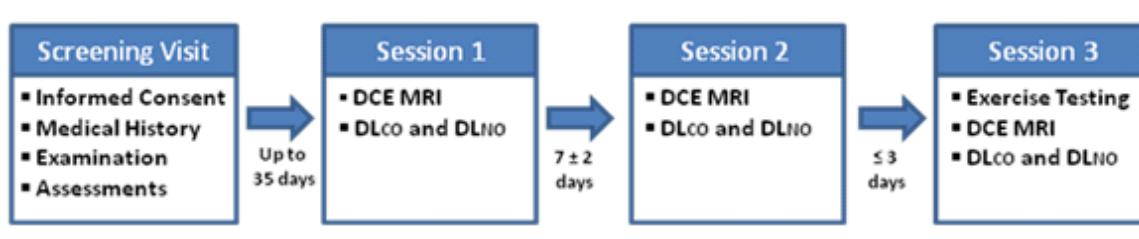
Objectives	Endpoints
Primary <ul style="list-style-type: none"> Explore the effect of standard of care treatment on DCE-MRI measures of pulmonary oedema and vascular permeability in patients with ADHF. 	<ul style="list-style-type: none"> Change in interstitial volume (v_e) and exchange rate (k^{trans}) measured using DCE-MRI in patients with ADHF during hospitalization and following the resolution of pulmonary oedema.
Exploratory <ul style="list-style-type: none"> Explore the effect of standard of care treatment on plasma volume and MRI physical properties influenced by the tissue microenvironment relaxation rate, and proton density in patients with ADHF. Explore the effect of standard of care treatment on ultrasound measures of pulmonary oedema in patients with ADHF. Explore the distribution of DCE-MRI measures of pulmonary physiology in ADHF patients both before and after standard of care treatment 	<ul style="list-style-type: none"> Change in Plasma volume (v_p), T_1 relaxation rate, and proton density measured using MRI in patients with ADHF during hospitalization and following the resolution of pulmonary oedema Change in B-lines measured using ultrasound in patients with ADHF during hospitalization and following the resolution of pulmonary oedema Interstitial volume (v_e) and exchange rate (k^{trans}), and other clinical and biochemical measures as listed above if data permit

Section 3.1 Study Schematic

PREVIOUS TEXT

Protocol waivers or exemptions are not allowed. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential.

The study design schematic is shown below:



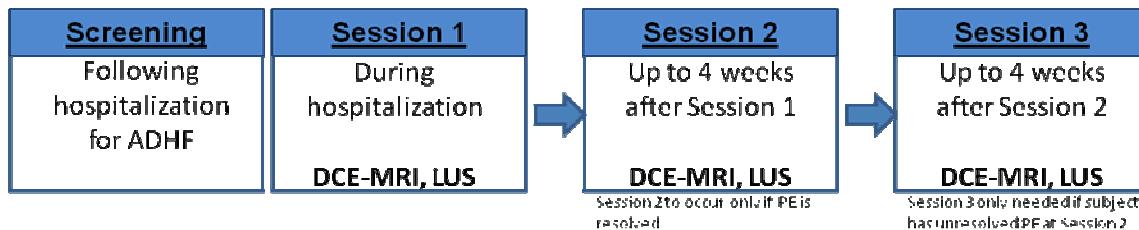
REVISED TEXT

Protocol waivers or exemptions are not allowed. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table, are essential.

For healthy volunteers and subjects with HF (Groups 1 and 2, respectively), the study design schematic is shown below:



For subjects with ADHF (Group 3), the study design is shown below:



Section 3.2 Study Design Detail

PREVIOUS TEXT

The study consists of four sessions. The first visit will be a Screening Visit during which an appropriately trained study team member will describe the study, answer any questions the potential participant may have about the study seek informed, written consent, and assess eligibility to enrol in the study. Within 35 days, subjects will return for the first scanning session, where subjects will undergo the baseline procedure. The second imaging session will occur approximately one week later to measure within-subject variability. A third imaging session (which will be conducted in 2 visits) will incorporate a bicycle exercise challenge prior to the MRI scan, and this third scan will be performed approximately one to three days after the second imaging session.

REVISED TEXT

For Groups 1 and 2, tThe study will consist of four sessions. The first visit will be a Screening Visit during which an appropriately trained study team member will describe the study, answer any questions the potential participant may have about the study seek informed, written consent, and assess eligibility to enrol in the study. Within 35 days, subjects will return for the first scanning session, where subjects will undergo the baseline procedure. The second imaging session will occur approximately one week later to measure within-subject variability. A third imaging session (which will be conducted in 2 visits) will incorporate a bicycle exercise challenge prior to the MRI scan, and this third scan will be performed approximately one to three days after the second imaging session.

For subjects with ADHF (Group 3), Screening will occur during hospitalization to assess eligibility and enrolment in the study. Session 1 will be conducted while the subject is still hospitalized with evidence of pulmonary oedema and receiving standard of care treatment. Session 2 will be conducted within 4 weeks of the first scan, when the signs of pulmonary oedema are considered to be resolved as judged by the Investigator. If a subject's pulmonary oedema has not resolved at Session 2, as judged by the Investigator using lung auscultation and/or chest x-ray, then the subject will be not be scanned by MRI at Session 2 and will be brought back for Session 3 up to 4 weeks after Session 2 for their second MRI.

Section 3.3 Risk Management

PREVIOUS TEXT

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

All inclusion criteria will be satisfied before patients undergo any procedures.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
Risk of exacerbation of dyspnoea in heart failure subjects when lying supine.	This is expected to be rapidly reversible on sitting upright, as the orthopnoea effect is dependent upon venous pressure	To minimize the risk, only patients with clinically stable heart failure (as defined in Section 4.2 will be recruited, and a trial of the supine position will be performed during the screening visit.	All procedures will be medically supervised. There is continuous monitoring of peripheral oxygen saturations and heart rate in the scanner control room, as well as direct vision down the magnet bore from the control room. The subject has a squeeze-bulb call bell to interrupt scanning. MRI compatible trolleys, wheel chairs and oxygen cylinders allow for removal from the scanner room into the foyer of the scanning suite where a full resuscitation trolley is available. All scanning staff members are trained in the emergency removal of subjects from the scanners.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
			In the event of significant dyspnoea, treatments available in the study area will include sitting upright and oxygen if required. If further therapies are required, best practice clinical management including transfer to hospital for further management will be undertaken.
Use of Gd in subjects with abnormal renal function may result in Nephrogenic Systemic Fibrosis/Nephrogenic Fibrosing Dermopathy (NSF/NFD).	Gadobutrol (Gd) (Gadovist; Bayer) at a dose less than or equal to 0.1mmol/kg will be used in the MRI protocol. It is excreted in the urine with approximately 50% and 90% of the dose excreted by 2 and 12 hours, respectively, post-injection. Approximately 200 million patients have had contrast enhanced Gd MRI examinations since such scans were first introduced 20 years ago [Thomsen, 2006]. The contrast agents are widely used off label for MRI angiography and cardiac MRI due to their low side effect profile and low risk of nephrotoxicity. From June 2006 to 12 April 2007, more than 215 individuals with NSF/NFD had been reported to the FDA [Cowper, ICNSFR Website, 2007]. All these individuals were exposed to Gd prior to developing NSF and all had moderate to end-stage renal disease prior to exposure to Gd: approximately 90% of the cases were dialysis dependent whilst the remaining 10% of subjects had chronic kidney disease or acute kidney injury [Perazella, 2007]. The incidence of NSF in dialysis and end-stage renal disease patients when exposed to Gd is in the range 3-5% [Marckmann, 2006; Kuo, 2008; However, there are no reports of NSF in subjects with normal renal function [Perazella, 2007; Deo, 2007; Chewning, 2007; Thomsen, 2006]. It is concluded that subjects dependent on dialysis and those with estimated glomerular filtration rate (eGFR) <30mL/min (chronic kidney disease stage 4/5) are at risk of developing NSF on exposure to Gd [Perazella, 2007].	As a result of this recent evidence, subjects with creatinine clearance levels < 60mL/min will be excluded from this study.	Gd contains unpaired electrons that interact with neighbouring water molecules and thereby generate MRI contrast. Free unbound Gd is extremely toxic, but when chelated to a ligand, its safety profile is dramatically improved, assisted by a 500-fold increase in renal excretion [Kramer, 2007]. The elimination half-time in patients with normal renal function is approximately 1.5 hours, but in patients with severely reduced kidney function (GFRs between 2-10), it is as long as 34 hours. The decreased elimination time may allow Gd to dissociate from its chelating agent. Free, unbound Gd may then deposit in tissue beds and incite a fibrotic process, perhaps via circulating fibrocytes. Patients with normal renal function appear to have negligible risk of developing NSF/NFD [Kramer, 2007].
Side effects from	Side effects of bicycle testing can	Inclusion criteria.	Medical oversight during the test.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
bicycle testing	include shortness of breath, light-headedness, drop in blood pressure, and abnormal heart rhythm. In rare cases, these side effects can be serious or life-threatening. Also possible are direct injuries such as bruises, sprains, and strains and indirect problems such worsening of pain from arthritis.	Medical history and PI discretion as to patient's ability to participate in the exercise test.	
Subject may not be able to enter the MRI machine	Certain interventions, prostheses, or foreign bodies might be incompatible with the MRI scanner.	Exclusion criteria and local MRI scanning facility criteria	All participants will be screened according to local hospital criteria and trial inclusion/exclusion before entering the MRI room to ensure they are able to have the MRI conducted.
Subject may not be able to complete MRI once started	MRI can be associated with claustrophobia. The radio fields used in MRI can cause mild nerve stimulation and tissue heating, both of which are strictly regulated by both software and hardware controls implemented by the scanner manufacturer that cannot be bypassed.	Exclusion criteria and medical history.	All scans will be conducted by experienced radiographers and radiologists in the nominated scanning sites. If a subject becomes claustrophobic, the subject will be withdrawn from the study if they are unable to tolerate it again.

REVISED TEXT

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

All inclusion criteria will be satisfied before patients undergo any procedures.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
Risk of exacerbation of dyspnoea in heart failure subjects when lying supine.	This is expected to be rapidly reversible on sitting upright, as the orthopnoea effect is dependent upon venous pressure	To minimize the risk, only patients with clinically stable heart failure (as defined in Section 4.2 will be recruited , and a trial of the supine position will be performed during the screening visit.	All procedures will be medically supervised. There is continuous monitoring of peripheral oxygen saturations and heart rate in the scanner control room, as well as direct vision down the magnet bore from the control room. The subject has a squeeze-bulb call bell to interrupt scanning. MRI compatible trolleys, wheel chairs and oxygen cylinders allow for removal from the scanner room into the foyer of the scanning suite where a full resuscitation trolley is available. All scanning staff members are trained in the emergency removal of subjects from the scanners.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
			In the event of significant dyspnoea, treatments available in the study area will include sitting upright and oxygen if required. If further therapies are required, best practice clinical management including transfer to hospital for further management will be undertaken.
Use of Gd in subjects with abnormal renal function may result in Nephrogenic Systemic Fibrosis/Nephrogenic Fibrosing Dermopathy (NSF/NFD).	Gadobutrol (Gd) (Gadovist; Bayer) at a dose less than or equal to 0.1mmol/kg will be used in the MRI protocol. It is excreted in the urine with approximately 50% and 90% of the dose excreted by 2 and 12 hours, respectively, post-injection. Approximately 200 million patients have had contrast enhanced Gd MRI examinations since such scans were first introduced 20 years ago [Thomsen, 2006]. The contrast agents are widely used off label for MRI angiography and cardiac MRI due to their low side effect profile and low risk of nephrotoxicity. From June 2006 to 12 April 2007, more than 215 individuals with NSF/NFD had been reported to the FDA [Cowper, ICNSFR Website, 2007]. All these individuals were exposed to Gd prior to developing NSF and all had moderate to end-stage renal disease prior to exposure to Gd: approximately 90% of the cases were dialysis dependent whilst the remaining 10% of subjects had chronic kidney disease or acute kidney injury [Perazella, 2007]. The incidence of NSF in dialysis and end-stage renal disease patients when exposed to Gd is in the range 3-5% [Marckmann, 2006; Kuo, 2008; However, there are no reports of NSF in subjects with normal renal function [Perazella, 2007; Deo, 2007; Chewning, 2007; Thomsen, 2006]. It is concluded that subjects dependent on dialysis and those with estimated glomerular filtration rate (eGFR) <30mL/min (chronic kidney disease stage 4/5) are at risk of developing NSF on exposure to Gd [Perazella, 2007].	As a result of this recent evidence, subjects with creatinine clearance levels < 60mL/min will be excluded from this study (Groups 1 and 2) and < 40mL/min in Group 3 .	Gd contains unpaired electrons that interact with neighbouring water molecules and thereby generate MRI contrast. Free unbound Gd is extremely toxic, but when chelated to a ligand, its safety profile is dramatically improved, assisted by a 500-fold increase in renal excretion [Kramer, 2007]. The elimination half-time in patients with normal renal function is approximately 1.5 hours, but in patients with severely reduced kidney function (GFRs between 2-10), it is as long as 34 hours. The decreased elimination time may allow Gd to dissociate from its chelating agent. Free, unbound Gd may then deposit in tissue beds and incite a fibrotic process, perhaps via circulating fibrocytes. Patients with normal renal function appear to have negligible risk of developing NSF/NFD [Kramer, 2007].
Side effects from bicycle testing	Side effects of bicycle testing can include shortness of breath, light-	Inclusion criteria. Medical history	Medical oversight during the test.

Potential risk	Summary of data	Impact- eligibility criteria	Strategy-monitoring/stopping criteria
	headedness, drop in blood pressure, and abnormal heart rhythm. In rare cases, these side effects can be serious or life-threatening. Also possible are direct injuries such as bruises, sprains, and strains and indirect problems such worsening of pain from arthritis.	and PI discretion as to patient's ability to participate in the exercise test.	
Subject may not be able to enter the MRI machine	Certain interventions, prostheses, or foreign bodies might be incompatible with the MRI scanner.	Exclusion criteria and local MRI scanning facility criteria	All participants will be screened according to local hospital criteria and trial inclusion/exclusion before entering the MRI room to ensure they are able to have the MRI conducted.
Subject may not be able to complete MRI once started	MRI can be associated with claustrophobia. The radio fields used in MRI can cause mild nerve stimulation and tissue heating, both of which are strictly regulated by both software and hardware controls implemented by the scanner manufacturer that cannot be bypassed.	Exclusion criteria and medical history.	All scans will be conducted by experienced radiographers and radiologists in the nominated scanning sites. If a subject becomes claustrophobic, the subject will be withdrawn from the study if they are unable to tolerate it again.
Within group 3, exposure to ionizing radiation from chest x-ray.	The dose from a single chest X-ray should not exceed 20 micro-sievert (Sv) and this corresponds to a lifetime risk of a fatal malignancy of about 1 in 1 million (ICRP 103). The study will minimize exposure by using x-rays acquired as part of clinical care whenever possible. It is expected that all subjects will require a single chest x-ray, but may have a maximum of 3 chest x-rays (up to maximum 60 micro-Sv within this study, lifetime risk of fatal malignancy of approximately 1 in 333,333).	Females of reproductive potential and all subjects under 50 years of age will be excluded from participation in group 3.	All procedures will be performed by a qualified technician.

Section 4.1 Number of Subjects

PREVIOUS TEXT

This study plans to enrol a sufficient number of subjects to have at least 24 subjects (12 HV and 12 subjects with HF) with evaluable MRI data. For each subject, the MRI data must be of sufficient quality to enable DCE-MRI modelling from both Session 1 and Session 2. Additionally, at least 6 HV and 6 subjects with HF are needed to complete the third scanning session with evaluable MRI data following exercise testing.

After 12 subjects (6 HV and 6 subjects with HF) have completed Sessions 1 and 2, an interim review will be performed. Following the interim review, the study team will

decide whether to (a) terminate the study if it is determined that the image quality is poor (e.g., due to excessive movement during scanning or related to specifics around lung tissue) or the procedures are not well tolerated in the HF group (e.g., inability to lie flat for the duration of the imaging session), or (b) continue the study to enrol a sufficient number of additional subjects to meet the designated numbers required (see above).

The interim decision will be based on the expert opinion of the Investigator in consultation with the GSK Study Team.

REVISED TEXT

With respect to Groups 1 and 2, tThis study plans to enrol a sufficient number of subjects to have at least 24 subjects (12 HV and 12 subjects with HF) with evaluable MRI data. For each subject, the MRI data must be of sufficient quality to enable DCE-MRI modelling from both Session 1 and Session 2. Additionally, at least 6 HV and 6 subjects with HF are needed to complete the third scanning session with evaluable MRI data following exercise testing.

After 12 subjects (6 HV and 6 subjects with HF) have completed Sessions 1 and 2, an interim review will be performed. Following the interim review, the study team will decide whether to (a) terminate the study if it is determined that the image quality is poor (e.g., due to excessive movement during scanning or related to specifics around lung tissue) or the procedures are not well tolerated in the HF group (e.g., inability to lie flat for the duration of the imaging session), or (b) continue the study to enrol a sufficient number of additional subjects to meet the designated numbers required (see above).

The interim decision will be based on the expert opinion of the Investigator in consultation with the GSK Study Team.

For Group 3, it is planned that a sufficient number of subjects with ADHF will be enrolled so that at least 5 subjects complete two scanning Sessions (1 and 2 or 1 and 3).

Section 4.2 Eligibility Criteria

PREVIOUS TEXT

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

4.2.1 Inclusion Criteria

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

4.2.1.1 Inclusion Criteria for Heart Failure Group

3. Established diagnosis of mild to moderate heart failure of any aetiology with symptoms defined as corresponding to the New York Heart Association (NYHA) class II or III

4.2.1.2 Inclusion Criteria for Healthy Volunteer Group

4. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, brief physical examination, clinical laboratory tests, and ECG

4.2.1.3 Inclusion Criteria for Both the Healthy Volunteer and Heart Failure Groups

5. Males or females over 18 years of age at the time of signing the informed consent
6. Body weight \geq 50kg and BMI within the range 18-40 kg/m² (inclusive)
7. Able to understand and comply with protocol requirements, instructions and protocol-stated restrictions and is willing to take part in the imaging sessions
8. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form

4.2.2 Exclusion Criteria

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

4.2.2.1 Exclusion Criteria for Heart Failure Group

5. History of known primary pulmonary disease requiring current medication or other therapy
6. Orthopnoea of sufficient severity to preclude supine scanning as determined at screening
7. Unstable angina within the past 3 months
8. Uncontrolled hypertension (resting systolic BP $>$ 160mmHg or resting diastolic BP $>$ 100mmHg)
9. Resting hypoxia while breathing room air (SaO₂ $<$ 88%)

4.2.2.2 Exclusion Criteria for Both the Healthy Volunteer and Heart Failure Groups

10. Current smoker, defined as having smoked in the preceding 6 months
11. Contraindication for MRI scanning (as assessed by local MRI safety questionnaire), which includes but is not limited to:
 - c. Intracranial aneurysm clips (except Sugita) or other metallic objects
 - d. Intra- orbital metal fragments that have not been removed

- e. Pacemakers or other implanted cardiac rhythm management devices and non-MRI compatible heart valves
- f. Inner ear implants
- g. History of claustrophobia

12. Pregnant females as determined by positive urine HCG test at screening or prior to any scanning session

13. Positive test for drugs of abuse, not due to current prescription drugs as determined by the GSK Medical Monitor and PI, and alcohol screen

14. Estimated creatinine clearance (Cockcroft-Gault) <60mL/minute

REVISED TEXT

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

4.2.1 Inclusion Criteria

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

4.2.1.1 Inclusion Criteria for Heart Failure Group (Group 2)

- 1. Established diagnosis of mild to moderate heart failure of any aetiology with symptoms defined as corresponding to the New York Heart Association (NYHA) class II or III

4.2.1.2 Inclusion Criteria for Healthy Volunteer Group (Group 1)

- 2. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, brief physical examination, clinical laboratory tests, and ECG

4.2.1.3 Inclusion Criteria for Both the Healthy Volunteer and Heart Failure Groups (Group 1 and 2)

- 1. Males or females over 18 years of age at the time of signing the informed consent
- 2. Body weight $\geq 50\text{kg}$ and BMI within the range $18\text{-}40\text{ kg/m}^2$ (inclusive)
- 3. Able to understand and comply with protocol requirements, instructions and protocol-stated restrictions and is willing to take part in the imaging sessions
- 4. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form

4.2.1.4 Inclusion Criteria for Subjects with ADHF (Group 3)

- 1. **Male subjects OR female subjects of non reproductive potential as defined as pre-menopausal females with a documented tubal ligation or hysterectomy, or post-menopausal defined as 12 months of spontaneous amenorrhea [in**

questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) > 40 MIU/mL and oestradiol < 40pg/mL (< 140 pmol/L) is confirmatory.

- 2. 50 years of age or over at the time of signing the informed consent**
- 3. Hospitalized for the management of acute decompensated HF**
- 4. Presence of dyspnoea at rest or with minimal activity**
- 5. Presence of at least one of the following signs:**
 - Tachypnea with respiratory rate ≥ 20 breaths/min
 - Rales or crackles audible on auscultation
- 6. Chest x-ray with evidence of pulmonary congestion/oedema performed approximately within the last 48 hours (if not available – an additional research CXR may be requested)**
- 7. Have received at least one treatment with an intravenous diuretic prior to the first MRI scan**
- 8. Body weight ≥ 50 kg and BMI within the range 18-40 kg/m² (inclusive)**
- 9. Able to understand and comply with protocol requirements, instructions and protocol-stated restrictions and is willing to take part in the imaging sessions**
- 10. Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form**

4.2.2 Exclusion Criteria

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

4.2.2.1 Exclusion Criteria for Heart Failure Group (Group 2)

- 1. History of known primary pulmonary disease requiring current medication or other therapy**
- 2. Orthopnoea of sufficient severity to preclude supine scanning as determined at screening**
- 3. Unstable angina within the past 3 months**
- 4. Uncontrolled hypertension (resting systolic BP > 160mmHg or resting diastolic BP > 100mmHg)**
- 5. Resting hypoxia while breathing room air (SaO₂ <88%)**

4.2.2.2 Exclusion Criteria for Both the Healthy Volunteer and Heart Failure Groups (Group 1 and 2)

6. Current smoker, defined as having smoked in the preceding 6 months
7. Contraindication for MRI scanning (as assessed by local MRI safety questionnaire), which includes but is not limited to:
 - h. Intracranial aneurysm clips (except Sugita) or other metallic objects
 - i. Intra- orbital metal fragments that have not been removed
 - j. Pacemakers or other implanted cardiac rhythm management devices and non-MRI compatible heart valves
 - k. Inner ear implants
 - l. History of claustrophobia
8. Pregnant females as determined by positive urine HCG test at screening or prior to any scanning session
9. Positive test for drugs of abuse, not due to current prescription drugs as determined by the GSK Medical Monitor and PI, and alcohol screen
10. Estimated creatinine clearance (Cockcroft-Gault) <60mL/minute

4.2.2.3 Exclusion Criteria for Subjects with ADHF (Group 3)

1. End-stage heart failure defined as requiring left ventricular assist devices, intra-aortic balloon pump or any type of mechanical support
2. Chronic or intermittent renal support therapy (hemodialysis, ultrafiltration, or peritoneal dialysis)
3. Ongoing or planned intravenous diuretic treatment within 1 hour of MRI scan appointment
4. History of known primary pulmonary disease requiring current medication or other therapy
5. Orthopnoea of sufficient severity to preclude supine scanning (as determined by a 15-minute test of lying supine with or without the use of oxygen)
6. Contraindication for MRI scanning (as assessed by local MRI safety questionnaire), which includes but is not limited to:
 - a. Intracranial aneurysm clips (except Sugita) or other metallic objects
 - b. Intra-orbital metal fragments that have not been removed
 - c. Pacemakers or other implanted cardiac rhythm management devices and non-MRI compatible heart valves
 - d. Inner ear implants
 - e. History of claustrophobia
7. Estimated creatinine clearance (Cockcroft-Gault) <40mL/minute
8. Contraindication to MRI contrast agents
9. Previous inclusion in a research and/or medical protocol involving nuclear medicine, PET or radiological investigations with significant radiation burden (a significant radiation burden being defined as 10 mSv in addition to natural background radiation, in the previous 3 years including the dose from this study).

Section 4.3.1.1 Female Subjects

PREVIOUS TEXT

No specific forms of contraception are required for female subjects of childbearing potential, though they will be informed that they will be withdrawn from the study if they become pregnant.

REVISED TEXT

For subjects in Groups 1 and 2, n~~o~~ No specific forms of contraception are required for female subjects of childbearing potential, though they will be informed that they will be withdrawn from the study if they become pregnant. **Female subjects under the age of 50 years or of childbearing potential are not eligible for Group 3.**

Section 4.3.2 Exercise

ADDED TEXT

In Groups 1 and 2, participants will be told to avoid strenuous exercise for 24 hours prior to each scanning session.

Section 4.3.3 Caffeine and Alcohol

ADDED TEXT

(GROUPS 1 AND 2) – to section title

Section 4.4 Withdrawal Criteria and Procedures

PREVIOUS TEXT

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

Subjects who withdraw or do not have evaluable DCE-MRI data from Sessions 1 and 2 will be replaced in order to meet the designated number of subjects (see Section 4.1).

Should a subject fail to attend the clinic for a required study visit, the site should attempt to contact the subject and re-schedule the missed visit as soon as possible. The site should also counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study based on previous non-compliance. In cases where the subject does not return for the rescheduled visit or cannot be reached to reschedule the missed visit, the site should make every effort to regain contact with the subject so that they can be withdrawn appropriately from the study.

REVISED TEXT

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

For Groups 1 and 2, subjects who withdraw or do not have evaluable DCE-MRI data from Sessions 1 and 2 will be replaced in order to meet the designated number of subjects (see Section 4.1). For subjects in group 3, who withdraw or do not have evaluable DCE-MRI scans from either Session 1 and 2 or Session 1 and 3 will be replaced in order to meet the designated number of subjects.

Should a subject fail to attend the clinic for a required study visit, the site should attempt to contact the subject and re-schedule the missed visit as soon as possible. The site should also counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study based on previous non-compliance. In cases where the subject does not return for the rescheduled visit or cannot be reached to reschedule the missed visit, the site should make every effort to regain contact with the subject so that they can be withdrawn appropriately from the study.

Section 4.5 Subject Completion

ADDED TEXT

A completed subject is defined as one who has completed all **sessions** of the study.

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

Section 5.1 Concomitant Medications and Therapies

PREVIOUS TEXT

Subjects in the healthy group may take all regularly prescribed and over-the-counter medication as planned throughout this study.

Subjects in the heart failure group will take all regularly prescribed and over the counter medication as planned - except for loop diuretics, aldosterone antagonists, and thiazide diuretics on the day of imaging. These subjects will take their dose of these medicines after imaging has completed on that day.

REVISED TEXT

Group 1: Subjects in the healthy group may take all regularly prescribed and over-the-counter medication as planned throughout this study.

Group 2: Subjects in the heart failure group will take all regularly prescribed and over the counter medication as planned - except for loop diuretics, aldosterone antagonists, and thiazide diuretics on the day of imaging. These subjects will take their dose of these medicines after imaging has completed on that day.

Group 3: Subjects who have been hospitalized for ADHF will receive standard of care treatment both during the hospitalization period and following discharge. Within 1 hour of a planned MRI scan appointment, subjects should not have any ongoing or planned intravenous diuretic treatment (i.e., any treatment should be given after the planned scan appointment). Current diuretic dose will be recorded at each session.

Section 6 Study Assessments and Procedures

ADDED TEXT AND TABLE

For Groups 1 and 2, the procedures and assessments related to exercise testing in Session 3, will be conducted on two separate visits as detailed in the Time and Events Table, Section 6.1.1. The visits do not need to be on consecutive days.

Table 6.1.1. Time and Event Table for Healthy Volunteers and Subjects with HF (Groups 1 and 2)

Table 6.1.2 Time and Event Table for Subjects with ADHF (Group 3)

PROCEDURE	SCREENING	SESSION 1	SESSION 2 ⁴	SESSION 3 ⁵
	Following Hospital Admission For ADHF	During Hospitalization For ADHF	Up To 4 Weeks After Session 1	Up To 4 Weeks After Session 2
Informed Consent	X			
Demography/Medical History/Concomitant Medications¹	X	X ⁸	X ⁸	X ⁸
Vital Signs including weight	X	X ²	X	X
Lung Auscultation	X	X ²	X	X
Orthopnoea Assessment	X			
Chest X-Ray³		X ²	X	X
Oximetry	X	X	X	X
Lung Ultrasound⁶		X ⁷	X	X
Magnetic Resonance Imaging (DCE-MRI)		X	X	X
Dyspnoea Score (5-Point Likert Scale)	X	X	X	X
Respiratory Rate	X	X	X	X
Creatinine	X	X	X	X
Haematocrit⁹	X	X	X	X
FSH and Estradiol¹⁰	X			
NT proBNP		X	X	X
Record Any SAEs and any AEs Related To Study Procedures		X	X	X

1. Information will be reviewed based on available hospital records
2. Assessment does not need to be repeated if Session 1 is conducted within 48 hours of Screening
3. At any designated visit, a chest X-ray need not be repeated if one has already been conducted as part of standard care
4. Session 2 will only be conducted when the pulmonary oedema is considered resolved. If pulmonary oedema is considered unresolved, only CXR and LUS may be collected.
5. Session 3 only in subjects deemed to not have pulmonary oedema resolved at session 2 by the Investigator.

6. Where feasible, Ultrasound may be performed to assess pulmonary oedema in addition to lung auscultation and / or CXR. The LUS may be completed at a separate visit (\pm 3 Days). Additional anatomic structures may be assessed during any ultrasound.
7. Multiple ultrasounds may be performed during hospitalization period of Session 1 up to one daily with a maximum of 3.
8. Sessions 1, 2 and 3 will include recording current diuretic dosage
9. Haematocrit values that are no more than 1 week old may be used
10. In women of non child bearing potential only

Section 6.3.1 Physical Examinations

ADDED TEXT

A brief physical examination will be performed at each session for both groups of subjects (**Groups 1 and 2**). In subjects with HF (**Group 2**), peripheral oedema (level above ankle, non-dependent limb) should be monitored at all sessions. Height will be measured only on the first visit (Screening), and weight will be measured at every session. **Lung auscultation will be performed on patients with ADHF in Group3 at Screening, Sessions 1, 2, and 3 by investigator or medically trained designee.**

Section 6.3.2 Vital Signs

ADDED TEXT

For Groups 1 and 2, additional measurements of heart rate and blood pressure to be made during the exercise testing will be detailed in the SPM.

Group 3 will include a weight measurement at Sessions 1, 2 and 3 (if needed).

Section 6.3.3 Electrocardiogram

ADDED TEXT

- **For Groups 1 and 2**, 12-lead ECGs will be obtained during the study using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.
- ECGs will be obtained in the semi-supine position after the subject has been resting for at least 5 minutes.

Section 6.3.4 Clinical Laboratory Assessments and Urinalysis

DELETED TEXT

eGFR (estimated Glomerular Filtration Rate)

ADDED TEXT

CrCl (estimated using Cockcroft-Gault)

Section 6.3.4.1 Pregnancy Testing

ADDED TEXT

For Groups 1 and 2, a standard urine pregnancy test will be performed at Screening and at every Session (first day of Session 3) in women of childbearing potential. If the test is positive, the subject will be withdrawn from the study. **For Group 3, FSH and estradiol will be testing if needed to confirm non child bearing potential status.**

Section 6.4 N-terminal pro-Brain-type Natriuretic Peptide (NT-pro-BNP)

ADDED TEXT

For subjects in Group 2, blood samples for NT-proBNP determinations will be drawn **at screening and in Session 3** before and after the maximal exercise test, before the constant workload exercise test and after MRI scanning only in subjects with HF. **For subjects participating in Group 3**, **blood samples for NT proBNP levels will be drawn according to the time and events table**. Record the date and time that each sample is collected. Details of the processing, storage, and shipping of samples are located in the SPM.

Section 6.5 Dyspnea Score

ADDED TEXT

After the MRI scan in Sessions 1 and 2, a standardised, validated dyspnoea 5-point Likert scale (Mebazza, 2010) will be completed **for Groups 1 and 2**. In Session 3 only, the score will be completed before and after the maximal exercise test, and before and after the constant workload exercise test (before scanning) and after MRI scanning.

For Group 3, the dyspnoea 5-point Likert scale will be completed at Screening and before and after the MRI scan in Sessions 1, 2, and 3 (if needed).

Details of the scale and administration are located in Appendix 2 (Section 11.2) and the SPM.

Section 6.6 Respiratory Rate

ADDED TEXT

Respiratory rate will be recorded over a 60-second period.

For Groups 1 and 2, respiratory rate will be measured after the MRI scan in Sessions 1 and 2. In Session 3 only, respiratory rate will be measured immediately before and after the maximal exercise test, and before and after the constant workload exercise test (before scanning), and after MRI scanning. Any additional measurements to be taken during the exercise testing procedure will be detailed in the SPM.

For Group 3, the respiratory rate will be measured at Screening and before the MRI scan in Sessions 1, 2 and 3 (if needed).

Section 6.7 Chest X Rays

ADDED SECTION

For subjects with ADHF in Group 3, a clinically indicated chest X-ray (CXR) will be performed to assess cardiogenic pulmonary oedema. CXRs used as part of standard clinical care may be used when a CXR is acquired within 48 hours of a scheduled

CXR per T&E Table Section 6.1.2. A maximum of 3 CXR scans will be performed as part of this study, each with a maximum of 20 microsievert. Additional details regarding the chest X-rays and the designation of resolution of pulmonary oedema may be found in the SPM.

Section 6.8 Lung Ultrasound

ADDED SECTION

For subjects with ADHF in Group 3, lung ultrasound may be used to assess pulmonary oedema, where feasible, specifically through measurement of the number of B lines. Additional anatomic structures may be assessed during any ultrasound. Additional details regarding the ultrasounds will be included within the SPM.

Section 6.9 Magnetic Resonance Imaging

ADDED TEXT

Subjects within Group 3 may receive medical oxygen during the exam provided they receive oxygen during all MRI sessions and delivery rate is kept constant during a scanning session and matched for both scanning sessions. Further details of scanning site training procedures and scanning protocols will be provided in a dedicated Imaging Manual.

Section 6.10 Exercise Test

ADDED TEXT

In Session 3, subjects **in Groups 1 and 2** will perform two exercise tests.

Section 6.11 DLco and DLno

ADDED TEXT

For Groups 1 and 2, DLco and DLno measurements will be made after each MRI scan has been completed and prior to the constant workload exercise test in Session 3, Visit 2.

Section 6.12.2 Definition of Adverse Events

PREVIOUS TEXT

An AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.

Since no investigational product will be administered in this study, information regarding the occurrence of adverse events will not be routinely collected. Medical occurrences

(non-serious events) that begin during the study may be recorded under Medical History/Current Medical Conditions. Non-serious events related to study procedures may also be recorded.

REVISED TEXT

An AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.

Since no investigational product will be administered in this study, information regarding the occurrence of adverse events will not be routinely collected. Medical occurrences (non-serious events) and non-serious events related to study procedures that begin during the study will be recorded in the medical notes.

Section 8 Data Analysis and Statistical Comparisons

ADDED TEXT

Section 8.1 Hypotheses Comparisons

Estimation approach will be used for the comparison of interests. No formal hypothesis testing will be performed. There are two comparisons of interest in this study to be performed **between Group 1 and Group 2**. The first is to estimate the feasibility of whether DCE-MRI can detect differences in DCE-MRI measures of pulmonary oedema or vascular permeability between HF and HV groups. The second comparison is to determine whether a burst of exercise activity can enhance the interstitial lung fluid (and/or exchange rate) from baseline.

Section 8.2 Sample Size Considerations

Sample size is based on feasibility. A similarly sized study using DCE-MRI was able to detect statistically significant differences in the lungs of smokers compared to HVs (Naish, 2008).

Section 8.4 Data Analysis Considerations

Anonymised data may be transferred within GSK or to external sites. In some cases, we may use pooled or anonymised individual image data to support scientific reports.

All imaging data will be anonymised and stored on a Picture Archiving and Communication System (PACS) and RIS.

Section 8.4 Interim Analysis

Interim analysis will be conducted as described in Section 3.1.

Section 8.5 Final Analyses

The formal statistical analysis described below will be performed for Groups 1 and 2. For the primary endpoints, contrast agent interstitial volume (v_e) and exchange rate (k^{trans}) data will be fitted separately using a mixed effect model with subject treated as a random effect, patient population (HF or HV) and scanning session as a fixed effect. Point estimates and associated 95% confidence intervals (CI) will be constructed to provide a plausible range of values for the true comparisons of interest such as the mean difference DCE-MRI measures of interests between HF and HV groups for the different scanning sessions of interest. If model assumptions of normality appear grossly violated, alternative methods (e.g., use of raw data with log transformation or non-parametric methods) will be considered.

For the secondary endpoint, the estimation of the within subject variability of DCE-MRI measures of pulmonary oedema and vascular permeability between study visits will be calculated based on the mixed model above using data from baseline scans by group.

For the exploratory analysis on the differences in plasma volume, relaxation rate, and proton density between HF and HV groups as measured using DCE-MRI, the similar analysis will be provided as above if data permit. The correlations of MRI measures with clinical and biochemical measures: dyspnoea score, NYHA grade, respiratory rate, NT-proBNP, exercise capacity, DLco, DLno, body weight, urine specific gravity, fractional excretion of sodium (FENa), blood urea to creatinine ratio and others will also be explored as data permit.

The distribution of DCE-MRI measures of pulmonary physiology (interstitial volume (v_e), exchange rate (k^{trans}) and others) in HF and HV groups both before and after exercise will be explored using empirical cdf/pdf and/or scatter plots for each subgroup of interest to help visualize the univariate distribution and the change.

For Group 3, DCE-MRI measures, lung ultrasound (B Line count), NT proBNP, dyspnoea score, and respiratory rate will be summarized by visit/session. Individual line plots will be provided for each endpoint of interest to visualize the change in these measurements during hospitalization and after standard treatment. For subjects in Group 3 who withdrew after Session 1, the DCE-MRI measures from this scan may be included in the analyses.

Section 9.7 Provision of Study Results to Investigators, Posting of Information on Publicly Available Clinical Trials Registers and Publication

DELETED TEXT

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