TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	A Randomized, Double-blind, Sponsor un-blinded, Placebo-controlled, Phase 2a Crossover Study to Evaluate the Effect of the TRPV4 Channel Blocker, GSK2798745, on Pulmonary Gas Transfer and Respiration in Patients with Congestive Heart Failure

Compound Number: GSK2798745

Development Phase: II

Effective Date: 19-APR-2016

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Author (s): PPD

Revision Chronology

GlaxoSmithKline Document Number	**Date**	**Version**
2014N221874_00	2015-MAY-19	Original
2014N221874_01	2015-JUL-29	Amendment No. 1
This amendment incorporates the changes requested during the FDA review of the IND.		
2014N221874_02	2016-APR-19	Amendment No. 2
This amendment incorporates minor changes to eligibility (NT proBNP, BMI).		

SPONSOR SIGNATORY

PPD

4/19/2016

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Date

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MONITOR/SPONSOR INFORMATION PAGE

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In some countries, the clinical trial sponsor may be the local GlaxoSmithKline Affiliate Company (or designee). If applicable, the details of the alternative Sponsor and contact person in the territory will be provided to the relevant regulatory authority as part of the clinical trial application.

Regulatory Agency Identifying Number: IND 125361

PROTOCOL AGREEMENT PAGE

For protocol number 201881

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

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

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

Investigator Name:	
Investigator Address:	
Investigator Phone Number:	

Investigator Signature	Date

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1. PROTOCOL SYNOPSIS FOR STUDY 201881

Rationale

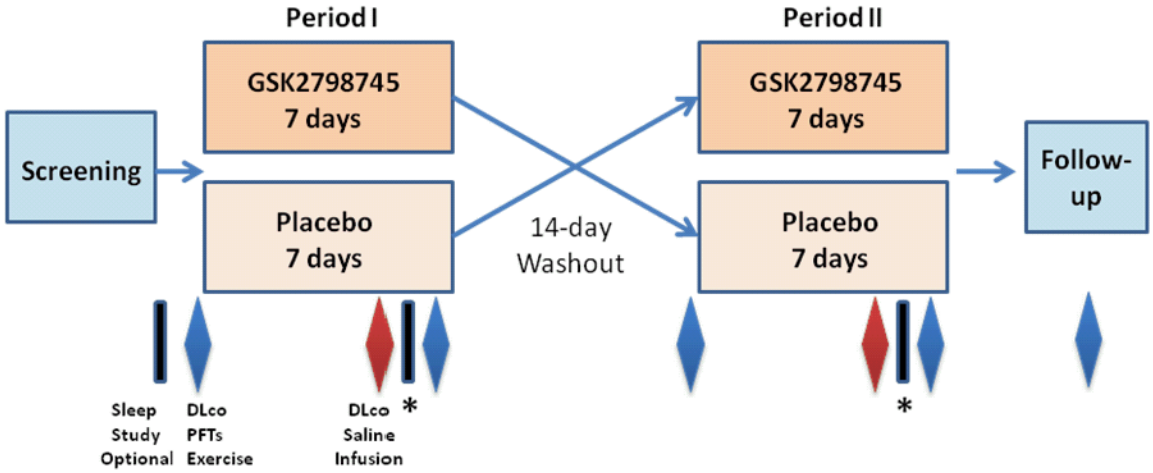
In blocking the transient receptor potential vanilloid 4 (TRPV4) ion channel and reducing pulmonary interstitial fluid, GSK2798745 may improve pulmonary function, respiration, and gas exchange as well as sleep-disordered breathing in patients with heart failure. Therefore, the current study is designed to investigate the effect of repeat administration of GSK2798745 on pulmonary gas exchange, respiration, and sleep architecture.

Objective(s)/Endpoint(s)

Objectives	Endpoints
Primary	
• To assess the effect of GSK2798745 on pulmonary gas transfer	• Change in the diffusing capacity for carbon monoxide (DLco)
Secondary	
• To assess the effect of GSK2798745 on alveolar-capillary conductance (D_M)	• Change in the diffusing capacity for nitric oxide (DLno), membrane conductance (D_M), and capillary blood volume (Vc)
• To assess the effect of GSK2798745 on pulmonary gas transfer following exercise and following an intravenous saline infusion	• Change in diffusing capacity for carbon monoxide (DLco)
• To evaluate the effect of GSK2798745 on ventilatory efficiency	• Change in the ventilatory efficiency (VE/VCO_2 slope) determined during a standardized 3-minute step test
• To evaluate the effect of GSK2798745 on pulmonary function	• Changes in spirometry measures including forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) as well as forced expiratory flows, FEF_{25-75} FEF_{50}, and FEF_{75} • Change in functional residual capacity (FRC) and end-expiratory lung volume (EELV) measured by body plethysmograph
• To assess the effect of GSK2798745 on respiratory rate	• Change in respiratory rate continuously measured by body sensor (Preventice BodyGuardian)
• To assess the effect of GSK2798745 on dyspnea	• Change in dyspnea score
• To evaluate the safety and tolerability of repeat administration of GSK2798745	• Clinical monitoring of vital signs, ECGs, and clinical laboratory safety data including liver function tests (LFTs) and troponin, as well

Objectives	Endpoints
	as reporting of adverse events (AEs)
• To assess the effect of GSK2798745 on quality of life	• Change in SF-36 acute score
• To characterize the pharmacokinetic profile of GSK2798745 in subjects with heart failure	• Area under the concentration time curve (AUC), maximum drug concentration (Cmax), time to maximum observed plasma concentration (tmax), and elimination half-life (T½)

Overall Design



Eligible Subjects Only

This is a randomized, double-blind, sponsor-unblinded, placebo-controlled, 2 by 2 crossover study in adults with heart failure. Approximately 14 days (up to 25 days) before the start of Period I, subjects will begin a screening evaluation to determine eligibility for entry into the study. Eligible subjects will be randomized to one of two treatment sequences. Subjects will receive either GSK2798745 2.4 mg or placebo once daily for a period of 7 days. After at least a 14-day washout period (preferably not greater than 30 days from last dose in Period I), subjects will participate in a second, identical 7-day treatment period and receive the alternate study medication.

The main objectives of the current study are to investigate the effect of repeat administration of GSK2798745 on pulmonary gas exchange and pulmonary function.

2. INTRODUCTION

GSK2798745 is a potent and selective transient receptor potential vanilloid 4 (TRPV4) channel blocker being developed for the treatment of heart failure.

2.1. Study Rationale

Patients with heart failure (HF) have a reduced capacity to perform exercise concurrent with numerous defects in ventilatory function including decreased lung compliance and abnormal gas transfer across the alveolar-vascular membrane. These elements can lead to the sensation of dyspnea.

In blocking the TRPV4 ion channel and reducing pulmonary interstitial fluid, GSK2798745 may improve pulmonary function, respiration, and gas exchange as well as sleep-disordered breathing in patients with heart failure. Therefore, the main objectives of the current study are to investigate the effect of repeat administration of GSK2798745 on pulmonary gas exchange and pulmonary function.

The overall resistance of the lung to gas transfer is presented by the inverse of the diffusing capacity of the lung to carbon monoxide (DLco). Changes in DLco and the diffusing capacity of the lung to nitric oxide (DLno) reflect the alveolar-capillary membrane conductance (D_M). DLco has been shown to be decreased in patients with chronic heart failure [Magini, 2015; Guazzi, 2008; Olsen, 2006; Agostoni, 2003]. Acute pulmonary congestion also causes a reduction in DLco, which is reversible. With exercise, patients with chronic heart failure demonstrate a reduced rate of increase in DLco and a decrease near peak exertion that is consistent with the limitation of alveolar-capillary recruitment and progressive interstitial edema [Olsen, 2006]. Moreover, there is a direct correlation between hemodynamic changes due to stressors [Andersen, 2015] and D_M, which has been shown to decrease after exercise [Agostoni, 2003] and infusion of saline [Guazzi, 1999]. Therefore, the effects of GSK2798745 treatment on gas exchange following exercise and intravenous saline infusion challenges will also be investigated.

Additionally, measures of pulmonary function including FEV_1, FVC and DLco have been shown to predict prognosis in patients with heart failure [Olson, 2013]. In this placebo-controlled crossover study, the pulmonary function of subjects will be characterized prior to treatment, after short-term treatment with GSK2798745, and again after the discontinuation of drug administration.

Further, polysomnography will be utilized to characterize sleep-disordered breathing patterns in these subjects with heart failure who participate in the optional sleep apnea sub-study. These patients frequently display sleep-disordered breathing often characterized by Cheyne-Stokes respiration (CSR) with central sleep apnea (CSA). This sleep-disordered breathing is characterized by recurrent cycles of apnea with a crescendo-decrescendo pattern of tidal volume. Although obstructive sleep apnea (OSA) is also prevalent, CSA is uniquely associated with heart failure and recognized as one of the independent risk factors for disease progression [Costanzo, 2015; Somers, 2008]. In those willing to participate in this aspect of the study, a baseline assessment of the apnea-hypopnea index will identify those subjects with sleep-disordered breathing for inclusion

into the sub-study. These subjects will undergo two additional sleep studies to determine whether TRPV4 channel blockade with GSK2798745 treatment (as compared to placebo) has an effect on sleep architecture.

2.2. Brief Background

Multiple lines of evidence from animal models have identified an important role for the TRPV4 Ca^{2+}-permeating ion channel in mediating the development of cardiogenic pulmonary edema. TRPV4 channels are expressed at the alveolar septal barrier, which functions to limit the movement of intravascular fluid into the interstitial and alveolar air spaces of the lung. The TRPV4 channel is activated in response to increased pulmonary venous pressure, resulting in structural changes in the endothelial cells that affect the integrity of the alveolar barrier; the enhanced permeability results in pulmonary edema and associated symptoms of dyspnea, orthopnea, paroxysmal nocturnal dyspnea, and reduced exercise tolerance.

Preclinical studies have demonstrated that TRPV4 channel blockade inhibits structural changes in endothelial cells, reduces pressure-dependent lung permeability, and prevents and resolves pulmonary edema in heart failure models. Furthermore, the TRPV4 channel is expressed in the endothelium of small pulmonary vessels in human lungs and appears to be increased in individuals with congestive heart failure [Thorneloe, 2012]. Based on the role of the TRPV4 channel in the mediation of cardiogenic pulmonary edema, it is postulated that blockade of this channel may provide therapeutic benefit in patients with heart failure (HF) and manifestations of increased extravascular lung fluid.

To date, GSK2798745 has been administered to healthy volunteers as single doses of 0.25, 1, 5, and 12.5 mg. No clinically significant safety concerns were noted. Additionally, repeat doses of GSK2798745 5 mg once daily were administered to healthy volunteers. No clinically significant safety concerns were observed with repeat administration. For detailed information please refer to the Investigator's Brochure for GSK2798745 [GlaxoSmithKline Document Number 2013N162862_01].

3. OBJECTIVE(S) AND ENDPOINT(S)

Objectives	Endpoints
Primary	
• To assess the effect of GSK2798745 on pulmonary gas transfer	• Change in the diffusing capacity for carbon monoxide (DLco)
Secondary	
• To assess the effect of GSK2798745 on alveolar-capillary conductance (D_M)	• Change in the diffusing capacity for nitric oxide (DLno), membrane conductance (D_M), and capillary blood volume (Vc)
• To assess the effect of GSK2798745 on pulmonary gas transfer following exercise and following an intravenous saline infusion	• Change in diffusing capacity for carbon monoxide (DLco)

Objectives	Endpoints
• To evaluate the effect of GSK2798745 on ventilatory efficiency	• Change in the VE/VCO_2 slope determined during a standardized 3-minute step test
• To evaluate the effect of GSK2798745 on pulmonary function	• Changes in spirometry measures including forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) as well as forced expiratory flows, FEF_{25-75} FEF_{50}, and FEF_{75} • Change in functional residual capacity (FRC) and end-expiratory lung volume (EELV) measured by body plethysmograph
• To assess the effect of GSK2798745 on dyspnea	• Change in dyspnea score
• To assess the effect of GSK2798745 on respiratory rate	• Change in respiratory rate continuously measured by body sensor (Preventice BodyGuardian)
• To evaluate the safety and tolerability of repeat administration of GSK2798745	• Clinical monitoring of vital signs, ECGs, and clinical laboratory safety data, including LFTs and troponin as well as reporting of adverse events (AEs)
• To assess the effect of GSK2798745 on quality of life	• Change in SF-36 acute score
• To characterize the pharmacokinetic profile of GSK2798745 in subjects with heart failure	• Area under the concentration time curve (AUC), maximum drug concentration (Cmax), time to maximum observed plasma concentration (tmax), and elimination half-life (T½)
Exploratory	
• To determine the effect of GSK2798745 on oxygen saturation	• Change in minimum O_2 saturation measured during polysomnography
• To assess effect of GSK2798745 on sleep-disordered breathing patterns	• Change in apnea-hypopnea index (AHI) determined by polysomnography • Change in the central, obstructive and mixed apnea indexes as determined by polysomnography
• To determine whether GSK2798745 affects sleep-disordered breathing leading to an	• Change in respiratory temporal dynamics

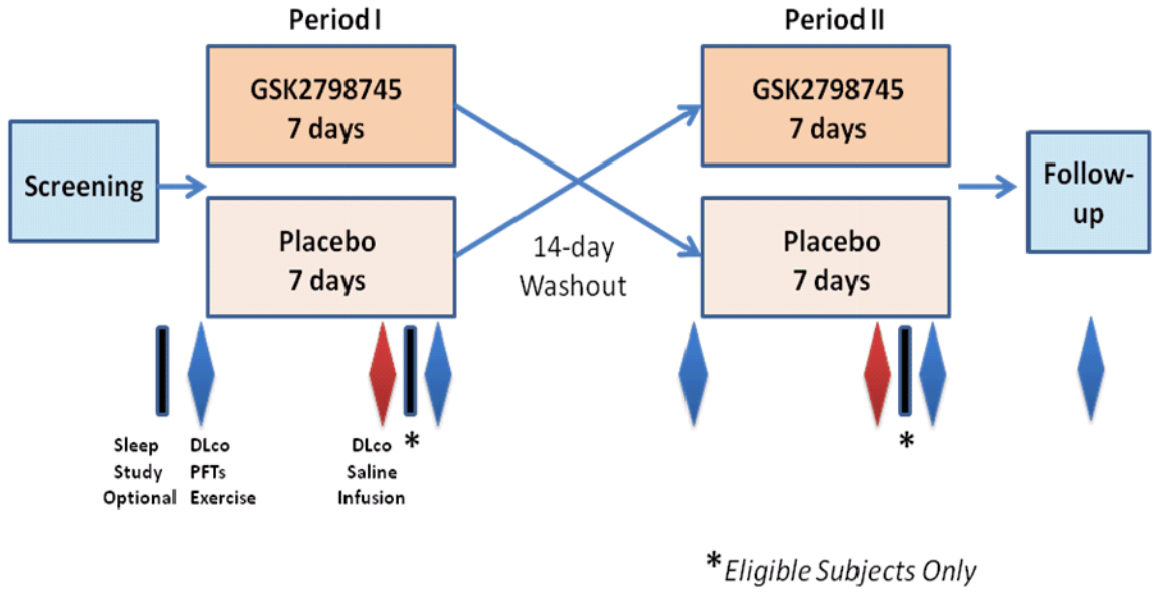
Objectives	Endpoints
improvement in sympathoadrenal activity	during in-house sleep apnea evaluation • Change in plasma and urinary catecholamine concentrations in subjects with sleep-disordered breathing
• To determine whether GSK2798745 affects electrocardiographic parameters	• Changes in time and frequency domain analyses of ECG: HR variability to define sympathetic activity
• To explore the impact of GSK2798745 on the exposure of HMG-CoA reductase inhibitors	• AUC of HMG-CoA reductase inhibitors and their key metabolites

4. STUDY DESIGN

4.1. Overall Design

This is a randomized, double-blind, sponsor-unblinded, placebo-controlled, 2 by 2 crossover study in adults with heart failure (Figure 1).

Figure 1 Study Schematic



4.2. Treatment Arms, Duration and Assessments

Approximately 14 days (up to 25 days) before the start of Period I, subjects will begin a screening evaluation to determine eligibility for entry into the study. Eligible subjects will be randomized to one of two treatment sequences. Subjects will receive either GSK2798745 2.4 mg or placebo once daily for a period of 7 days. After at least a 14-day

washout period (preferably not greater than 30 days from last dose in Period I), subjects will participate in a second, identical 7-day treatment period and receive the alternate study medication.

During both study periods, the first 6 subjects will remain in the unit (i.e. clinical research unit, CRU) overnight for the first 2 days (Days 1 and 2) to allow for continuous safety monitoring. Subsequently, these initial subjects will remain in the unit for a minimum of 4 hours after the administration of study medication (Days 3 through 7). All subjects will be equipped with the Preventice BodyGuardian for continuous remote monitoring of ECG, heart rate, and respiration rate (Day -1 to Day 7). Subjects may remain in the unit for the duration of each of the 7 day treatment periods for convenience.

After the first 6 randomized subjects have completed the two-day, in-house safety monitoring during Periods I and II, subsequent subjects enrolled into the study will remain in the unit for a minimum of 4 hours after the administration of study medication on all study days (Days 1 through 7). However, subjects may remain in the unit for the duration of each of the 7 day treatment periods as determined on a case by case basis with the Investigator(s). This is not a requirement for successful completion of the study, but is mainly for purposes of subject convenience only.

Prior to the start of each study period, all subjects will undergo an assessment of pulmonary function testing (PFTs), gas transfer measurements including DLco, DLno and Vc, breath-by-breath measurements of minute ventilation, O_2 consumption and VCO_2 production during a metered 3-minute step exercise to establish ventilatory efficiency (V_E/VCO_2), and a dyspnea assessment. Additionally, a measurement of N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) concentration, clinical laboratory tests, patient-reported health status (SF-36 Acute), and general safety assessments will be obtained.

Only for subjects willing to participate in the sleep apnea sub-study, they will undergo a sleep study to ascertain whether sleep-disordered breathing patterns are apparent prior to the <u>first</u> study period (Period I). Subjects with sleep-disordered breathing, defined as an apnea-hypopnea index (AHI) >5 based on polysomnography, will undergo a repeat sleep study after 6 days of treatment in each of the two study periods.

Pharmacokinetic profiles will be obtained during both study periods. On Day 4 and Day 7 of each study period, the assessments of pulmonary function will be repeated. On Day 5 of each study period, subjects will receive an intravenous saline infusion, and DLco and DLno measurements will be performed before and after the procedure. On Day 5 of each study period, subjects will remain in the unit for a minimum of 4 hours after completion of the intravenous saline infusion. On Day 7 of each study period, the step-exercise challenge and gas transfer measurements, dyspnea assessment, measurement of NT-proBNP concentration, clinical laboratory tests, patient-reported health status (SF-36 Acute), and general safety assessments will be repeated.

All subjects will return for a Follow-up Visit approximately 2 weeks after completion of the second study period; pulmonary and safety assessments will be repeated at this visit.

The duration of participation in this study is expected to be approximately 8 weeks from Screening to the Follow-up Visit.

4.3. Type and Number of Subjects

A sufficient number of subjects with heart failure will be enrolled so that 12 subjects complete the two study periods and critical assessments. If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigators.

After the first 12 subjects complete both study periods, an interim analysis will be performed to assess the intra-subject variability: (a) if the actual variability is less than or equal to the assumed value, the study will be completed with 12 subjects; or (b) if the actual variability is greater than the assumed value, additional subjects may need to be enrolled.

If an insufficient number of subjects participate in the sleep sub-study, additional subjects who meet the criterion for the sleep apnea sub-study may be enrolled to undergo the polysomnography investigation at the discretion of the Sponsor and in consultation with the Investigators.

In addition, another (separate) cohort of subjects may be enrolled to allow for the evaluation of an additional dose level at the discretion of the Sponsor in consultation with the Investigators.

4.4. Design Justification

The crossover design has been selected to provide greater statistical power with a smaller number of subjects. Each subject serves as his or her own matched control avoiding issues of comparability of groups with regard to confounding variables such as age, sex, and disease severity. The use of a placebo control allows for a more rigorous assessment of the effects of the drug treatment on physiologic measures and symptom assessments. Subjects selected for this study have chronic heart failure and are stable on standard treatment to ensure that their disease status does not change significantly during the course of the study. Additionally, the limited 7-day treatment period is not expected to alter the disease status of the subjects. The 14-day washout period alleviates the possibility of carryover effects from one period to the other. Given the short duration of the study, it is expected that subjects will complete the study in order to have data in each period of the crossover for all subjects.

4.5. Dose Justification

Based on the no observed adverse effect level (NOAEL) of 3mg/kg/day established in the 3-month dog study and a ≥30-fold safety margin from that NOAEL exposure, the daily exposure will not intentionally exceed a Cmax of 0.049 µg/mL and/or an AUC of 0.448 µg*hr/mL on an individual basis. The planned dosage of GSK2798745 will be 2.4 mg administered once daily as a capsule formulation without any meal restrictions. This dosage was selected on the basis of pharmacokinetic and safety profiles in healthy

volunteers who received repeat administration in the first time in human study, TR4117387. The dose may be adjusted based on emerging data.

For detailed information please refer to the Investigator's Brochure for GSK2798745 [GlaxoSmithKline Document Number 2013N162862_01]

4.6. Benefit:Risk Assessment

Summaries of findings from both clinical and non-clinical studies conducted with GSK2798745 can be found in the Investigator's Brochure. The following section outlines the risk assessment and mitigation strategy for this protocol:

4.6.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) GSK2798745		
Vascular lesions	Dogs (4-week study): At 30mg/kg/day, 2 males had arterial lesions • One male: Heart – Coronary artery inflammation; Thymus – Arteriole inflammation with fibroplasia • One male: Epididymides – Artery degeneration/necrosis with inflammation Dogs (12-week study): At 10mg/kg/day, 1 male and 1 female had arterial lesions • One male: Epididymides – Arteriole degeneration/necrosis with lymphocytic inflammation • One female: Bladder – Arteriole degeneration/necrosis with lymphocytic inflammation	The arterial lesions noted in heart, thymus, epididymides, and urinary bladder cannot be monitored directly. There is currently no human translation biomarker or understanding of the underlying mechanism. Since these effects cannot be monitored directly in clinical studies, a coverage of ≥30 fold will be maintained from the no-effect dose (3mg/kg/day); exposure will not exceed the average daily AUC of 0.448 hr*ug/mL and/or Cmax of 0.049 ug/mL on an individual basis.
Myocardial toxicity	Dogs (4-week study): At 30mg/kg/day, myofiber degeneration/necrosis & inflammation (2 animals) .	Subjects with history of acute coronary syndromes (including unstable angina), coronary angioplasty, or stenting within the past 6 months will be excluded. Troponin levels will be monitored throughout the study. A safety margin of ≥50 fold will be maintained from the no-effect dose (10mg/kg/day) observed in dogs.
Mortality/moribund	Dogs (4-week study): At 30 mg/kg/day one male terminated	Weight and adverse events reported by subjects will be

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
condition; poor viability	early (Day 6) due to poor clinical condition. Another male had transient whole body shaking on Days 8 and 9. Dogs (13-week study): At 10 mg/kg/day one male was terminated early (Day 74) due to welfare reasons. Rats (micronucleus and comet study): mortality occurred following 1 to 3 doses at ≥600mg/kg/day	monitored.
Gastrointestinal and/or hepatic toxicity	GI toxicity - ≥3mg/kg/day in dogs and at 30 and 300mg/kg/day in rats, consisting of mucosal erosion/ulceration in the stomach and/or duodenum. Hepatic Toxicity: Biliary epithelial hypertrophy/hyperplasia and periductal mixed inflammatory cell infiltrate into the liver was observed at 300mg/kg/day in rat (7-day study) and focal hepatocellular degeneration in 1 male dog at 30mg/kg/day (4-week study)	Subjects with active ulcer disease or GI bleeding will be excluded. Assessment of fecal occult blood will be performed prior to Day 1 of the first study period and following Day 7 of each study period. Subjects will be monitored for GI intolerance and sequential clinical chemistry analysis including liver enzymes.
Testicular toxicity	Rats (4-week study): Spermatid retention at ≥60mg/kg/day. Not associated with degenerative changes in testes or epididymides. No spermatogenic abnormalities were observed in dogs	A safety margin of ≥40 fold will be maintained from the no effect dose (10 mg/kg/day) in rats.
Skeletal muscle toxicity	Rat (4-week study): Myofiber necrosis: myofiber degeneration/regeneration; fibroplasia, at 300mg/kg/day in the soleus muscle	CPK levels will be monitored throughout the study.
Seizures and	Rats (micronucleus and comet study): Convulsions observed at	Subjects with a history of seizure disorder or stroke within

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
convulsions	≥600mg/kg/day. Convulsions were not related to Cmax, nor occurred at any predictable time from dose administration. Dogs: No CNS findings at 12mg/kg/day in the dog 7-day EEG/CV study. In other compounds in the same series, convulsions have been observed.	the last 5 years will be excluded from the study.
Low food consumption	Dogs (4-week study): 30mg/kg/day reduced food consumption. Two males were terminated early (Day 10) due to extremely reduced food consumption. Rats (4-week study): 300mg/kg/day had decreased food consumption.	Weight and appetite will be monitored.
Effects on macrophages (Phospholipid accumulation)	Rats (4-week study): ≥60mg/kg/day in the lung (prominent alveolar macrophages); 300mg/kg/day in the mesenteric lymph node (increased cellularity of sinus macrophages) and thymus (macrophage vacuolation; increased thymus weight). Consistent with phospholipid accumulation (phospholipidosis) based on ultrastructural appearance of mesenteric lymph nodes at 300mg/kg/day. Findings were not associated with degenerative changes.	A safety margin of ≥40 fold will be maintained from the no effect dose (10 mg/kg/day) in rats.
Study Procedures		
Exercise	Side effects can include shortness of breath, light-headedness, decrease in blood pressure, or 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 as worsening of pain from arthritis.	Subjects will have continuous breath-by-breath monitoring of vital signs as well as monitoring of blood pressure. A medically trained professional will be directing the exercise testing.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
NaCl Infusion	Side effects of a peripheral infusion of NaCl may include pain or redness at the infusion site and peripheral vein trauma (vein thrombosis, or extravasation of saline). Subjects may also experience symptoms such as generalized swelling, increase in urine output, dyspnea, or exacerbation of congestive heart failure.	The infusion will be performed by a medically trained professional and subjects will be monitored during and after the infusion.

Other Theoretical Risks:

Potential effects on vasoregulation: TRPV4 mediates prostaglandin release from isolated human endothelial cells and *in vivo* in rats, supporting the potential forTRPV4 blockade to modulate blood pressure via prostaglandin release. No effect of GSK2798745 on blood pressure was observed in preclinical studies. Blood pressure will be monitored throughout the study.

Genetic deletion of TRPV4 in mice has been shown to effect hearing. TRPV4 knockout (KO) mice at age 8 weeks exhibited normal hearing thresholds but at age 24 weeks, had delayed-onset hearing loss; additionally, the cochlea was found to be vulnerable to acoustic injury with sound overexposure [Tabuchi, 2005]. Patients with Charcot-Marie-Tooth disease Type 2C (CMT2C), an autosomal dominant axonal neuropathy related to TRPV4 gene mutations, demonstrate symptoms that include hearing loss caused by nerve damage in the inner ear (sensorineural hearing loss). These are predominantly gain of function TRPV4 abnormalities, in which the hearing loss is sporadic among family members; and relegated to some TRPV4 defects but not in others. Although the exact mechanism is unclear, it has been suggested that the TRPV4 channel plays an important role in peripheral nerve function and that the alterations in TRPV4 in CMT2C may be due to increased channel activity leading to excessive calcium influx and a calcium overload. However, these findings are academic, and have not been observed in any drug induced model. There is potential for benefit with GSK2798745, in that with cells (HEK293) expressing the CMT2C mutant channel, inhibitors of the TRPV4 channel were found to block the increased intracellular calcium concentrations and resultant cell death [Landouré, 2010]. Despite the very low risk that hearing will be affected, audiometry will be conducted during the study at baseline, Day 7 and at Follow-up. A repeat dose investigative mouse hearing study with a TRPV4 antagonist is planned to further characterize this theoretical risk.

Genetic deletion of TRPV4 in mice has also been shown to alter bone metabolism. Specifically, congenital TRPV4 KO mice displayed impaired bone resorption due to decreased osteoclast number and activity; osteoblast and osteocyte numbers were not affected [Masuyama, 2008; Mizoguchi, 2008; van der Eerden, 2013]. These changes in osteoclast function resulted in increased bone mass and intracortical porosity along with reduced bone elasticity and hind-limb unloading-induced bone loss. Consistent with a role for TRPV4 in modulating osteoclast function, TRPV4 KO mice exhibited altered serum Collagen Type I C-Telopeptide (CTX) and urinary deoxypyridinoline (DPD), specific markers of bone resorption, while no changes in procollagen type 1 amino-terminal propeptide (P1NP), a specific marker of bone formation, were observed. In addition, no differences in serum calcium or parathyroid hormone (PTH) were noted, suggesting no major changes in external calcium balance. Consistent with these latter findings, rats treated for 7 days with the TRPV4 inhibitor GSK2193874 exhibited no changes in plasma calcium or urinary calcium excretion [Thorneloe, 2012]. Osteoclast function, however, has not been assessed in any drug-induced model. As a result of the findings in TRPV4 KO mice, associations between genetic variants in the TRPV4 gene locus with fracture risk were assessed. Whereas risk of osteoporotic fracture was 1.9 times higher in men homozygous for an intronic SNP in the TRPV4 gene (T-allele of rs1861809) in the Rotterdam Study population, these results were not replicated in other

cohorts and no trends were observed for women [van der Eerden, 2013]. In spite of the low risk that GSK2798745 will alter bone metabolism in mature adult patients, fasted serum CTX will be assessed at baseline and on Day 7 of each study period.

Taking into account the measures taken to minimize the risk to subjects participating in this study, including a 30-fold exposure margin from the NOAEL in dogs, the potential risks identified in association with GSK2798745 are justified by the anticipated benefits that may be afforded to subjects with heart failure.

5. SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in the Investigator's Brochure.

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.

5.1. Inclusion Criteria

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

AGE
1. ≥ 21 years of age at the time of signing the informed consent form

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2. Diagnosis of heart failure (New York Heart Association Class II-IV) for a minimum of 3 months prior to screening
3. Clinically stable with no changes in optimized guidance-directed medications and no hospitalizations for heart failure for at least 1 month prior to Screening
4. NT-proBNP >400 pg/mL measured within 6 months prior to OR at Screening
5. Average DLco measurements outside the normal range (% Predicted DLco < 80%) during the Screening Period

WEIGHT
6. Body mass index (BMI) ≥18 and ≤45 kg/m^2

SEX
7. Male or female of non-childbearing potential ➢ Male patients with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives after the last dose of study medication. a. Vasectomy with documentation of azoospermia. b. Male condom plus partner use of one of the contraceptive options below: • Contraceptive subdermal implant that meets the standard operating procedure (SOP) effectiveness criteria including a <1% rate of failure per year, as stated in the product label • Intrauterine device or intrauterine system that meets the SOP effectiveness criteria including a <1% rate of failure per year, as stated in the product label [Hatcher, 2007a] • Oral contraceptive, either combined or progestogen alone [Hatcher, 2007a] • Injectable progestogen [Hatcher, 2007a] • Contraceptive vaginal ring [Hatcher, 2007a] • Percutaneous contraceptive patches [Hatcher, 2007a] These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception. ➢ A female subject is eligible to participate if at least one of the following conditions applies: a. Non-reproductive potential defined as: • Pre-menopausal females with one of the following: • Documented tubal ligation • Documented hysteroscopic tubal occlusion procedure with follow-up confirmation of bilateral tubal occlusion • Hysterectomy • Documented bilateral oophorectomy b. Postmenopausal defined as 12 months of spontaneous amenorrhea. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment.

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

5.2. Exclusion Criteria

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

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
1. History of acute coronary syndromes including unstable angina or myocardial infarction within 6 months of screening
2. Coronary revascularization including angioplasty and stenting within 6 months of Screening
3. History of stroke or seizure disorder within 5 years of Screening
4. Diagnosis of asthma
5. Diagnosis of chronic obstructive pulmonary disease (COPD) with FEV_1 <50% of predicted measured within 4 weeks of Screening
6. History of a condition that required radiation therapy to the thorax
7. History of any type of malignancy within the past five years with the exception of successfully treated basal cell cancer of the skin
8. Active ulcer disease or gastrointestinal bleeding at the time of screening
9. Current or chronic history of liver disease, known hepatic impairment, or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones)
10. Alanine transaminase (ALT) >2x Upper Limit of Normal (ULN) and bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%)
11. QTc > 450 msec or QTc > 480 msec in subjects with Bundle Branch Block • The QTc is the QT interval corrected for heart rate according to Bazett's formula (QTcB), Fridericia's formula (QTcF), and/or another method, machine-read or manually over-read. • The *same* QT correction formula *must* be used for *each individual subject* throughout the study. This formula may not be changed or substituted once the subject has been enrolled. • For purposes of data analysis, QTcB, QTcF, another QT correction formula, or a composite of available values of QTc will be used as specified in the Reporting and Analysis Plan (RAP).
12. History or current evidence of any serious or clinically significant gastrointestinal, renal, endocrine, neurologic, hematologic or other condition that is uncontrolled on permitted therapies or that would, in the opinion of the investigator or the

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
GlaxoSmithKline (GSK) medical monitor, make the subject unsuitable for inclusion in this study

CONCOMITANT MEDICATIONS
13. Use of medications specified for the treatment of COPD including short- and long-acting bronchodilators (β-agonists and anticholinergics) and inhaled glucocorticoids as well as oxygen therapy 14. Use of a listed prohibited medication (Section 6.10.2) within the restricted timeframe relative to the first dose of study medication 15. Use of a strong inhibitors or inducers of cytochrome P450 (CYP) 3A or p-glycoprotein (see Section 6.10.2)

RELEVANT HABITS
16. Current smoker 17. History of alcohol abuse within 6 months of the study in the opinion of the investigator(s).

CONTRAINDICATIONS
18. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or Medical Monitor, contraindicates their participation

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
19. Presence of hepatitis B surface antigen (HBsAg), positive hepatitis C antibody test result at screening or within 3 months of screening. For potent immunosuppressive agents, subjects with presence of hepatitis B core antibody (HBcAb) should also be excluded 20. A positive pre-study drug/alcohol screen (excluding prescribed medications) 21. Use of another investigational product in a clinical study within the following time period prior to the first administration of study medication in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer) 22. Exposure to more than 4 investigational medicinal products within 12 months prior to the first administration of study medication

5.3. Eligibility for Optional Sleep Apnea Sub-Study

Subjects eligible for the main study will be eligible for the sleep apnea sub-study, if the following inclusion criteria are met:

- An apnea-hypopnea index (AHI) ≥5 events/hour based on polysomnography conducted prior to the first study period (Period I)
- Agreement to participate in the sub-study by signing a separate informed consent form

5.4. Screening/Baseline Failures

Screen failures are defined as subjects who consent to participate in the clinical trial but are not subsequently randomized. In order to ensure transparent reporting of screen failure subjects, meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and respond to queries from Regulatory authorities, a minimal set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and any Serious Adverse Events.

5.5. Withdrawal/Stopping Criteria

The following actions must be taken in relation to a subject who fails to attend the clinic for a required study visit:

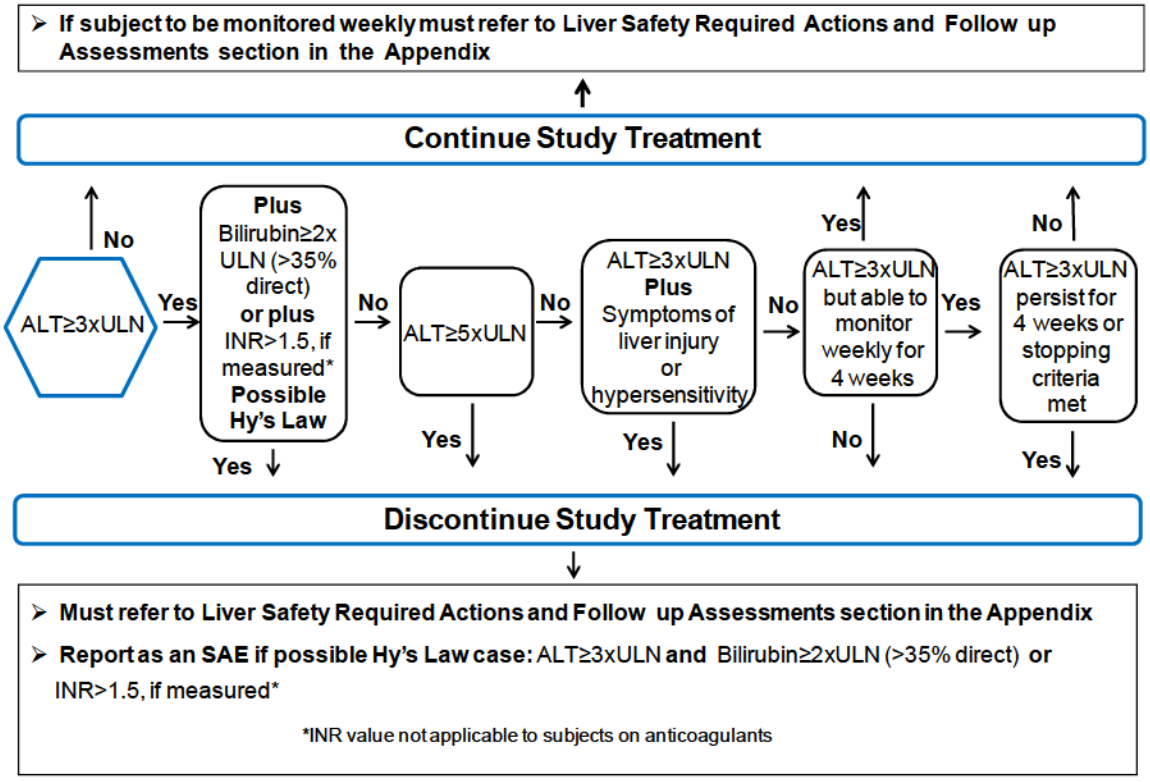
- The site must attempt to contact the subject and re-schedule the missed visit as soon as possible.
- The site must counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- In cases where the subject is deemed 'lost to follow up', the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and if necessary a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, only then will he/she be considered to have withdrawn from the study with a primary reason of "Lost to Follow-up".

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. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records. If a subject is withdrawn from study treatment, that subject will be withdrawn from the study.

5.5.1. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure subject safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf

Phase II Liver Chemistry Stopping and Increased Monitoring Algorithm



Liver Safety Required Actions and Follow up Assessments Section can be found in Appendix 2

5.5.1.1. Study Treatment Restart or Rechallenge

Study treatment restart or rechallenge after liver chemistry stopping criteria are met by any subject participating in this study is not allowed.

5.5.2. QTc Stopping Criteria

- The same QT correction formula must be used for each individual subject to determine eligibility for and discontinuation from the study. This formula may not be changed or substituted once the subject has been enrolled.
- For example, if a subject is eligible for the protocol based on QTcB, then QTcB must be used for discontinuation of this individual subject as well.
- Once the QT correction formula has been chosen for a subject's eligibility, the same formula must continue to be used for that subject for all QTc data being collected for data analysis. Safety ECGs and other non-protocol specified ECGs are an exception.
- The QTc should be based on single or averaged QTc values of triplicate electrocardiograms obtained over a brief (e.g., 5-10 minutes) recording period.

A subject who meets either of the bulleted criteria below will be withdrawn from the study:

- QTc > 500 msec OR Uncorrected QT > 600 msec
- Change from baseline of QTc > 60 msec

For patients with underlying **bundle branch block**, follow the discontinuation criteria listed below:

Baseline QTc with Bundle Branch Block	Discontinuation QTc with Bundle Branch Block
< 450 msec	> 500 msec
450 – 480 msec	≥ 530 msec

5.5.3. Other Dose Adjustment/Stopping Safety Criteria

For an individual study participant, additional stopping criteria include, but are not limited to:

- Significant changes to medication (e.g., diuretics) as judged by the Principal Investigator in consultation with the Medical Monitor if necessary
- An emergency room (ER) visit or hospitalization for treatment of an episode of acute decompensated heart failure (ADHF)
- Severe signs or symptoms or significant changes in any of the safety assessments (e.g., ECG, vital signs, laboratory tests) that put the safety of the individual at risk, as judged by the Principal Investigator in consultation with the Medical Monitor if necessary.

5.5.3.1. Troponin Stopping Criteria

If after screening, the local cardiac troponin (cTn) is > 2 times the subject's baseline value and > ULN for the assay, it is recommended that subject undergo repeat troponin testing and urgent evaluation if symptoms suggestive of cardiac ischemia are present.

Asymptomatic Subject:

If the second value of cTn is below the ULN for the assay or less than 2 times the subject's baseline value, the subject can continue in the study with close follow-up of symptoms. ECG and further cTn measurements should be performed as clinically indicated.

If the second value of cTn remains > ULN and > 2 times the subject's baseline value, treatment with GSK2798745 should be interrupted. The subject should be evaluated by a cardiologist and undergo any clinically appropriate testing. Any re-start of study treatment must be discussed with the GSK Medical Monitor.

Symptomatic Subject:

Cardiology consultation should be obtained immediately for any subject with new signs or symptoms suggestive of cardiac ischemia, including chest pain, increased shortness of breath, and diaphoresis. GSK2798745 should be discontinued permanently, and the subject should be withdrawn from the study.

5.5.3.2. Digoxin Stopping Criteria

For subjects who are taking digoxin, concentrations will be monitored prior to administration of study medication daily. If the digoxin concentrations are observed to be significantly lower or higher following study drug administration, the dose of digoxin may be titrated if possible in consultation with the Medical Monitor and Investigator. In case the digoxin dose cannot be titrated, the subject may be withdrawn from the study or continue in the study with the withdrawal of the digoxin treatment, as clinically appropriate. Any follow-up and safety observations will be collected and monitored in these subjects.

5.6. Subject and Study Completion

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

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

6. STUDY TREATMENT

6.1. Investigational Product and Other Study Treatment

The term 'study treatment' is used throughout the protocol to describe the product received by the subject as indicated in the protocol design.

	Study Treatment	
Product name:	GSK2798745	Placebo
Formulation description:	Granule filled capsule	Placebo blend filled capsule
Dosage form:	Capsule	Capsule
Unit dose strength(s)/Dosage level(s):	2.4 mg[1]	NA
Route of Administration	Oral	Oral
Dosing instructions:	Dose with 240mL water	Dose with 240mL water.
Physical description:	Size one White Opaque capsule containing white to almost white solid	Size one White Opaque capsule containing white to almost white solid
Method for individualizing dosage:	Pharmacy to assemble	Pharmacy to assemble

1. In the event of a dose adjustment, dosage levels for capsules filled with GSK2798745 granules will be accordingly adjusted.

6.2. Treatment Assignment

Subjects will be assigned to study treatment in accordance with the randomization schedule generated in RandAll NG by Clinical Statistics prior to the start of the study, using validated internal software.

Subjects will be randomized into the study by means of an interactive web response system (IWRS) i.e. RAMOS NG, to receive one the two treatment regimens shown below in Table 1.

Table 1 Treatment Sequences

Sequence	Period 1	Period 2
AP	GSK2798745 2.4mg	Placebo
PA	Placebo	GSK2798745 2.4mg

6.3. Blinding

This will be a double-blind study where the sponsor and clinical pharmacist are un-blinded and the following will apply.

- The investigator or treating physician may unblind a subject's treatment assignment **only in the case of an emergency** OR in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject as judged by the investigator.
- Investigators have direct access to the subject's individual study treatment via the IWRS (RAMOS NG).
- It is preferred (but not required) that the investigator first contacts the Medical Monitor or appropriate GSK study personnel to discuss options **before** unblinding the subject's treatment assignment.
- If GSK personnel are not contacted before the unblinding, the investigator must notify GSK as soon as possible after unblinding, but without revealing the treatment assignment of the unblinded subject, unless that information is important for the safety of subjects currently in the study.
- The date and reason for the unblinding must be fully documented in the CRF
- A subject may continue in the study if that subject's treatment assignment is unblinded by the discretion of the Investigator in consultation with the Medical Monitor.
- GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any subject with a Serious Adverse Event (SAE). If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the subject's treatment assignment, may be sent to investigators in accordance with local regulations and/or GSK policy.

6.4. Packaging and Labeling

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

6.5. Preparation/Handling/Storage/Accountability

A description of the methods and materials required for preparation of GSK2798745 or placebo capsules will be detailed in a Technical Agreement. The capsules will be extemporaneously prepared as per instructions that will be reviewed and approved by GSK prior to use.

- Only subjects enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure environmentally controlled and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the

investigator and authorized site staff. Please see Study Reference Manual (SRM) for additional information regarding storage.

- The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e. receipt, reconciliation and final disposition records).
- Further guidance and information for final disposition of unused study treatment are provided in the SRM.
- Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff. Take adequate precautions to avoid direct eye or skin contact and the generation of aerosols or mists. In the case of unintentional occupational exposure notify the monitor, Medical Monitor and/or GSK study contact.
- A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

Precaution will be taken to avoid direct contact with the study treatment. A MSDS describing occupational hazards and recommended handling precautions will be provided to the investigator. In the case of unintentional occupational exposure notify the monitor, Medical Monitor and/or GSK study contact.

6.6. Compliance with Study Treatment Administration

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

6.7. Treatment of Study Treatment Overdose

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

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

1. Contact the Medical Monitor immediately.
2. Closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities until GSK2798745 can no longer be detected systemically (at least 5 days for GSK2798745).

3. Obtain a plasma sample for pharmacokinetic (PK) analysis within 1 day from the date of the last dose of study treatment if requested by the Medical Monitor (determined on a case-by-case basis).
4. Document the quantity of the excess dose as well as the duration of the overdosing in the CRF.

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

6.8. Treatment after the End of the Study

The investigator is responsible for ensuring that consideration has been given to the post-study care of the subject's medical condition, whether or not GSK is providing specific post-study treatment.

6.9. Lifestyle and/or Dietary Restrictions

6.9.1. Monitoring in the Unit

The first six subjects who participate in the study will be admitted to the unit to be monitored for the first two days (Day 1 and Day 2) following the first administration of study medication in both study periods. Subsequently, these subjects will remain in the unit for a minimum of 4 hours after the administration of study medication on each of the remaining 5 days (Days 3 through 7) of each study period. On Day 5, subjects will remain in the unit for a minimum of 4 hours after the completion of the saline infusion.

For the remainder of subjects participating in the study, they will remain in the unit for a minimum of 4 hours after the administration of study medication on each of the 7 days of each study period.

Following the completion of all assessments on each study day, subjects may return home. For subjects who cannot return home each evening for multiple reasons including but not limited to distance from clinical site or transportation issues, housing will be provided or they may opt to remain in the unit for the duration of the study period. Housing should be consistent in both study periods. All subjects will be continuously monitored remotely (see Section 7.4.7).

6.9.2. Meal and Dietary Restrictions

Subjects are not permitted to consume red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days prior to the first dose of study medication until their discharge from the unit after their last dose of study medication in each period.

Three meals per day will be provided by dietary department either in the unit or as a boxed meal(s) to go.

No meal restrictions will be placed with regard to drug administration. Subjects will be allowed to consume a standard breakfast prior to drug administration.

6.9.3. Caffeine and Alcohol

During each study period, it is recommended that subjects minimize ingestion of caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate) for 24 hours prior to the start of dosing until completion of all assessments.

During each study period, subjects will abstain from alcohol for 24 hours prior to the start of dosing until completion of all assessments.

6.9.4. Activity

Subjects should refrain from strenuous activity 48 hours prior to each study period and throughout the study (except when in the exercise portion of the study). Subjects may participate in light recreational activities during studies (e.g., watch television, read).

6.10. Concomitant Medications and Non-Drug Therapies

6.10.1. Permitted Medications and Non-Drug Therapies

Acetaminophen at doses of ≤ 3 grams/day is permitted for use any time during the study.

The patients may stay on all regular prescription medications unless specified in Section 6.10.2. Digoxin is permitted, but with close monitoring to maintain the therapeutic window. Other concomitant medication may be considered on a case by case basis by the Investigator in consultation with the GSK Medical Monitor.

6.10.2. Prohibited Medications and Non-Drug Therapies

Except for the permitted medications noted above (Section 6.10.1), subjects must abstain from taking non-prescription drugs (including vitamins and dietary or herbal supplements), within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose of study medication until completion of the follow-up visit, unless in the opinion of the Investigator and sponsor the medication will not interfere with the study.

Subjects must provide to the investigator information/list of all medications they are currently taking.

GSK2798745 has weak CYP3A4 inhibition potential. There is a likelihood that the concentrations of drugs that are substrates of CYP3A4 may be increased. HMG-CoA reductase inhibitors such as atorvastatin and simvastatin are examples of CYP3A4 substrates that are likely to be taken by the eligible patients as part of their medications. The concentrations of these statins will be monitored during the study. The investigators may also consider substitutions of these medications.
Subjects should avoid using drugs that are strong inhibitors or inducers of Cytochrome P450 (CYP) 3A4 or p-glycoprotein (P-gp) because they may alter GSK2798745 concentrations. The list of background therapy/drugs may be modified based on emerging data. These include, but are not limited to, those listed in Table 2; consider therapeutic substitutions for these medications.

GSK2798745 systemic concentrations will be monitored to detect any drug interactions with moderate CYP3A4 inhibitors or P-gP inhibitors (see SRM for a detailed list).

It is strongly recommended that patients avoid using drugs that are sensitive substrates of Cytochrome P450 (CYP) 3A4 and/or P-gp or that have a low therapeutic index because concentrations of these substrates may be increased by GSK2798745. If co-administration of medications with interaction potential with GSK2798745 is necessary, investigators should monitor subjects for loss of efficacy or consider substitutions of these medications. See SRM for a detailed list. The list may be modified based on emerging data. All concomitant medications may be reviewed by the Medical Monitor and it will be up to the discretion of the Investigator and if necessary the Medical Monitor, whether the medication can be continued and/or the subject can participate in the study.

Table 2 Strong inducers/inhibitors of CYP3A and P-gp

Antiretrovirals:	atazanavir, danoprevir, elvitegravir, fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, telaprevir, tipranavir
Antibiotics:	clarithromycin, telithromycin, troleandomycin, rifampin
Oral antifungals:	ketoconazole, itraconazole, voriconazole
Antidepressant	nefazadone
Immunosuppressant	cyclosporine
Anti-Epileptic	carbamazepine, phenytoin

7. STUDY ASSESSMENTS AND PROCEDURES

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

This section lists the procedures and parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Table Section 7.1

- Assessments scheduled for the same nominal time, should occur in the following order:

  1. 12-lead ECG
  2. Vital signs
  3. Blood samples

Note: The timing of the assessments should allow the blood draw to occur at the exact time each day.

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

7.1. Time and Events Table

Procedure	Screening Period	Treatment Periods I and II (Day)										Follow-up
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	8	9	14 ± 4 days after last dose of study medication
Informed consent	X											
Inclusion and exclusion criteria	X											
Demography	X											
Full physical examination including height & weight	X											
Brief physical exam including weight		X				X			X			X
Medical history/Medication history	X											
Hepatitis B & C screen	X											
Echocardiogram[16]	X											
Urine drug and alcohol test	X	X										
Clinical lab assessments[14]	X	X				X			X			X
NT-proBNP	X	X							X			
Serum CTX[11]		X							X			
Fecal Occult Blood Test[12]	X								X			
Pharmacokinetic sample[1]			X	X	X	X	X	X	X	X	X	
Digoxin sample[2]		X	X	X	X	X	X	X	X			X
Troponin	X	X		X		X			X			X
DLco/DLno[10]	X[15]	X				X	X		X			X
12-lead ECG/ time & frequency domains[3]	X[9]	X[9]				X			X			X

Procedure	**Screening Period**	**Treatment Periods I and II (Day)**										**Follow-up**
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	8	9	14 ± 4 days after last dose of study medication
Vital signs	X	X	X	X	X	X	X	X	X			X
Weight			X	X	X		X	X				
Genetic sample		For subjects who provide consent, sample collected after start of treatment										
Study treatment[4]			X	X	X	X	X	X	X			
AE/SAE review		X	←==========→									X
Concomitant med review		X	←==========→									X
Pulmonary function tests		X				X			X			X
Step exercise test/breath-by-breath gas exchange		X							X			X
Dyspnea assessment		X					X		X			X
Orthopnea							X					
SF-36 Acute		X							X			X
Polysomnography including oximetry (Sleep Apnea Clinic)[5,13]		←==========→						←==========→				
Plasma and 8-hour urine catecholamines[6]		X						X				
Saline infusion							X					
Admission to unit[7]			X									
Discharge from unit[7]					X							
Meals[8]		X	X	X	X	X	X	X	X			
Appetite Assessment (SNAQ)		X				X			X			X
CSSRS (Suicidality)		X							X			X
Audiometry		X[13]							X[17]			X
BodyGuardian Monitor (Preventice)		←==========Continuous==========→										

1. Pharmacokinetic samples to determine GSK2798745 and any metabolite(s) concentrations will be obtained at the following times on Day 1 of each treatment period: Predose, 0.5, 1, 1.5, 2, 3, 5, 8 and 12 hours and at the following times on Day 7 of each treatment period: Predose, 0.5, 1, 1.5, 2, 3, 5 and 10 hours. On Day 2 of each treatment period, samples will be collected predose and at 12 hours post dose. On Days 3 through 6, PK samples will be collected predose. On Days 8 and 9, samples will be collected at 24 and 48 hours, respectively, after the last dose of study medication administered on Day 7. The time points may be modified based on emerging data. Some of these timepoints will also be utilized to collect samples to determine concentrations of other medications (e.g., atorvastatin and/or simvastatin). See the SRM for detailed information.
2. Digoxin concentrations will be monitored only in those subjects who are taking digoxin.
3. Time and frequency domains assessed by ECG obtained on Day -1 and Day 7.
4. Subjects will remain in the unit for a minimum of 4 hours after each administration of study medication. [Note: The first 6 subjects enrolled will remain in the unit for 52 hours after the first dose of study medication in each period]
5. Only for subjects participating in the sub-study: At Day -1 of the first study period only, subjects will undergo an overnight sleep study. Only those subjects who are participating in the sub-study with sleep disordered breathing will undergo additional sleep studies on Day 6 of both study Periods I and II.
6. Only for subjects participating in the sub-study: Urinary norepinephrine (NE) and epinephrine (E) concentrations will be determined from 8-hour collections obtained during the overnight sleep study; urinary creatinine will also be measured to normalize the NE and E concentrations. A blood sample to determine plasma concentrations of NE and E will be obtained the morning after the sleep study between the hours of 9:00 am and 10:00 am after the subject has been supine for 20 minutes in a quiet room.
7. The first 6 subjects enrolled will remain in the unit for two days (Day 1 and Day 2) after the first dose of study medication in each period. Subjects may remain in the unit for the duration of each of the 7 day treatment periods for convenience. Housing should be consistent in both study periods.
8. Three meals per day will be provided by the dietary department either in the unit or as a boxed meal(s) to take home.
9. ECGs to be performed in triplicate
10. On the days when exercise or the saline infusion is performed, DLco/DLno will be completed just prior to and after both exercise and the saline infusion.
11. Blood sample for analysis of Serum CTX must be collected when the subject is fasting. See Section 7.4.9.1
12. Fecal Occult Blood Test (FOBT) cards will be provided to subjects at the end of the screening visit and must be completed and sent back to the laboratory prior to the baseline visit according to the laboratory's standard collection procedures. Similarly, subjects will be given FOBT cards at the end of the Day 7 visit of each study period with completion instructions. See Section 7.4.9.2
13. Day -1 audiometry and sleep study assessments may be completed 7 days prior to Day -1.
14. Clinical laboratory assessments will be collected as a fasting sample.
15. The screening visit will only collect % Predicted DLco (DLco single breath)
16. If a subject has had an echocardiogram completed within the last 6 months prior to screening, it does not need to be repeated
17. Day 7 Audiometry may be completed with a window of ± 2 days

7.2. Screening and Critical Baseline Assessments

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

Medical/medication/alcohol/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5. Cardiovascular medical history/risk factors will also be assessed at screening, including the following items pertaining to medical and medication history:

- Onset and type of symptoms
- Years since diagnosis of HF
- New York Heart Association (NYHA) class
- LVEF (%)
- Years since diagnosis of sleep apnea (if applicable)
- Degree of exercise intolerance: distance/ stairs/time prior to breathlessness
- Presence of orthopnea and/or paroxysmal nocturnal dyspnea
- Peripheral edema: presence and height beyond ankle; recent weight gains, level of peripheral pitting edema
- Significant past medical history including onset, etiology, and results of any recent relevant investigations of heart failure, as applicable
- Medication history

Procedures conducted as part of the subject's routine clinical management and obtained prior to signing of informed consent may be utilized for Screening or baseline purposes provided the procedure meets the protocol-defined criteria and has been performed in the timeframe of the study.

During the Screening Period prior to the first study period (Period I), subjects will have the following assessments performed to determine eligibility for enrollment:

- NT-proBNP
- % Predicted DLco
- Polysomnography (eligibility into sub-study)

7.3. Pharmacodynamic Endpoints

7.3.1. DLco and DLno

The diffusing capacity of the lung to carbon monoxide (DLco) or nitric oxide (DLno) is a measure of the ability of a gas to transfer from the alveoli across the alveolar epithelium and the capillary endothelium to the red blood cells. Changes in DLco and DLno reflect the alveolar-capillary membrane conductance (D_M). Since nitric oxide has a greater

affinity for hemoglobin, the diffusion of nitric oxide is mainly limited by the transfer of gas across the alveolar capillary membrane. Measurements are made simultaneously.

Acute pulmonary congestion causes a reduction in DLco. In patients with heart failure, DLco is decreased and serves as a predictor of disease progression. An impairment in the diffusing capacity of the lung may be related to the symptoms and exercise intolerance associated with heart failure.

DLco will be measured during the Screening Period to determine eligibility for enrollment into the study. Subjects must have a % Predicted DLco <80%.

During each study period, DLco and DLno will be measured just prior to and after the 3-minute step test on Days -1, and 7 and at the Follow-up Visit. Measurements will also be made just prior to and after an intravenous saline infusion on Day 5.

Details of the pulmonary testing methodology including diffusing capacity and measured components are included in the SRM. Refer to the instruction manual(s) for the equipment for further details.

7.3.2. Submaximal Exercise Test

Blockade of the TRPV4 channel with GSK2798745 may decrease pulmonary edema resulting in an increase in exercise capacity and/or oxygen uptake.

Subjects will be asked to participate in a submaximal exercise test that consists of 3 parts: a 2-minute resting baseline, a 3-minute step exercise, and a 1-minute recovery period [Woods, 2011]. Throughout the test, breathing pattern, gas exchange, and heart rate will be monitored using a simplified gas analysis system (i.e. SHAPE Medical Systems, Inc). Respiratory exchange ratio (RER) and the Borg Rating of Perceived Exertion (RPE) measures will be utilized to ensure subjects perform progressive exercise while maintaining a submaximal level throughout the exercise period. Minute ventilation, breath frequency, tidal volume, oxygen consumption, carbon dioxide production, RER and end tidal CO_2 will be obtained from the breath-by-breath gas measurements. The ventilation/carbon dioxide production (VE/VCO_2) slope and other variables will be derived from this data.

The exercise protocol will be completed on Day -1 and Day 7 of each study period. DLco/DLno measurements will be obtained before and after the exercise challenge. Additional details of the exercise protocol for the 3-minute step test are provided in the SRM.

7.3.3. Intravenous Saline Infusion

In patients with chronic HF, intravenously infused saline elicited a reduction of D_M [Puri, 1999] suggesting that the abnormal pulmonary diffusion in this population may have a variable component that could be amenable to therapeutic intervention. Similarly, an infusion of saline into the pulmonary artery significantly reduced both DLco and D_M in patients with chronic HF but not in control subjects [Guazzi, 1999]; the changes in D_M were inversely related to VE/VCO_2 [Guazzi, 2001]. The hemodynamic effects of volume

expansion with saline loading has been shown to be associated with increases in left and right heart filling pressures and pulmonary artery pressures in patients with HF [Andersen, 2015]. In this latter study, no patient developed dyspnea or any evidence of pulmonary congestion despite increase in cardiac filling pressures.

All subjects will undergo an intravenous 0.9% NaCl infusion on Day 5 of both study periods in the presence of a cardiologist. The procedure will be conducted only if the subject is without any signs of obvious congestion as determined by the Investigator. Subjects will be carefully monitored both during and after the infusion and will be provided intravenous diuretics if necessary. Details of this procedure are described in Appendix 9.

DLco and DLno measurements will be obtained prior to the initiation of the infusion and after the completion of the infusion. Additional details are included in the SRM.

7.3.4. Pulmonary Function Tests

Measures of pulmonary function have been shown to predict prognosis in patients with HF [Olson, 2013].

Spirometry measures will include forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) as well as forced expiratory flows, FEF_{25-75} FEF_{50}, and FEF_{75}

Functional residual capacity (FRC) and end-expiratory lung volume (EELV) will be measured by body plethysmography.

7.3.5. Dyspnea

Dyspnea will be assessed using a standardised, validated 5-point scale [Mebazaa, 2010] on Days -1 and 7 of each study period as well as at the Follow-up Visit. Additional details regarding the scale and the assessment are provided in Appendix 6 and the SRM.

7.3.6. Polysomnography

Only subjects participating in the sleep apnea substudy will undergo overnight polysomnography during the Screening Period. Consenting subjects who meet all the eligibility criteria for the main study and have an apnea/hypopnea index (AHI) ≥5 events/hour based on polysomnography will participate in subsequent sleep studies on Day 6 of both study periods.

Multiple recordings will be monitored including electroencephlogram (EEG), electrooculogram (EOG), electromyogram (EMG), electrocardiogram (ECG), and oximetry. Sleep stages and arousals, respiratory rate, and O_2 saturation will be measured. Disordered breathing events will be classified as apneas or hypopneas and as either obstructive or central.

Additional details outlining the polysomnography procedure are detailed in the SRM.

7.3.7. Health Survey: SF-36 Acute

The SF-36 Acute health survey is validated questionnaire, which provides a profile of functional health and well-being. Eight multifunctional scales are employed as well as a single-item evaluation of changes in health status and two summary component scales. The SF-36 Acute has a 1-week recall period for 6 of the 8 scales, which is appropriate for the length of this study. The SF-36 Acute will be administered on Days -1 and 7 of each study period as well as at the Follow-up Visit. Additional details regarding the administration of the scale are provided in Appendix 8 and the SRM.

7.4. Safety

Planned time points for all safety assessments are listed in the Time and Events Table (Section 7.1). Additional time points for safety tests (such as vital signs, physical examinations and clinical laboratory safety tests) may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

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

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

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

7.4.1.1. Time period and Frequency for collecting AE and SAE information

- AEs and SAEs will be collected from the start of Study Treatment until the follow-up contact (see Section 7.4.1.3), at the timepoints specified in the Time and Events Table (Section 7.1).
- Medical occurrences that begin prior to the start of study treatment but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF.
- Any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact.
- All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Appendix 5.
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

NOTE: The method of recording, evaluating and assessing causality of AEs and SAEs plus procedures for completing and transmitting SAE reports to GSK are provided in Appendix 5

7.4.1.2. Method of Detecting AEs and SAEs

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

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

7.4.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs, and non-serious AEs of special interest (as defined in Section 4.6.1) will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up (as defined in Section 5.5). Further information on follow-up procedures is given in Appendix 4.

7.4.1.4. Cardiovascular and Death Events

For any cardiovascular events detailed in Appendix 4 and all deaths, whether or not they are considered SAEs, specific Cardiovascular (CV) and Death sections of the CRF will be required to be completed. These sections include questions regarding cardiovascular (including sudden cardiac death) and non-cardiovascular death.

The CV CRFs are presented as queries in response to reporting of certain CV MedDRA terms. The CV information should be recorded in the specific cardiovascular section of the CRF within one week of receipt of a CV Event data query prompting its completion.

The Death CRF is provided immediately after the occurrence or outcome of death is reported. Initial and follow-up reports regarding death must be completed within one week of when the death is reported.

7.4.1.5. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to GSK of SAEs and non-serious AEs related to study treatment (even for non- interventional post-marketing studies) is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a product under clinical investigation are met.

GSK has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. GSK will comply with country specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Board (IRB)/Independent Ethics Committee (IEC) 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 a 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.

7.4.2. Pregnancy

- Details of all pregnancies in female partners of male subjects will be collected after the start of dosing and until the follow up visit.
- If a pregnancy is reported then the investigator should inform GSK within 2 weeks of learning of the pregnancy and should follow the procedures outlined in Appendix 5.

7.4.3. Physical Examinations

- A complete physical examination will include, at a minimum, assessment of the head, eyes, ears, nose, throat, skin, thyroid, lymph nodes, extremities, and the cardiovascular, respiratory, gastrointestinal and neurological systems. Height and weight will also be measured and recorded.
- A brief physical examination will include, at a minimum assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen). Peripheral edema (height and severity above ankle, non-dependant limb), as well as jugular venous distention and should be assessed. Weight will also be measured and recorded.
- Weight will also be measured once daily as indicated in the Time and Events Table.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

7.4.4. Vital Signs

- Vital signs will be measured in a semi-supine position after 5 minutes rest and will include temperature, systolic and diastolic blood pressures, heart rate, respiratory rate, and pulse oximetry.

7.4.5. Electrocardiogram (ECG)

- Triplicate 12-Lead ECGs will be obtained during the Screening Period and at baseline (Day -1) and single 12-lead ECGs will be obtained on Days 4 and 7 and the Follow-up Visit using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF or QTcB intervals.
- Refer to Section 5.5.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- Resting and stationary measures will also be collected to determine heart rate variability and time domains

7.4.6. Echocardiogram

A transthoracic echocardiogram will be performed during the Screening Period, unless the subject has had an echocardiogram completed within the 6 months prior to screening. Images will be obtained in standard views (e.g., long axis parasternal, short axis parasternal, and apical 2, 3, 4 and 5 chamber). The time to acquire images should not exceed 30 minutes.

7.4.7. Remote Monitoring

Continuous remote monitoring will be performed during each 7-day study period utilizing the Preventice BodyGuardian Remote Monitoring System. The cardiac monitoring system is a portable, wireless body sensor that measures ECG, heart rate, respiratory rate, and activity level. Data will be stored and transmitted to a 24-hour monitoring center. Subjects will be instructed to wear the sensor throughout each study period.

7.4.8. Audiometry

A hearing assessment will be conducted prior to the administration of study medication and at the end of each study period as described in the Time and Events Table. Audiometry will be performed by authorized, trained staff. Details of the audiometry testing may be found in the SRM.

7.4.9. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 3, must be conducted in accordance with the Laboratory Manual (if applicable), and Protocol Time and Events Schedule. All laboratory assessments will be collected as fasting samples. Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/centre number, and visit date. Details for the preparation and shipment of samples (if applicable) will be provided by the laboratory and are detailed in the SRM. Reference ranges for all safety parameters will be provided to the study staff by the local laboratory.

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification) the results must be recorded in the CRF.

Refer to the SRM for appropriate processing and handling of samples to avoid duplicate and/or additional blood draws.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 3.

Table 3 Clinical Laboratory Assessments

Laboratory Assessments	**Parameters**			
Hematology	Platelet Count	*RBC Indices*:	*WBC count with Differential*:	
	RBC Count	MCV	Neutrophils	
	Hemoglobin	MCH	Lymphocytes	
	Hematocrit	MCHC	Monocytes	
	WBC Count (absolute)		Eosinophils	
	Reticulocyte count		Basophils	
Clinical Chemistry	BUN	Potassium	AST (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	ALT (SGPT)	Total Protein
	Glucose, fasting	Calcium	Alkaline phosphatise	Albumin
	CPK	Uric Acid	GGT	
	Chloride			
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal) • Urinary norepinephrine and epinephrine (during sleep study)			
Other Screening Tests	• Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Digoxin concentrations (only in subjects treated with Digoxin) • Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • HMG CoA Reductase Inhibitor concentration (only in subjects taking medication)			
Biomarker(s)/ Other Assessments	• N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) • Norepinephrine and Epinephrine (substudy only) • Serum Collagen Type I C-Telopeptide (CTX), fasting • Fecal Occult Blood Test (FOBT) • Troponin			
NOTES : Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 5.5.1 and Appendix 2				

All laboratory tests with values that are considered clinically significantly abnormal during participation in the study or within 14 days after the last dose of study treatment should be repeated until the values return to normal or baseline. If such values do not return to normal within a period judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

7.4.9.1. Serum Collagen Type I Telopeptide (CTX)

Blood samples will be collected at the Baseline Visit (Day -1) and on Day 7 of each study period. Approximately 1 mL will be collected as a fasting sample from the subject at the start of each of these visits.

7.4.9.2. Fecal Occult Blood Test (FOBT)

Based on the gastric erosions found in a preclinical model (dogs at ≥3 mg/kg), FOBT will be performed to assess any possible drug-related GI blood loss. Due to concomitant anti-platelet therapy that many patients with heart failure receive, along with the common incidence of intestinal mucosal edema and associated diarrhea, findings of fecal blood would not be unexpected in this population. Subjects will be tested for the presence of fecal occult blood prior to the start of treatment period I (between Screening and Baseline) and after each of the two treatment periods.

Once subjects have signed the consent form and have been enrolled into the study based on meeting the eligibility criteria, they will be provided 3 FOBT cards at the end of the screening visit. Study staff will provide the subjects with instructions for completing the tests and subsequent shipment of the tests back to the laboratory. This assessment will be completed between Screening and Baseline (Day -1). Additionally, subjects will be provided 3 FOBT cards on Day 7 of each treatment period with the same instructions for completion and shipment for laboratory analysis.

7.4.10. Suicidal Risk Monitoring

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

Subjects being treated with GSK2798745 should be assessed and monitored appropriately for suicidality and unusual changes in behaviour. Consideration should be given to discontinuing GSK2798745 in subjects who experience signs of suicidal ideation or behaviour.

The C-SSRS is a measure of suicidal ideation and behavior, with demonstrated predictive validity and reliability. Improved assessments of suicidal ideation and behavior are necessary to better understand the relationship between suicidal AEs and medication treatment. The FDA recommends the use of suicidality assessment instruments that map

to the Columbia Classification Algorithm for Suicide Assessment (C-CASA). One such instrument is the C-SSRS. Sections of the C-SSRS include suicidal ideation, intensity of ideation, suicidal behavior, and actual suicide attempt(s). The C-SSRS assesses lifetime and current suicidal thoughts and behaviors across these categories based on an increasing severity of a 1- to 5-rating scale. The semi-structured questionnaire is completed by a trained and experienced neurologist, psychiatrist, or neuropsychologist, or another trained and experienced person approved by the Sponsor, who may be the Principal Investigator or a sub-investigator for the study. See Appendix 7 and the SRM for details of the scale.

7.5. Pharmacokinetics

7.5.1. Blood Sample Collection

Blood samples for pharmacokinetic analysis of GSK2798745 will be collected at the time points indicated in Section 7.1 Time and Events Table.

Approximately 2 mL of blood will be collected in EDTA tubes. The actual date and time of each blood sample collection will be recorded. The timing and volume of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure adequate PK monitoring of GSK2798745 and, if possible, any relevant GSK2798745 metabolites.

Where possible, PK monitoring of relevant co-administered drugs and their metabolites will also be undertaken (atorvastatin and simvastatin). Approximately 2 mL of blood will be collected into sodium heparin tubes. The actual date and time of each blood sample collection will be recorded. The volume of such samples may be altered and additional time points may be added to ensure adequate statin PK monitoring.

Additional collection, processing, storage and shipping procedures are provided in the Study Reference Manual (SRM).

7.5.2. Sample Analysis

Plasma analysis will be performed under the control of Platform Technologies and Science-Drug Metabolism and Pharmacokinetics (PTS-DMPK)/ GlaxoSmithKline, the details of which will be included in the Study Reference Manual (SRM). Concentrations of GSK2798745, and co-administered statins and corresponding metabolites (atorvastatin and metabolites, and simvastatin and metabolites) will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Plasma samples may be analyzed for metabolite M1 dependent upon the availability of an assay. Until this time, GSK will store the remaining plasma from the PK plasma samples for future possible metabolite analysis.

7.6. Biomarker(s)

Blood samples will be taken for NT-proBNP measurement during the Screening Period and at the timepoints indicated in the Time and Event Table, Section 7.1.

Details of sample collection, processing, storage, and shipment requirements are provided in the SRM.

7.7. Genetics

Information regarding genetic research is included in Appendix 3.

8. DATA MANAGEMENT

- For this study subject data will be entered into GSK defined CRFs, transmitted electronically to GSK or designee 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.
- CRFs (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.
- Original CRFs will be retained by GSK, while the investigator will retain a copy. Subject initials will not be collected or transmitted to GSK according to GSK policy.

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

This is a double-blind study with the sponsor un-blinded, which allows data to be reviewed on an on-going basis by the sponsor.

9.1. Hypotheses

An estimation approach will be used for the comparison between GSK2798745 treatment and placebo for the change from baseline in DLco at Day 4 and Day 7. Point estimates and associated 95% confidence intervals for the differences in means will be constructed to provide a plausible range of values for the true comparisons of interest.

9.2. Sample Size Considerations

The sample size was determined using an estimation approach in order to provide sufficient precision for the estimation of the DLco comparison between the treatment and placebo groups. A power statement has been calculated to put the sample size in a familiar context.

Based on the DLco measurements reported by [Olsen, 2006], the baseline mean DLco at rest is 15.3 mL/mmHg/min. The within-subject variability (SDw) additionally provided by the Mayo Clinic Investigators is 1.9. The corresponding change from baseline variance (SDw) estimation is 1.5. If we assume the baseline values for HF subjects in the planned study will be similar to the values at rest in the HF group described in this reference, then a 15% increase from baseline would equate to an effect size of 2.3.

In a two-period crossover design (in which a subject receives placebo in one period and an active dose in the other period) with a sample size of 12 subjects, the estimates of the half-widths of the 95% confidence intervals for the difference in the mean change from baseline in DLco (active-placebo) is expected to be approximately 1.36 or less . That is, if the observed difference in the change from baseline for GSK2798745 and placebo is 2.3, then the 95% CI would be (0.94, 3.66).

In addition, 12 subjects would allow the detection of a 2.3 magnitude difference in the mean change from baseline for DLco under assumption that the SDw for change from baseline for DLco is 1.5, using a two-sided t-test with alpha=0.05 with more than 90% power.

9.2.1. Sample Size Assumptions

If a subject is withdrawn from the study prior to completing both study periods, a subject may be enrolled as a replacement so that a total of 12 subjects complete all assessments in the two study period.

9.2.2. Sample Size Sensitivity

If the within-subject standard deviation is higher than expected, the precision of the 95% CI and the power of a two-sided t-test to detect an effect size of 2.3 (mean difference

between the active dose and placebo) with a 5% significance level would be as follows (with a two-period crossover design with 12 subjects):

SDw	Power with Effect Size of 2.3 mL/mmHg/min	Precision of the 95% Confidence Interval
1.9	76%	1.73
2.4	56%	2.2

9.2.3. Sample Size Re-estimation or Adjustment

When 12 subjects have completed both periods, an analysis will be performed to determine whether additional subjects (up to a maximum of 24 subjects) should be enrolled based on the estimate of within-subject variance for the change from baseline in DLco.

9.3. Data Analysis Considerations

9.3.1. Analysis Populations

All Subjects Population

The 'All Subjects Population' is defined as all randomized subjects who receive at least one dose of study medication.

Per Protocol Population

The 'Per Protocol Population' will consist of any Analysis Population subjects who are compliant with protocol-specific criteria. Subjects with specified protocol deviations will be excluded.

9.3.2. Interim Analysis

Safety, tolerability and efficacy data will be reviewed in-stream by the sponsor. These reviews may include individual subject data, summaries by treatment group and graphical displays. Subjects and all site personnel (except the un-blinded pharmacist) will be blinded to subject randomization. The decision to continue or stop recruitment will be made based on the evaluation of all available safety and efficacy data.

After 12 subjects complete the study, a sample size re-estimation will be performed based on the estimate of within-subject variance for the change from baseline in the DLco measurement. If the within-subject variance for the change from baseline in DLco is assumed reasonable, the study will be considered completed. Otherwise, additional subjects up to a total of 24 may be enrolled into the study. The final analyses and reporting of the study will be performed after all patients have completed the central formal study.

In addition, a Bayesian predictive approach will be used to assess the futility of the percent change from baseline in DLco at Day 7 to help determine whether additional patients are needed after the sample size re-evaluation. However, this alone will not be used as a basis for determining futility.

The futility criteria for the change from baseline in DLco will be based on the predictive probability at the interim that the end of the study difference in the change from baseline in DLco between GSK2798745 treatment and placebo will meet the definition of a positive outcome.

9.4. Key Elements of Analysis Plan

Final analysis will be provided when all subjects have finished the study and the database has been frozen. The construction of analysis data sets will be performed in accordance with all applicable GlaxoSmithKline standards and procedures.

Adjustment for multiplicity of treatment group comparisons and endpoints is not planned. All available data will be used in the analyses as defined in the populations above. Missing values will not be imputed.

9.4.1. Primary Analyses

Profiles for DLco will be summarized and presented graphically by treatment group as appropriate for the data. The change from baseline in DLco at Day 4, Day 5 and Day 7 will be calculated and summarized in tabular format and/or graphically by treatment. For the change from baseline in DLco, a statistical analysis will be performed using a mixed effect model with repeated measures with a fixed effect term for treatment, period and day, with a random effect for subject, day in the repeated statement with subject being subject by period, and baseline as a covariate, if data permit. Point estimates and associated 95% confidence intervals will be constructed for the differences between the active treatment and placebo.

Full details of analysis will be specified in the RAP.

9.4.2. Secondary Analyses

For other diffusing capacity pharmacodynamic (PD) endpoints such as DLno, D_M and Vc, similar analyses as in Section 9.4.1 will be provided, as data permit. For these PD endpoints, which may be $\log_e$ transformed prior to analysis to satisfy model assumptions, point estimates and their associated 95% confidence intervals will be back-transformed to provide point estimates and 95% confidence intervals for the ratios.

For pulmonary function measurement endpoints such as FVC, FEV1, FEF_{25-75} FEF_{50}, and FEF_{75}, FRC and EELV, similar analysis as in Section 9.4.1 will be provided, as data permit. For these PD endpoints, which may be $\log_e$ transformed prior to analysis to satisfy model assumptions, point estimates and their associated 95% confidence intervals will be back-transformed to provide point estimates and 95% confidence intervals for the ratios.

For respiratory rate measurement and dyspnea score, similar analysis as in Section 9.4.1 will be provided, as data permit.

In terms of DLco and DLno changes from baseline after saline challenge on Day 5 and after exercise on Day 7, the change from baseline for the challenge (after-before) will be calculated and summarized in tabular format by treatment. A statistical analysis will be performed using a mixed effect model with a fixed effect term for treatment, period and sequence, random effect term for subject-within-sequence, and baseline as a covariate, if data permit. Point estimates and associated 95% confidence intervals will be constructed for the differences between the active treatment and placebo.

In terms of ventilatory efficiency, scatter plots for Ve/VCO2 at Baseline and Day 7 will be performed and the slope or correlation of Ve/VO2 provided. Summary statistics for Ve and VCO2 and change from baseline measurement will be provided.

Details on the analysis of other endpoints will be provided in RAP.

Pharmacokinetic analyses will be the responsibility of the Clinical Pharmacology Modeling & Simulation Department within GlaxoSmithKline. Plasma GSK2798745 concentration-time data will be analyzed by non-compartmental methods with the latest available version of WinNonlin software. Calculations will be based on the actual sampling times recorded during the study. From the plasma concentration-time data, the pharmacokinetic parameters such as Cmax and AUC may be determined, as data permit.

The details of the PK analysis will be listed in the RAP.

9.4.3. Other Analyses

Safety data will be presented in tabular and/or graphical format and summarized descriptively according to GSK's Integrated Data Standards Library (IDSL) standards. No formal statistical analyses are planned for safety data. Safety data will be listed and summarized. All subjects who received an active dose of study medication will be included in the evaluation of clinical safety and tolerability. Clinical monitoring and laboratory safety will be reviewed by the Investigator and vital signs will be summarized by treatment.

9.4.4. Exploratory Analyses

Pharmacokinetic analyses for the statins and any statin metabolites will be the responsibility of the Clinical Pharmacokinetics Modeling & Simulation Department within GlaxoSmithKline. Calculations will be based on the actual sampling times recorded during the study. The systemic concentrations of any HMG-CoA reductase inhibitors (e.g., simvastatin, atorvastatin) and their metabolites will be summarized, as data permit. The details of the PK analysis will be listed in the RAP.

Data from the polysomnography sub-study will be presented in tablular and/or graphical format and summarized descriptively.

10. STUDY GOVERNANCE CONSIDERATIONS

10.1. Posting of Information on Publicly Available Clinical Trial Registers

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

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

Prior to initiation of a 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, and with GSK policy.

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

- IRB/IEC review and favorable opinion/approval of the study protocol and amendments as applicable
- Signed informed consent to be obtained for each subject before participation in the study (and for amendments as applicable)
- Investigator reporting requirements (e.g. reporting of AEs/SAEs/protocol deviations to IRB/IEC)
- GSK will provide full details of the above procedures, either verbally, in writing, or both.
- Signed informed consent must be obtained for each subject prior to participation in the study
- The IEC/IRB, and where applicable the regulatory authority, approve the clinical protocol and all optional assessments, including genetic research.
- Optional assessments (including those in a separate protocol and/or under separate informed consent) and the clinical protocol should be concurrently submitted for approval unless regulation requires separate submission.
- Approval of the optional assessments may occur after approval is granted for the clinical protocol where required by regulatory authorities. In this situation, written approval of the clinical protocol should state that approval of optional assessments is being deferred and the study, with the exception of the optional assessments, can be initiated.

10.3. Quality Control (Study Monitoring)

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

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

- Data are authentic, accurate, and complete.
- Safety and rights of 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

10.4. Quality Assurance

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

10.5. Study and Site Closure

- Upon completion or premature discontinuation of the study, the GSK monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK Standard Operating Procedures.
- 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 the reasons for taking such action with the investigator or the head of the medical institution (where applicable). When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action.

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

10.6. Records Retention

- Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.
- The records must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site 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 ensure 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, GSK standards/procedures, and/or institutional requirements.
- 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 is no longer associated with the site.

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

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

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

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

The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.

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

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations

ADHF	Acute decompensated heart failure
AE	Adverse Event
AHI	Apnea-hypopnea index
ALT	Alanine transaminase
AST	Aspartate aminotransferase
AUC	Area under the curve
BMI	Body mass index
Ca^{2+}	Calcium
C-CASA	Columbia Classification Algorithm for Suicide Assessment
CHF	Chronic heart failure
CI	Confidence intervals
Cmax	Maximum concentration
CMT2C	Charcot-Marie-Tooth disease Type 2C
CNS	Central nervous system
CO_2	Carbon dioxide
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disease
CPK	Creatine phosphokinase
CRF	Case report form
CRU	Clinical research unit
CSA	Central sleep apnea
CSR	Cheyne-Strokes respiration
C-SSRS	Columbia Suicide Severity Rating Scale
cTn	Troponin (cardiac)
CTX	Collagen Type I Telopeptide
CV	Cardiovacsular
CYP	Cytochrome P450 enzyme
DLco	Diffusing capacity of the lung for carbon monoxide
DLno	Diffusing capacity of the lung for nitric oxide
D_M	Alveolar-caplillary membrane conductance
DMPK	Drug Metabolism and Pharmacokinetics
DNA	Deoxyribonucleic acid
DPD	Deoxypyridinoline
E	Epinephrine
ECG	Electrocardiogram
EEG	Electroencephalography
EELV	End expiratory lung volume
EMG	Electromyogram
EOG	Electrooculogram
ER	Emergency Room

FDA	Food and Drug Administration
FEF	Forced expiratory flows
FEV_1	Forced expiratory volume in 1 second
FOBT	Fecal Occult Blood Test
FRC	Forced residual capacity
FSH	Follicle stimulating hormone
FVC	Forced vital capacity
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilence
GSK	GlaxoSmithKline
HBcAb	Hepatitis B core antibody
HbsAg	Hepatitis B surface antigen
HEK	Human embryonic kidney cells
HF	Heart failure
HR	Heart rate
HRT	Hormone Replacement Therapy
IDSL	Integrated Data Standards Library
IEC	Independent ethics committee
IgM	Immunoglobulin
INR	International normalized ratio
IP	Investigational Product
IRB	Institutional review board
IWRS	Interactive web response system
Kg	Kilogram
KO	Knockout
LDH	Lactate dehydrogenase
LFT	Liver function tests
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
MSDS	Material Safety Data Sheet
Na	Sodium
NaCl	Sodium chloride
NE	Norepinephrine
nM	Nanomolar
NO	Nitric oxide
NOAEL	No observed adverse event level
NYHA	New York Heart Association
O_2	Oxygen
OSA	Obstructive sleep apnea
P1NP	Procollagen type 1 amino-terminal propeptide
PD	Pharmacodynamic
PFT	Pulmonary function tests
P-gp	P-glycoprotein
PGx	Pharmacogenomics
PK	Pharmacokinetics

PTH	Parathyroid hormone
PTS-DMPK	Platform Technologies and Sciences-Drug Metabolism and Pharmacokinetics
QTc	QT interval corrected
QTcB	QT interval corrected according to Bazett's formula
QTcF	QT interval corrected according to Fridericia's formular
RAP	Reporting and analysis plan
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
SAE	Serious Adverse Event
SDw	Within-subject variability
SOP	Standard Operating Procedure
SRM	Study reference manual
t½	Half-life
tmax	Time to maximum observed plasma concentration
TRPV4	Transient receptor potential vanilloid 4
µg	Micrograms
ULN	Upper limit of normal
VCO_2	Volume of carbon dioxide production
Vc	Capillary blood volume
VE	Ventilation
VO_2	Volume of oxygen
WBC	White blood cells

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
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12.2. Appendix 2: Liver Safety Required Actions and Follow up Assessments

Phase II liver chemistry stopping and increased monitoring criteria have been designed to assure subject safety and evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance). http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf.

Phase II liver chemistry stopping criteria and required follow up assessments

Liver Chemistry Stopping Criteria – Liver Stopping Event	
ALT-absolute	ALT ≥ 5xULN
ALT Increase	ALT ≥ 3xULN persists for ≥4 weeks
Bilirubin[1, 2]	ALT ≥ 3xULN **and** bilirubin ≥ 2xULN (>35% direct bilirubin)
INR2	ALT ≥ 3xULN **and** INR>1.5, if INR measured
Cannot Monitor	ALT ≥ 3xULN and cannot be monitored weekly for 4 weeks
Symptomatic[3]	ALT ≥ 3xULN associated with symptoms (new or worsening) believed to be related to liver injury or hypersensitivity

Required Actions and Follow up Assessments following ANY Liver Stopping Event	
Actions	**Follow Up Assessments**
• **Immediately** discontinue study treatment • Report the event to GSK **within 24 hours** • Complete the liver event CRF and complete an SAE data collection tool if the event also meets the criteria for an SAE[2] • Perform liver event follow up assessments • Monitor the subject until liver chemistries resolve, stabilize, or return to within baseline (see **MONITORING** below) • **Do not restart/rechallenge** subject with study treatment unless allowed per protocol and GSK Medical Governance approval **is** granted • If restart/rechallenge **not allowed per protocol or not granted**, permanently discontinue study treatment and may continue subject in the study	• Viral hepatitis serology[4] • Blood sample for pharmacokinetic (PK) analysis, obtained 24 hours after last dose[5] • Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). • Fractionate bilirubin, if total bilirubin≥2xULN • Obtain complete blood count with differential to assess eosinophilia • Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE report form • Record use of concomitant medications on the concomitant medications report

Liver Chemistry Stopping Criteria – Liver Stopping Event	
for any protocol specified follow up assessments **MONITORING:** **<u>For bilirubin or INR criteria:</u>** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24 hrs** • Monitor subjects twice weekly until liver chemistries resolve, stabilize or return to within baseline • A specialist or hepatology consultation is recommended **<u>For All other criteria:</u>** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24-72 hrs** • Monitor subjects weekly until liver chemistries resolve, stabilize or return to within baseline	form including acetaminophen, herbal remedies, other over the counter medications. • Record alcohol use on the liver event alcohol intake case report form **<u>For bilirubin or INR criteria:</u>** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG or gamma globulins). • Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009]). **NOTE: not required in China** • Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease: complete Liver Imaging and/or Liver Biopsy CRF forms.

1. Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study treatment for that subject if ALT ≥ 3xULN **and** bilirubin ≥ 2xULN.. Additionally, if serum bilirubin fractionation testing is unavailable, **record presence of detectable urinary bilirubin on dipstick**, indicating direct bilirubin elevations and suggesting liver injury.
2. All events of ALT ≥ 3xULN **and** bilirubin ≥ 2xULN (>35% direct bilirubin) or ALT ≥ 3xULN **and** INR>1.5, if INR measured which may indicate severe liver injury (possible 'Hy's Law'), **must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis)**; INR measurement is not required and the threshold value stated will not apply to subjects receiving anticoagulants
3. New or worsening symptoms believed to be related to liver injury (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, or jaundice) or believed to be related to hypersensitivity (such as fever, rash or eosinophilia)
4. Includes: Hepatitis A IgM antibody; Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); Hepatitis E IgM antibody
5. PK sample may not be required for subjects known to be receiving placebo or non-GSK comparator treatments.) Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in

the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the SRM.

Phase II liver chemistry increased monitoring criteria with continued therapy

Liver Chemistry Increased Monitoring Criteria – Liver Monitoring Event	
Criteria	**Actions**
ALT ≥3xULN and <5xULN **and** bilirubin <2xULN, **without** symptoms believed to be related to liver injury or hypersensitivity, **and** who can be monitored weekly for 4 weeks	• Notify the GSK medical monitor **within 24 hours** of learning of the abnormality to discuss subject safety. • Subject can continue study treatment • Subject must return weekly for repeat liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) until they resolve, stabilise or return to within baseline • If at any time subject meets the liver chemistry stopping criteria, proceed as described above • If, after 4 weeks of monitoring, ALT <3xULN and bilirubin <2xULN, monitor subjects twice monthly until liver chemistries normalize or return to within baseline.

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12.3. Appendix 3: Genetic Research

Genetic Research Objectives and Analyses

The objectives of the genetic research are to investigate the relationship between genetic variants and:

- Response to medicine, including GSK2798745 or any concomitant medicines;
- Heart Failure susceptibility, severity, and progression and related conditions

Genetic data may be generated while the study is underway or following completion of the study. Genetic evaluations may include focused candidate gene approaches and/or examination of a large number of genetic variants throughout the genome (whole genome analyses). Genetic analyses will utilize data collected in the study and will be limited to understanding the objectives highlighted above. Analyses may be performed using data from multiple clinical studies to investigate these research objectives.

Appropriate descriptive and/or statistical analysis methods will be used. A detailed description of any planned analyses will be documented in a Reporting and Analysis Plan (RAP) prior to initiation of the analysis. Planned analyses and results of genetic investigations will be reported either as part of the clinical RAP and study report, or in a separate genetics RAP and report, as appropriate.

Study Population

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

Study Assessments and Procedures

A key component of successful genetic research is the collection of samples during clinical studies. Collection of samples, even when no *a priori* hypothesis has been identified, may enable future genetic analyses to be conducted to help understand variability in disease and medicine response.

- A 6 ml blood sample will be taken for Deoxyribonucleic acid (DNA) extraction. A Blood sample is collected at the baseline visit, after the subject has been randomized and provided informed consent for genetic research. Instructions for collection and shipping of the genetic sample are described in the laboratory manual. The DNA from the blood sample may undergo quality control analyses to confirm the integrity of the sample. If there are concerns regarding the quality of the sample, then the sample may be destroyed. The blood sample is taken on a single occasion unless a duplicate sample is required due to an inability to utilize the original sample.

The genetic sample is labelled (or "coded") with the same study specific number used to label other samples and data in the study. This number can be traced or linked back to the subject by the investigator or site staff. Coded samples do not carry personal identifiers (such as name or social security number).

Samples will be stored securely and may be kept for up to 15 years after the last subject completes the study, or GSK may destroy the samples sooner. GSK or those working with GSK (for example, other researchers) will only use samples collected from the study for the purpose stated in this protocol and in the informed consent form. Samples may be used as part of the development of a companion diagnostic to support the GSK medicinal product.

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

Informed Consent

Subjects who do not wish to participate in the genetic research may still participate in the study. Genetic informed consent must be obtained prior to any blood being taken.

Subject Withdrawal from Study

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

- Continue to participate in the genetic research in which case the genetic DNA sample is retained
- Discontinue participation in the genetic research and destroy the genetic DNA sample

If a subject withdraws consent for genetic research or requests sample destruction for any reason, the investigator must complete the appropriate documentation to request sample destruction within the timeframe specified by GSK and maintain the documentation in the site study records.

Genotype data may be generated during the study or after completion of the study and may be analyzed during the study or stored for future analysis.

- If a subject withdraws consent for genetic research and genotype data has not been analyzed, it will not be analyzed or used for future research.
- Genetic data that has been analyzed at the time of withdrawn consent will continue to be stored and used, as appropriate.

Screen and Baseline Failures

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

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

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

GSK may share genetic research data with other scientists to further scientific understanding in alignment with the informed consent. GSK does not inform the subject, family members, insurers, or employers of individual genotyping results that are not known to be relevant to the subject's medical care at the time of the study, unless required by law. This is due to the fact that the information generated from genetic studies is generally preliminary in nature, and therefore the significance and scientific validity of the results are undetermined. Further, data generated in a research laboratory may not meet regulatory requirements for inclusion in clinical care.

12.4. Appendix 4: Definition of and Procedures for Recording, Evaluating, Follow-Up and Reporting of Adverse Events

12.4.1. Definition of Adverse Events

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

Events <u>meeting</u> AE definition include:
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgement of the investigator. • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present prior to the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae). • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE. • The signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" also constitutes an AE or SAE.

Events NOT meeting definition of an AE include:
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure 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.

12.4.2. Definition of Serious Adverse Events

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

Serious Adverse Event (SAE) is defined as 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

Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:
from baseline is not considered an AE.
d. Results in disability/incapacity 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. Other situations: • Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. • Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse
g. Is associated with liver injury <u>and</u> impaired liver function defined as: • ALT ≥ 3xULN and total bilirubin* ≥ 2xULN (>35% direct), **or** • ALT ≥ 3xULN and INR** > 1.5. * Serum bilirubin fractionation should be performed if testing is available; if unavailable, measure urinary bilirubin via dipstick. If fractionation is unavailable and ALT ≥ 3xULN and total bilirubin ≥ 2xULN, then the event is still to be reported as an SAE. ** INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants. If INR measurement is obtained, the value is to be recorded on the SAE form.
• Refer to Appendix 2 for the required liver chemistry follow-up instructions

12.4.3. Definition of Cardiovascular Events

Cardiovascular Events (CV) Definition:
Investigators will be required to fill out the specific CV event page of the CRF for the following AEs and SAEs: • Myocardial infarction/unstable angina • Congestive heart failure • Arrhythmias • Valvulopathy • Pulmonary hypertension • Cerebrovascular events/stroke and transient ischemic attack • Peripheral arterial thromboembolism • Deep venous thrombosis/pulmonary embolism • Revascularization

12.4.4. Recording of AEs and SAEs

AEs and SAE Recording:
• When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event. • The investigator will then record all relevant information regarding an AE/SAE in the CRF • It is **not** acceptable for the investigator to send photocopies of the subject's medical records to GSK in lieu of completion of the GSK, AE/SAE CRF page. • There may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK. • The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms. • Subject-completed Value Evidence and Outcomes questionnaires and the collection of AE data are independent components of the study. • Responses to each question in the Value Evidence and Outcomes questionnaire will be treated in accordance with standard scoring and statistical procedures detailed by

AEs and SAE Recording:
the scale's developer. • The use of a single question from a multidimensional health survey to designate a cause-effect relationship to an AE is inappropriate.

12.4.5. Evaluating AEs and SAEs

Assessment of Intensity
The investigator will make an assessment of intensity for each AE and SAE reported during the study and will assign it to one of the following categories: • Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities. • Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities • Severe: An event that prevents normal everyday activities. - an AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. • An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality
• The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE. • A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. • The investigator will use clinical judgment to determine the relationship. • Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated. • The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of his/her assessment. • For each AE/SAE the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. • There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important**

Assessment of Causality
that the investigator always make an assessment of causality for every event prior to the initial transmission of the SAE data to GSK. • The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE data collection tool accordingly. • The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs
• The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE. • The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals. • 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 CRF. • The investigator will submit any updated SAE data to GSK within the designated reporting time frames.

12.4.6. Reporting of SAEs to GSK

SAE reporting to GSK via electronic data collection tool
• Primary mechanism for reporting SAEs to GSK will be the electronic data collection tool • If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the Medical Monitor. • Site will enter the serious adverse event data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool (InForm system) will be taken off-line to prevent the entry of new data or changes to existing data • If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, the site can report this information on a paper SAE form or to the Medical Monitor by telephone.

SAE reporting to GSK via electronic data collection tool
• Contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

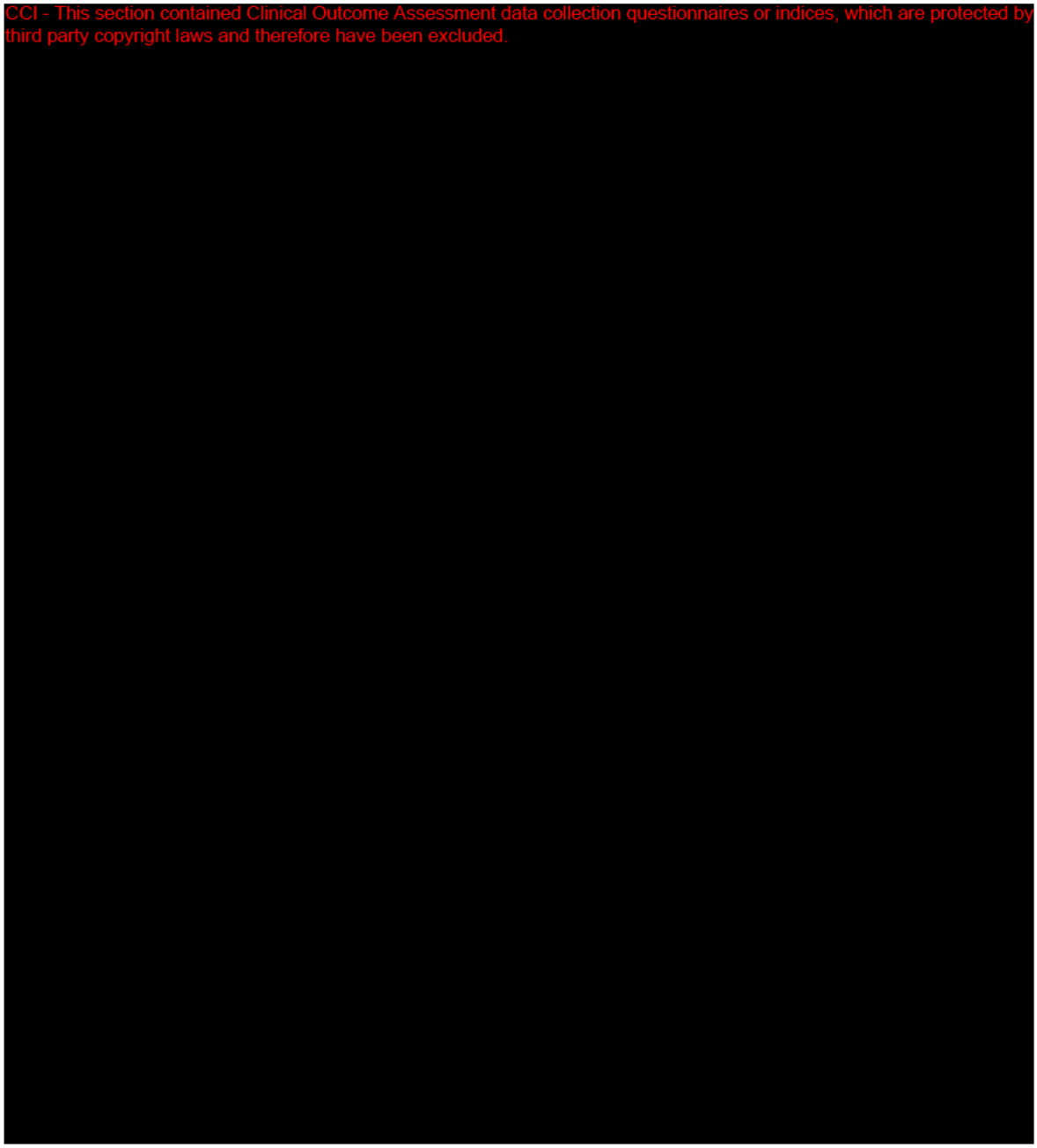
12.5. Appendix 5: Collection of Pregnancy Information

- Investigator will attempt to collect pregnancy information on any female partner of a male study subject who becomes pregnant while participating in this study. This applies only to subjects who are randomized to receive study medication.
- After obtaining the necessary signed informed consent from the female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of the partner's pregnancy
- Partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK.
- Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

12.6. Appendix 6: Dyspnea 5-Point Likert Scale

12.7. Appendix 7: Columbia Suicide-Severity Rating Scale (C-SSRS)

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.



COLUMBIA SUICIDE-SEVERITY

RATING SCALE (C-SSRS)

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.

12.8. Appendix 8: SF-36 Acute

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.

12.9. Appendix 9: Intravenous Saline Infusion

Rapid intravenous saline infusion has been employed to elicit fluid extravasation from the vasculature to the pulmonary interstitium. This methodology has been previously utilized in studies with healthy volunteers [Synder, 2006] and patients with heart failure [Fujimoto, 2013; Robbins, 2014; Andersen, 2015].

Subject Evaluation Prior to Infusion

On Day 5 of both study periods, the subject will be admitted to the unit and undergo routine weight measurement and vital sign assessments including temperature, systolic and diastolic blood pressures, heart rate, respiratory rate, and pulse oximetry to assess O_2 saturation. The subject will also undergo a pulmonary examination.

While in a sitting position, a dyspnea assessment, utilizing a standardized 5-point Likert scale, will be obtained to document the subject's current status. A subject who is not severely or very severely short of breath will undergo an orthopnea test (placed supine with head ≤ 20 degrees relative to horizontal). After an equilibration period of 2 minutes, the 5-point scale will be repeated. A subject who reports a worse dyspnea score when supine compared to sitting will be categorized as having orthopnea. Subjects categorized as having orthopnea will not be eligible to participate in the saline infusion.

Study treatment will be administered after all planned pre-dose assessments for Day 5 are completed.

Saline Infusion Procedure

A subject who is judged by the Investigator to be in a stable condition and without obvious congestion will then undergo the intravenous saline infusion procedure. This procedure will be conducted in the unit, in the presence of a cardiologist and monitoring personnel. A crash cart will be accessible, and intravenous diuretic will be available, if necessary.

A venous catheter will be inserted while the subject is semi-recumbent (approximately 45°). The subject will remain in a semi-recumbent position throughout the procedure.

DLco and DLno measurements and simple spirometry assessments will be obtained prior to the initiation of the infusion.

0.9% normal saline will be infused intravenously at a rate of approximately 150 mL/min to a total volume of 500 mL. During the saline infusion, blood pressure, heart rate, respiratory rate, and O_2 saturation will be continuously monitored, and any physical symptoms recorded.

DLco and DLno measurements and spirometry assessments will be obtained after the completion of the infusion. Additional details are provided in the SRM.

Post-infusion Monitoring

Following the completion of the saline infusion procedure, the subject will remain in the unit for the next 4 hours. Blood pressure, heart rate, respiratory rate, and O_2 saturation will be continuously monitored, and any physical symptoms recorded. The subject will also be assessed for dyspnea and orthopnea, as described above, at hourly intervals.

After the 4-hour period, the subject will be eligible to leave the unit if heart rate and respiratory rate are within 20% of the baseline value, O_2 saturation is within 2% of the baseline value, and if the dyspnea score is the same as the baseline score.

The subject will continue to be monitored by the Preventice BodyGuardian Remote Monitoring System as described in Section 7.4.7 of the protocol; ECG, heart rate, respiratory rate and activity level will be measured continuously.

Any worsening of heart failure that requires treatment in the 48 hours subsequent to the saline infusion will be reported as a serious adverse event.

Individual Stopping Criteria During the Saline Infusion Procedure

If the subject demonstrates one of the following changes during the saline infusion, the infusion should be discontinued:

- Decrease in O2 saturation of >4% compared to baseline
- Increase in respiratory rate of >20% compared to baseline
- Any increase in dyspnea perceived by the patient

If the subject has met one of these criteria during or immediately after the infusion, the subject should be carefully monitored and given intravenous diuretic treatment, intravenous or sublingual nitrates, or other treatment as required.

The subject may continue in the study. However, if this response occurred in the first study period, the subject should not undergo a second saline infusion in the subsequent study period.

Discontinuation of Saline Infusion Procedure

If during the conduct of the study, **two** subjects meet the individual stopping criteria during the infusion or demonstrate a worsening of heart failure in the 48 hours after completion of the infusion, this challenge procedure will be removed from the study design. No other subjects will undergo this assessment.

Reporting of Adverse Outcomes Associated with the Saline Infusion

Any of the following outcomes must be reported to the sponsor as a serious adverse event:

- Treatment with intravenous diuretic subsequent to the saline infusion

- Need for continued monitoring in the unit beyond the 4-hour mandated observation period
- Any worsening of heart failure that requires treatment during the 48-hour monitoring period after the saline infusion procedure

These events will be reported by the investigator as serious adverse events in the InForm electronic data collection tool. They will be reported by the sponsor to the FDA as an Expedited Report via electronic submission in eCTD format to the IND, as a 7-day or 15-day IND safety report, as applicable per timeframe specified in 21 CFR 312.32(c)(1). The completed report will also be provided to the IRB.

12.10. Appendix 10: Protocol Changes

Protocol Amendment 1

Summary of Amendment Changes

This amendment incorporates the changes requested during the FDA review of the IND.

List of Specific Changes

Section 4.2 Treatment Arms, Duration and Assessments

PREVIOUS TEXT

Pharmacokinetic profiles will be obtained during both study periods. On Day 4 and Day 7 of each study period, the assessments of pulmonary function will be repeated. On Day 6 of each study period, subjects will receive an intravenous saline infusion, and DLco and DLno measurements will be performed before and after the procedure. On Day 7 of each study period, the step-exercise challenge and gas transfer measurements, dyspnea assessment, measurement of NT-proBNP concentration, clinical laboratory tests, patient-reported health status (SF-36 Acute), and general safety assessments will be repeated.

REVISED TEXT

Pharmacokinetic profiles will be obtained during both study periods. On Day 4 and Day 7 of each study period, the assessments of pulmonary function will be repeated. On Day **5** ~~6~~ of each study period, subjects will receive an intravenous saline infusion, and DLco and DLno measurements will be performed before and after the procedure. On Day 7 of each study period, the step-exercise challenge and gas transfer measurements, dyspnea assessment, measurement of NT-proBNP concentration, clinical laboratory tests, patient-reported health status (SF-36 Acute), and general safety assessments will be repeated.

Section 4.6.1 Risk Assessment

PREVIOUS TEXT

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) GSK2798745		
Vascular lesions	Dogs (4-week study): At 30mg/kg/day, 2 males had arterial lesions • One male: Heart – Coronary artery inflammation; Thymus –	The arterial lesions noted in heart, thymus, epididymides, and urinary bladder cannot be monitored directly. There is currently no

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	Arteriole inflammation with fibroplasia • One male: Epididymides – Artery degeneration/necrosis with inflammation Dogs (12-week study): At 10mg/kg/day, 1 male and 1 female had arterial lesions • One male: Epididymides – Arteriole degeneration/necrosis with lymphocytic inflammation • One female: Bladder – Arteriole degeneration/necrosis with lymphocytic inflammation Rats: No vascular lesions observed	human translation biomarker or understanding of the underlying mechanism. Since these effects cannot be monitored directly in clinical studies, a coverage of ≥30 fold will be maintained from the no-effect dose (3mg/kg/day); exposure will not exceed the average daily AUC of 0.448 hr*ug/mL and/or Cmax of 0.049 ug/mL on an individual basis.
Myocardial toxicity	Dogs (4-week study): At 30mg/kg/day, myofiber degeneration/necrosis & inflammation (2 animals) Rats: No myocardial toxicity observed	Subjects with history of acute coronary syndromes (including unstable angina), coronary angioplasty, or stenting within the past 6 months will be excluded. Troponin levels will be monitored throughout the study. A safety margin of ≥50 fold will be maintained from the no-effect dose (10mg/kg/day) observed in dogs.
Mortality/moribund condition; poor viability	Dogs (4-week study): At 30 mg/kg/day one male terminated early (Day 6) due to poor clinical condition. Another male had transient whole body shaking on Days 8 and 9. Dogs (13-week study): At 10 mg/kg/day one male was terminated early (Day 74) due to welfare reasons. Rats (micronucleus and comet study): mortality occurred following 1 to 3 doses at ≥600mg/kg/day	Weight and adverse events reported by subjects will be monitored. .

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Gastrointestinal and/or hepatic toxicity	GI toxicity - ≥3mg/kg/day in dogs and at 30 and 300mg/kg/day in rats, consisting of mucosal erosion/ulceration in the stomach and/or duodenum. Hepatic Toxicity: Biliary epithelial hypertrophy/hyperplasia and periductal mixed inflammatory cell infiltrate into the liver was observed at 300mg/kg/day in rat (7-day study) and focal hepatocellular degeneration in 1 male dog at 30mg/kg/day (4-week study)	Subjects with active ulcer disease or GI bleeding will be excluded. Subjects will be monitored for GI intolerance and sequential clinical chemistry analysis including liver enzymes.

REVISED TEXT

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) GSK2798745		
Vascular lesions	Dogs (4-week study): At 30mg/kg/day, 2 males had arterial lesions • One male: Heart – Coronary artery inflammation; Thymus – Arteriole inflammation with fibroplasia • One male: Epididymides – Artery degeneration/necrosis with inflammation Dogs (12-week study): At 10mg/kg/day, 1 male and 1 female had arterial lesions • One male: Epididymides – Arteriole degeneration/necrosis with lymphocytic inflammation • One female: Bladder – Arteriole degeneration/necrosis	The arterial lesions noted in heart, thymus, epididymides, and urinary bladder cannot be monitored directly. There is currently no human translation biomarker or understanding of the underlying mechanism. Since these effects cannot be monitored directly in clinical studies, a coverage of ≥30 fold will be maintained from the no-effect dose (3mg/kg/day); exposure will not exceed the average daily AUC of 0.448 hr*ug/mL and/or Cmax of 0.049 ug/mL on an individual basis.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	with lymphocytic inflammation ~~**Rats: No vascular lesions observed**~~	
Myocardial toxicity	Dogs (4-week study): At 30mg/kg/day, myofiber degeneration/necrosis & inflammation (2 animals) ~~**Rats: No myocardial toxicity observed**~~	Subjects with history of acute coronary syndromes (including unstable angina), coronary angioplasty, or stenting within the past 6 months will be excluded. Troponin levels will be monitored throughout the study. A safety margin of ≥50 fold will be maintained from the no-effect dose (10mg/kg/day) observed in dogs.
Mortality/moribund condition; poor viability	Dogs (4-week study): At 30 mg/kg/day one male terminated early (Day 6) due to poor clinical condition. Another male had transient whole body shaking on Days 8 and 9. Dogs (13-week study): At 10 mg/kg/day one male was terminated early (Day 74) due to welfare reasons. Rats (micronucleus and comet study): mortality occurred following 1 to 3 doses at ≥600mg/kg/day	Weight and adverse events reported by subjects will be monitored. .

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Gastrointestinal and/or hepatic toxicity	GI toxicity - ≥3mg/kg/day in dogs and at 30 and 300mg/kg/day in rats, consisting of mucosal erosion/ulceration in the stomach and/or duodenum. Hepatic Toxicity: Biliary epithelial hypertrophy/hyperplasia and periductal mixed inflammatory cell infiltrate into the liver was observed at 300mg/kg/day in rat (7-day study) and focal hepatocellular degeneration in 1 male dog at 30mg/kg/day (4-week study)	Subjects with active ulcer disease or GI bleeding will be excluded. **Assessment of fecal occult blood will be performed prior to Day 1 of the first study period and following Day 7 of each study period.** Subjects will be monitored for GI intolerance and sequential clinical chemistry analysis including liver enzymes.

Genetic deletion of TRPV4 in mice has also been shown to alter bone metabolism. Specifically, congenital TRPV4 KO mice displayed impaired bone resorption due to decreased osteoclast number and activity; osteoblast and osteocyte numbers were not affected [Masuyama, 2008; Mizoguchi, 2008; van der Eerden, 2013]. These changes in osteoclast function resulted in increased bone mass and intracortical porosity along with reduced bone elasticity and hind-limb unloading-induced bone loss. Consistent with a role for TRPV4 in modulating osteoclast function, TRPV4 KO mice exhibited altered serum Collagen Type I C-Telopeptide (CTX) and urinary deoxypyridinoline (DPD), specific markers of bone resorption, while no changes in procollagen type 1 amino-terminal propeptide (P1NP), a specific marker of bone formation, were observed. In addition, no differences in serum calcium or parathyroid hormone (PTH) were noted, suggesting no major changes in external calcium balance. Consistent with these latter findings, rats treated for 7 days with the TRPV4 inhibitor GSK2193874 exhibited no changes in plasma calcium or urinary calcium excretion [Thorneloe, 2012]. Osteoclast function, however, has not been assessed in any drug-induced model. As a result of the findings in TRPV4 KO mice, associations between genetic variants in the TRPV4 gene locus with fracture risk were assessed. Whereas risk of osteoporotic fracture was 1.9 times higher in men homozygous for an intronic SNP in the TRPV4 gene (T-allele of rs1861809) in the Rotterdam Study population, these results were not replicated in other cohorts and no trends were observed for women [van der Eerden, 2013]. In spite of the low risk that GSK2798745 will alter bone metabolism in mature adult patients, fasted serum CTX will be assessed at baseline and on Day 7 of each study period.

Section 5.1 Inclusion Criteria

ADDED TEXT

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
9. Diagnosis of heart failure **(New York Heart Association Class II-IV)** for a minimum of 3 months prior to screening 10. Clinically stable with no changes in optimized guidance-directed medications and no hospitalizations for heart failure for at least 1 month prior to Screening 11. NT-proBNP >1000 pg/mL measured within 6 months prior to **OR at** Screening 12. Average DLco measurements outside the normal range (% Predicted DLco < 80%) during the Screening Period

Section 6.9.1 Monitoring in the Clinical Research Unit

ADDED TEXT

The first six subjects who participate in the study will be admitted to the Clinical Research Unit to be monitored for the first two days (Day 1 and Day 2) following the first administration of study medication in both study periods. Subsequently, these subjects will remain in the unit for a minimum of 4 hours after the administration of study medication on each of the remaining 5 days (Days 3 through 7) of each study period. **On Day 5, subjects will remain in the unit for a minimum of 4 hours after the completion of the saline infusion.**

Section 7.1 Time and Events Table

PREVIOUS TEXT

Procedure	Screening Period	Treatment Periods I and II (Day)								Follow-up
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	14 ± 4 days after last dose of study medication
Informed consent	X									
Inclusion and exclusion criteria	X									
Demography	X									
Full physical examination including height and weight	X									
Brief physical examination including weight		X				X			X	X
Medical history/Medication history	X									
Hepatitis B and Hepatitis C screen	X									
Echocardiogram	X									
Urine drug and alcohol breath test	X	X								
Clinical laboratory assessments	X	X				X			X	X
NT-proBNP	X	X							X	
Pharmacokinetic sample[1]		X	X	X	X	X	X	X	X	
Digoxin sample[2]		X	X	X	X	X	X	X	X	X

Procedure	**Screening Period**	**Treatment Periods I and II (Day)**								**Follow-up**
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	14 ± 4 days after last dose of study medication
Troponin	X	X		X		X			X	X
DLco/DLno[10]	X	X				X		X	X	X
12-lead ECG/ time and frequency domains[3]	X[9]	X[9]				X			X	X
Vital signs	X	X	X	X	X	X	X	X	X	X
Weight			X	X	X		X	X		
Genetic sample			For subjects who provide consent, sample collected after start of treatment							
Study treatment[4]			X	X	X	X	X	X	X	
AE/SAE review		X	←==========						==========→	X
Concomitant medication review		X	←==========						==========→	X
Pulmonary function tests		X				X			X	X
Step exercise test/breath-by-breath gas exchange		X							X	X
Dyspnea assessment		X							X	X
SF-36 Acute		X							X	X
Polysomnography including oximetry (Sleep Apnea Clinic)[5]		←==========						←==========		
Plasma and 8-hour urine catecholamines[6]		X						X		
Saline infusion								X		
Admission to CRU[7]			X							
Discharge from CRU[7]					X					

Procedure	Screening Period	Treatment Periods I and II (Day)								Follow-up
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	14 ± 4 days after last dose of study medication
Meals[8]		X	X	X	X	X	X	X	X	
Appetite Assessment (SNAQ)		X				X			X	X
CSSR (Suicidality Assessment)		X							X	X
Audiometry		X							X	X
BodyGuardian Monitor (Preventice)		←==========Continuous==========→								

1. Pharmacokinetic samples to determine GSK2798745 and any metabolite(s) concentrations will be obtained at the following times on Day 1 of each treatment period: Predose, 0.5, 1, 1.5, 2, 3, 5, 8 and 12 hours and at the following times on Day 7 of each treatment period: Predose, 0.5, 1, 1.5, 2, 3 and 5 hours. On Day 2 of each treatment period, samples will be collected predose and at 12 hours post dose. On days 3 through 6, PK samples will be collected predose. The time points may be modified based on emerging data. Some of these timepoints may also be utilized to collect samples to determine concentrations of other medications (e.g., atorvastatin and/or simvastatin). See the SRM for detailed information.
2. Digoxin concentrations will be monitored only in those subjects who are taking digoxin
3. Time and frequency domains assessed by ECG obtained on Day -1 and Day 7
4. Subjects will remain in the CRU for a minimum of 4 hours after each administration of study medication. [Note: The first 6 subjects enrolled will remain in the CRU for 52 hours after the first dose of study medication in each period]
5. Only for subjects participating in the sub-study: At Day -1 of the first study period only, subjects will undergo an overnight sleep study. Only those subjects who are participating in the sub-study with sleep disordered breathing will undergo additional sleep studies on Day 6 of both study Periods I and II.
6. Only for subjects participating in the sub-study: Urinary norepinephrine (NE) and epinephrine (E) concentrations will be determined from 8-hour collections obtained during the overnight sleep study; urinary creatinine will also be measured to normalize the NE and E concentrations. A blood sample to determine plasma concentrations of NE and E will be obtained the morning after the sleep study between the hours of 9:00 am and 10:00 am after the subject has been supine for 20 minutes in a quiet room.
7. The first 6 subjects enrolled will remain in the CRU for two days (Day 1 and Day 2) after the first dose of study medication in each period.
8. Three meals per day will be provided by the dietary department either in the CRU or as a boxed meal(s) to take home.
9. ECGs to be performed in triplicate
10. On the days where exercise or the saline infusion is performed, DLco/DLno will be completed just prior to and after both exercise and the saline infusion.

REVISED TEXT

Procedure	**Screening Period**	**Treatment Periods I and II (Day)**										**Follow-up**
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	**8**	**9**	14 ± 4 days after last dose of study medication
Informed consent	X											
Inclusion and exclusion criteria	X											
Demography	X											
Full physical examination including height & weight	X											
Brief physical exam including weight		X				X			X			X
Medical history/Medication history	X											
Hepatitis B & C screen	X											
Echocardiogram	X											
Urine drug and alcohol breath test	X	X										
Clinical lab assessments[14]	X	X				X			X			X
NT-proBNP	X	X							X			
Serum CTX[11]		**X**							**X**			
Fecal Occult Blood Test[12]	**X**								**X**			
Pharmacokinetic sample[1]		X	X	X	X	X	X	X	X	**X**	**X**	
Digoxin sample[2]		X	X	X	X	X	X	X	X			X
Troponin	X	X		X		X			X			X
DLco/DLno[10]	X	X				X	**X**	~~**X**~~	X			X
12-lead ECG/ time & frequency domains[3]	X[9]	X[9]				X			X			X

Procedure	**Screening Period**	**Treatment Periods I and II (Day)**										**Follow-up**
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	**8**	**9**	14 ± 4 days after last dose of study medication
Vital signs	X	X	X	X	X	X	X	X	X			X
Weight			X	X	X		X	X				
Genetic sample		For subjects who provide consent, sample collected after start of treatment										
Study treatment[4]			X	X	X	X	X	X	X			
AE/SAE review		X	←==========→									X
Concomitant med review		X	←==========→									X
Pulmonary function tests		X				X			X			X
Step exercise test/breath-by-breath gas exchange		X							X			X
Dyspnea assessment		X					X		X			X
Orthopnea							X					
SF-36 Acute		X							X			X
Polysomnography including oximetry (Sleep Apnea Clinic)[5,13]		←==========→						←==========→				
Plasma and 8-hour urine catecholamines[6]		X						X				
Saline infusion							**X**	X				
Admission to CRU[7]			X									
Discharge from CRU[7]					X							
Meals[8]		X	X	X	X	X	X	X	X			
Appetite Assessment (SNAQ)		X				X			X			X
CSSRS (Suicidality)		X							X			X
Audiometry		X[13]							X			X
BodyGuardian Monitor (Preventice)		←==========Continuous==========→										

1. Pharmacokinetic samples to determine GSK2798745 and any metabolite(s) concentrations will be obtained at the following times on Day 1 of each treatment period: Predose, 0.5, 1, 1.5, 2, 3, 5, 8 and 12 hours and at the following times on Day 7 of each treatment period: Predose, 0.5, 1, 1.5, 2, 3, ~~and~~ 5 **and 10** hours. On Day 2 of each treatment period, samples will be collected predose and at 12 hours post dose. On Days 3 through 6, PK samples will be collected predose. **On Days 8 and 9, samples will be collected at 24 and 48 hours, respectively, after the last dose of study medication administered on Day 7.** The time points may be modified based on emerging data. Some of these timepoints may also be utilized to collect samples to determine concentrations of other medications (e.g., atorvastatin and/or simvastatin). See the SRM for detailed information.
2. Digoxin concentrations will be monitored only in those subjects who are taking digoxin
3. Time and frequency domains assessed by ECG obtained on Day -1 and Day 7
4. Subjects will remain in the CRU for a minimum of 4 hours after each administration of study medication. [Note: The first 6 subjects enrolled will remain in the CRU for 52 hours after the first dose of study medication in each period]
5. Only for subjects participating in the sub-study: At Day -1 of the first study period only, subjects will undergo an overnight sleep study. Only those subjects who are participating in the sub-study with sleep disordered breathing will undergo additional sleep studies on Day 6 of both study Periods I and II.
6. Only for subjects participating in the sub-study: Urinary norepinephrine (NE) and epinephrine (E) concentrations will be determined from 8-hour collections obtained during the overnight sleep study; urinary creatinine will also be measured to normalize the NE and E concentrations. A blood sample to determine plasma concentrations of NE and E will be obtained the morning after the sleep study between the hours of 9:00 am and 10:00 am after the subject has been supine for 20 minutes in a quiet room.
7. The first 6 subjects enrolled will remain in the CRU for two days (Day 1 and Day 2) after the first dose of study medication in each period.
8. Three meals per day will be provided by the dietary department either in the CRU or as a boxed meal(s) to take home.
9. ECGs to be performed in triplicate
10. On the days where exercise or the saline infusion is performed, DLco/DLno will be completed just prior to and after both exercise and the saline infusion.
11. **Blood sample for analysis of Serum CTX must be collected when the subject is fasting. See Section 7.4.9.1**
12. **Fecal Occult Blood Test (FOBT) cards will be provided to subjects at the end of the screening visit and must be completed and sent back to the laboratory prior to the baseline visit according to the laboratory's standard collection procedures. Similarly, subjects will be given FOBT cards at the end of the Day 7 visit of each study period with completion instructions. See Section 7.4.9.2**
13. **Day -1 audiometry and sleep study assessments may be completed 7 days prior to Day -1.**
14. **Clinical laboratory assessments will be collected as a fasting sample.**

Section 7.3.3 Intravenous Saline Infusion

PREVIOUS TEXT

In patients with chronic HF, intravenously infused saline elicited a reduction of D_M [Puri, 1999] suggesting that the abnormal pulmonary diffusion in this population may have a variable component that could be amenable to therapeutic intervention. Similarly, an infusion of saline into the pulmonary artery significantly reduced both DLco and D_M in patients with chronic HF but not in control subjects [Guazzi, 1999]; the changes in DM were inversely related to VE/VCO2 [Guazzi, 2001]. The hemodynamic effects of volume expansion with saline loading has been shown to be associated with increases in left and right heart filling pressures and pulmonary artery pressures in patients with HF [Andersen, 2015]. This intervention has not been associated with any significant clinical safety signal in stable HF patients.

All subjects will undergo an intravenous 0.9% NaCl infusion on Day 6 of both study periods in the presence of a cardiologist and only if determined by the Investigator that subject is without any signs of obvious congestion. Subjects will be carefully monitored both during and after the infusion and will be provided intravenous diuretics if necessary. Details of this procedure are described in the SRM. DLco and DLno measurements will be obtained prior to the initiation of the infusion and after the completion of the infusion.

REVISED TEXT

In patients with chronic HF, intravenously infused saline elicited a reduction of D_M [Puri, 1999] suggesting that the abnormal pulmonary diffusion in this population may have a variable component that could be amenable to therapeutic intervention. Similarly, an infusion of saline into the pulmonary artery significantly reduced both DLco and D_M in patients with chronic HF but not in control subjects [Guazzi, 1999]; the changes in DM were inversely related to VE/VCO2 [Guazzi, 2001]. The hemodynamic effects of volume expansion with saline loading has been shown to be associated with increases in left and right heart filling pressures and pulmonary artery pressures in patients with HF [Andersen, 2015]. **In this latter study, no patient developed dyspnea or any evidence of pulmonary congestion despite increase in cardiac filling pressures.** ~~**This intervention has not been associated with any significant clinical safety signal in stable HF patients.**~~

All subjects will undergo an intravenous 0.9% NaCl infusion on Day **5** ~~**6**~~ of both study periods in the presence of a cardiologist **specializing in heart failure**. ~~**and**~~ **The procedure will be conducted** only if ~~**determined by the Investigator**~~ the subject is without any signs of obvious congestion **as determined by the Investigator**. Subjects will be carefully monitored both during and after the infusion and will be provided intravenous diuretics if necessary. Details of this procedure are described in **Appendix 9** ~~**the SRM**~~.

DLco and DLno measurements will be obtained prior to the initiation of the infusion and after the completion of the infusion.

Section 7.4.9 Clinical Laboratory Assessments

PREVIOUS TEXT

All protocol required laboratory assessments, as defined in Table 3, must be conducted in accordance with the Laboratory Manual, and Protocol Time and Events Schedule. Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/centre number, and visit date. Details for the preparation and shipment of samples will be provided by the laboratory and are detailed in the SRM. Reference ranges for all safety parameters will be provided to the site by the laboratory responsible for the assessments.

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification) the results must be recorded in the CRF.

Refer to the SRM for appropriate processing and handling of samples to avoid duplicate and/or additional blood draws.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 3.

Table 3 Clinical Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet Count		*RBC Indices*:	*WBC count with Differential:*
	RBC Count		MCV	Neutrophils
	Hemoglobin		MCH	Lymphocytes
	Hematocrit		MCHC	Monocytes
	WBC Count (absolute)			Eosinophils
	Reticulocyte count			Basophils
Clinical Chemistry	BUN	Potassium	AST (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	ALT (SGPT)	Total Protein
	Glucose, fasting	Calcium	Alkaline phosphatise	Albumin
	CPK	Uric Acid	GGT	Troponin
	Chloride			
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal) • Urinary norepinephrine and epinephrine (during sleep study)			
Other Screening	• Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Digoxin concentrations (only in subjects treated with Digoxin)			

Laboratory Assessments	**Parameters**
Tests	• Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • HMG CoA Reductase Inhibitor concentration (only in subjects taking medication)
Biomarker(s)/ Other Assessments	• N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) • Norepinephrine and Epinephrine
NOTES : Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 5.5.1 and Appendix 2	

REVISED TEXT

All protocol required laboratory assessments, as defined in Table 3, must be conducted in accordance with the Laboratory Manual **(if applicable),** and Protocol Time and Events Schedule. **All laboratory assessments will be collected as fasting samples.** Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/centre number, and visit date. Details for the preparation and shipment of samples **(if applicable)** will be provided by the laboratory and are detailed in the SRM. Reference ranges for all safety parameters will be provided to the **study staff** ~~**site**~~ by the **local** laboratory ~~**responsible for the assessments**~~.

If additional non-protocol specified laboratory assessments are performed at the institution's local laboratory and result in a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification) the results must be recorded in the CRF.

Refer to the SRM for appropriate processing and handling of samples to avoid duplicate and/or additional blood draws.

Haematology, clinical chemistry, urinalysis and additional parameters to be tested are listed in Table 3.

Table 3 Clinical Laboratory Assessments

Laboratory Assessments	**Parameters**			
Hematology	Platelet Count	*RBC Indices*:	*WBC count with Differential*:	
	RBC Count	MCV	Neutrophils	
	Hemoglobin	MCH	Lymphocytes	
	Hematocrit	MCHC	Monocytes	
	WBC Count (absolute)		Eosinophils	
	Reticulocyte count		Basophils	
Clinical Chemistry	BUN	Potassium	AST (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	ALT (SGPT)	Total Protein
	Glucose, fasting	Calcium	Alkaline phosphatise	Albumin
	CPK	Uric Acid	GGT	Troponin
	Chloride			
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal) • Urinary norepinephrine and epinephrine (during sleep study)			
Other Screening Tests	• Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Digoxin concentrations (only in subjects treated with Digoxin) • Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • HMG CoA Reductase Inhibitor concentration (only in subjects taking medication)			
Biomarker(s)/ Other Assessments	• N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) • Norepinephrine and Epinephrine • **Serum Collagen Type I C-Telopeptide (CTX), fasting** • **Fecal Occult Blood Test (FOBT)**			
NOTES : Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 5.5.1 and Appendix 2				

ADDED SECTIONS

Section 7.4.9.1 Serum Collagen Type I Telopeptide (CTX)

Blood samples will be collected at the Baseline Visit (Day -1) and on Day 7 of each study period. Approximately 1 mL will be collected as a fasting sample from the subject at the start of each of these visits.

Section 7.4.9.2 Fecal Occult Blood Test (FOBT)

Based on the gastric erosions found in a preclinical model (dogs at ≥3 mg/kg), FOBT will be performed to assess any possible drug-related GI blood loss. Due to concomitant anti-platelet therapy that many patients with heart failure receive, along with the common incidence of intestinal mucosal edema and associated diarrhea, findings of fecal blood would not be unexpected in this population. Subjects will be tested for the presence of fecal occult blood prior to the start of study period I (between Screening and Baseline) and after each of the two study periods.

Once subjects have signed the consent form and have been enrolled into the study based on meeting the eligibility criteria, they will be provided 3 FOBT cards at the end of the screening visit. Study staff will provide the subjects with instructions for completing the tests and subsequent shipment of the tests back to the laboratory. This assessment will be completed between Screening and Baseline (Day -1). Additionally, subjects will be provided 3 FOBT cards on Day 7 of each study period with the same instructions for completion and shipment for laboratory analysis.

Section 7.5.2 Sample Analysis

PREVIOUS TEXT

Once the plasma has been analyzed for GSK2798745 any remaining plasma may be analyzed for other compound-related metabolites and the results reported under a separate PTS-DMPK GlaxoSmithKline protocol.

REVISED TEXT

Plasma samples may be analyzed for metabolite M1 dependent upon the availability of an assay. Until this time, GSK will store the remaining plasma from the PK plasma samples for future possible metabolite analysis. ~~Once the plasma has been analyzed for GSK2798745 any remaining plasma may be analyzed for other compound-related metabolites and the results reported under a separate PTS-DMPK GlaxoSmithKline protocol.~~

Section 11 References

ADDED TEXT

Fujimoto N, Borlaug BA, Gregory D. Lewis GD, Hastings JL, Shafer KM, Bhella PS, Carrick-Ranson G, Levine BD. Hemodynamic responses to rapid saline loading: the impact of age, sex, and heart failure. Circulation. 2013;**127(1):55-62.**

Masuyama R, Vriens J, Voets T, Karashima Y, Owsianik G, Vennekens R, et al. TRPV4-mediated calcium influx regulates terminal differentiation of osteoclasts. Cell Metab. 2008;8:257-65.

Mizoguchi F, Mizuno A, Hayata T, Nakashima K, Heller S, Ushida T, et al. Transient receptor potential vanilloid 4 deficiency suppresses unloading-induced bone loss. J Cell Physiol. 2008;216:47-53.

Robbins IM, Hemnes AR, ME, Brittain EL, Zhao DX, Piana RN, Fong PP, Newman JH. High prevalence of occult pulmonary venous hypertension revealed by fluid challenge in pulmonary hypertension. Circ Heart Fail. 2014;7(1):116-22.

Synder EM, Beck KC, Hulsebus ML, Breen JF, Hoffman EA, Johnson BJ. Short-term hypoxic exposure at rest and during exercise reduces lung water in healthy humans. J Appl Physiol. 2006; 101: 1623–1632.

van der Eerden BC, Oei L, Roschger P, Fratzl-Zelman N, Hoenderop JG, van Schoor NM, et al. TRPV4 deficiency causes sexual dimorphism in bone metabolism and osteoporotic fracture risk. Bone. 2013;57:443-54.

ADDED APPENDIX

APPENDIX 9: Intravenous Saline Infusion

Rapid intravenous saline infusion has been employed to elicit fluid extravasation from the vasculature to the pulmonary interstitium. This methodology has been previously utilized in studies with healthy volunteers [Snyder, 2006] and patients with heart failure [Fujimoto, 2013; Robbins, 2014; Andersen, 2015].

Subject Evaluation Prior to Infusion

On Day 5 of both study periods, the subject will be admitted to the clinical research unit (CRU) and undergo routine weight measurement and vital sign assessments including temperature, systolic and diastolic blood pressures, heart rate, respiratory rate, and pulse oximetry to assess O_2 saturation. The subject will also undergo a pulmonary examination.

While in a sitting position, a dyspnea assessment, utilizing a standardized 5-point Likert scale, will be obtained to document the subject's current status. A subject, who is not severely or very severely short of breath, will undergo an orthopnea test (placed supine with head ≤ 20 degrees relative to horizontal). After an equilibration

period of 2 minutes, the 5-point scale will be repeated. A subject who reports a worse dyspnea score when supine compared to sitting will be categorized as having orthopnea. Subjects categorized with having orthopnea will not be eligible to participate in the saline infusion.

Study treatment will be administered after all planned pre-dose assessments for Day 5 are completed.

Saline Infusion Procedure

A subject, who is judged by the Investigator to be in a stable condition and without obvious congestion, will then undergo the intravenous saline infusion procedure. This procedure will be conducted in the clinical research unit, which is adjacent to the catheterization laboratory, in the presence of a cardiologist and monitoring personnel. A crash cart will be accessible, and intravenous diuretic will be available, if necessary.

An 18-gauge venous catheter will be inserted into a prominent antecubital vein while the subject is semi-recumbent (approximately 45°). The subject will remain in a semi-recumbent position throughout the procedure.

DLco and DLno measurements and simple spirometry assessments will be obtained prior to the initiation of the infusion.

Prewarmed 0.9% normal saline will be infused intravenously at a rate of 150 mL/min to a total volume of 500 mL. During the saline infusion, blood pressure, heart rate, respiratory rate, and O_2 saturation, will be continuously monitored, and any physical symptoms recorded.

DLco and DLno measurements and spirometry assessments will be obtained after the completion of the infusion.

Post-infusion Monitoring

Following the completion of the saline infusion procedure, the subject will remain in the clinical research unit for the next 4 hours. Blood pressure, heart rate, respiratory rate, and O_2 saturation, will be continuously monitored, and any physical symptoms recorded. The subject will also be assessed for dyspnea and orthopnea, as described above, at hourly intervals.

After the 4-hour period, the subject will be eligible to leave the CRU if heart rate and respiratory rate are within 20% of the baseline value, O_2 saturation is within 2% of the baseline value, and if the dyspnea score is the same as the baseline score.

The subject will continue to be monitored by the Preventice BodyGuardian Remote Monitoring System as described in Section 7.4.7 of the protocol; ECG, heart rate, respiratory rate and activity level will be measured continuously.

Any worsening of heart failure that requires treatment in the 48 hours subsequent to the saline infusion will be reported as a serious adverse event.

Individual Stopping Criteria During the Saline Infusion Procedure

If the subject demonstrates one of the following changes during the saline infusion, the infusion should be discontinued:

- **Decrease in O2 saturation of >4% compared to baseline**
- **Increase in respiratory rate of >20% compared to baseline**
- **Any increase in dyspnea perceived by the patient**

If the subject has met one of these criteria during or immediately after the infusion, the subject should be carefully monitored and given intravenous diuretic treatment, intravenous or sublingual nitrates, or other treatment as required.

The subject may continue in the study. However, if this response occurred in the first study period, the subject should not undergo a second saline infusion in the subsequent study period.

Discontinuation of Saline Infusion Procedure

If during the conduct of the study, two subjects meet the individual stopping criteria during the infusion or demonstrate a worsening of heart failure in the 48 hours after completion of the infusion, this challenge procedure will be removed from the study design. No other subjects will undergo this assessment.

Reporting of Adverse Outcomes Associated with the Saline Infusion

Any of the following outcomes must be reported to the sponsor as a serious adverse event:

- **Treatment with intravenous diuretic subsequent to the saline infusion**
- **Need for continued monitoring in the CRU beyond the 4-hour mandated observation period**
- **Any worsening of heart failure that requires treatment during the 48-hour monitoring period after the saline infusion procedure**

These events will be reported by the investigator as serious adverse events in the InForm electronic data collection tool. They will be reported by the sponsor to the FDA as an Expedited Report via electronic submission in eCTD format to the IND, as a 7-day or 15-day IND safety report, as applicable per timeframe specified in 21 CFR 312.32(c)(1). The completed report will also be provided to the IRB.

Protocol Amendment 2

Summary of Amendment Changes

This amendment incorporates minor changes to eligibility (NT proBNP, BMI).

List of Specific Changes

Title

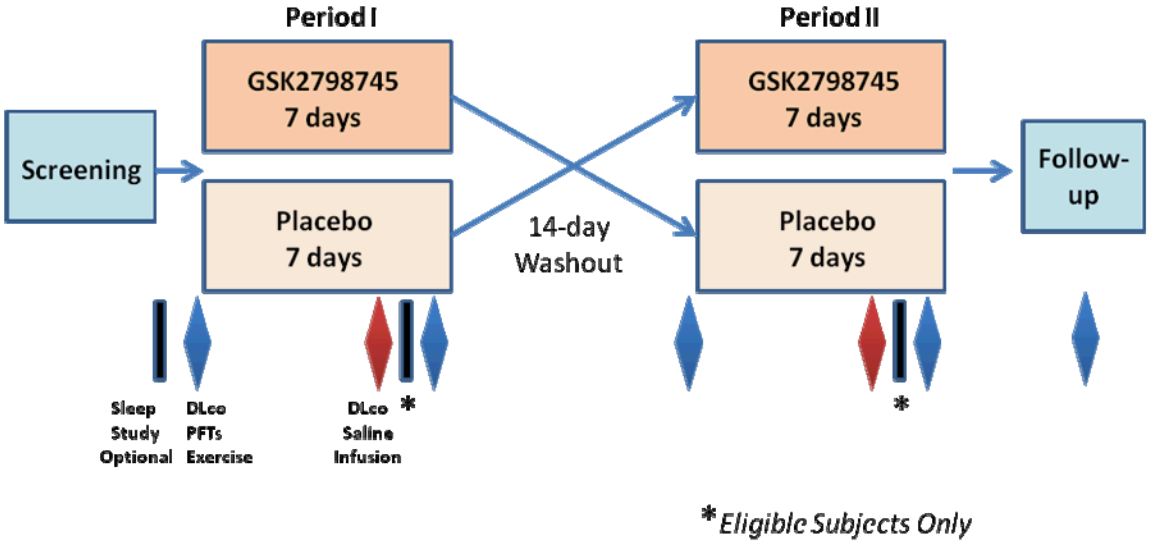
ADDED TEXT

A Randomized, Double-blind, **Sponsor un-blinded,** Placebo-controlled, Phase 2a Crossover Study to Evaluate the Effect of the TRPV4 Channel Blocker, GSK2798745, on Pulmonary Gas Transfer and Respiration in Patients with Congestive Heart Failure

Section 1

PREVIOUS TEXT

Overall Design



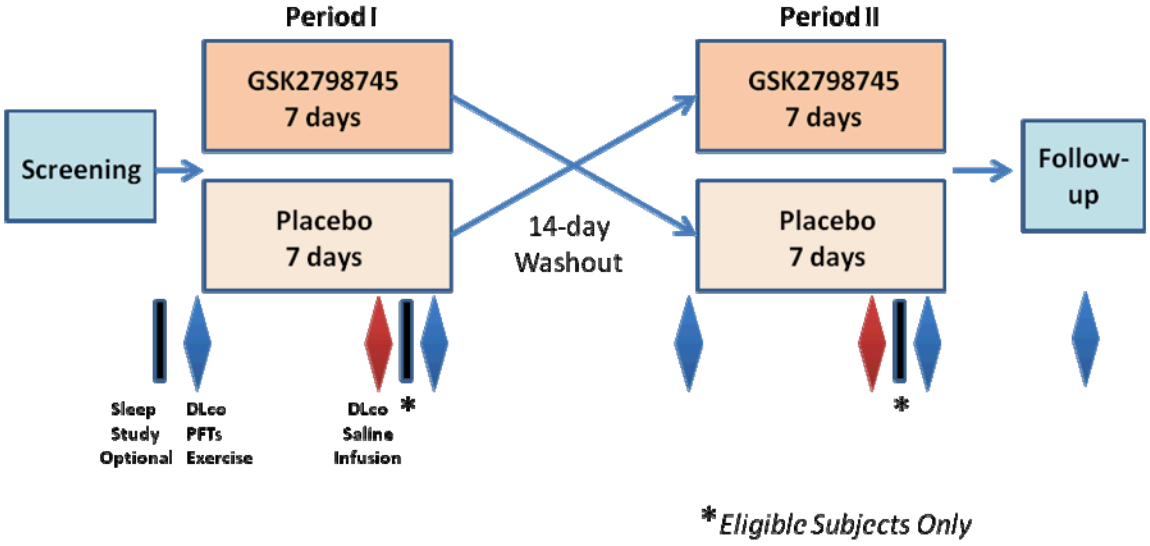
This is a randomized, double-blind, sponsor-unblinded, placebo-controlled, 2 by 2 crossover study in adults with heart failure. The study will be conducted at a single center.

Within 14 days (up to 25 days) of the start of Period I, subjects will begin a screening evaluation to determine eligibility for entry into the study. Eligible subjects will be randomized to one of two treatment sequences. Subjects will receive either GSK2798745 or placebo once daily for a period of 7 days. After at least a 14-day washout period

(preferably not greater than 30 days from last dose in Period I), subjects will participate in a second, identical 7-day study period and receive the alternate study medication.

REVISED TEXT

Overall Design



This is a randomized, double-blind, sponsor-unblinded, placebo-controlled, 2 by 2 crossover study in adults with heart failure. ~~**The study will be conducted at a single center.**~~

~~**Within**~~ **Approximately** 14 days (up to 25 days) ~~**of**~~ **before** the start of Period I, subjects will begin a screening evaluation to determine eligibility for entry into the study. Eligible subjects will be randomized to one of two treatment sequences. Subjects will receive either GSK2798745 **2.4 mg** or placebo once daily for a period of 7 days. After at least a 14-day washout period (preferably not greater than 30 days from last dose in Period I), subjects will participate in a second, identical 7-day ~~**study**~~ **treatment** period and receive the alternate study medication.

Section 4

PREVIOUS TEXT

4.1 Overall Design

This is a randomized, double-blind, sponsor-unblinded, placebo-controlled, 2 by 2 crossover study in adults with heart failure (Figure 1). The study will be conducted at a single center.

REVISED TEXT

4.1 Overall Design

This is a randomized, double-blind, sponsor-unblinded, placebo-controlled, 2 by 2 crossover study in adults with heart failure (Figure 1). ~~**The study will be conducted at a single center.**~~

PREVIOUS TEXT

4.2 Treatment Arms, Duration and Assessments

Within 14 days (up to 25 days) of the start of Period I, subjects will begin a screening evaluation to determine eligibility for entry into the study. Eligible subjects will be randomized to one of two treatment sequences. Subjects will receive either GSK2798745 or placebo once daily for a period of 7 days. After at least a 14-day washout period (preferably not greater than 30 days from last dose in Period I), subjects will participate in a second, identical 7-day study period and receive the alternate study medication.

During both study periods, the first 6 subjects will remain in the clinical research unit (CRU) overnight for the first 2 days (Days 1 and 2) to allow for continuous safety monitoring. Subsequently, subjects will remain in the research unit for a minimum of 4 hours after the administration of study medication (Days 3 through 7). Subjects will be equipped with the Preventice BodyGuardian for continuous remote monitoring of ECG, heart rate, and respiration rate (Day -1 to Day 7).

After the first 6 subjects have completed the two-day, in-house safety monitoring during Periods I and II, subsequent subjects enrolled into the study will remain in the research unit for a minimum of 4 hours after the administration of study medication on all study days (Days 1 through 7).

Prior to the start of each study period, all subjects will undergo an assessment of pulmonary function testing (PFTs), gas transfer measurements including DLco, DLno and Vc, breath-by-breath measurements of minute ventilation, O_2 consumption and VCO_2 production during a metered 3-minute step exercise to establish ventilatory efficiency (V_E/VCO_2), and a dyspnea assessment. Additionally, a measurement of N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) concentration, clinical laboratory tests, patient-reported health status (SF-36 Acute), and general safety assessments will be obtained.

Only for subjects willing to participate in the sleep apnea sub-study, they will undergo a sleep study to ascertain whether sleep-disordered breathing patterns are apparent prior to the <u>first</u> study period (Period I). Subjects with sleep-disordered breathing, defined as an apnea-hypopnea index (AHI) >5 based on polysomnography, will undergo a repeat sleep study after 6 days of treatment in each of the two study periods.

Pharmacokinetic profiles will be obtained during both study periods. On Day 4 and Day 7 of each study period, the assessments of pulmonary function will be repeated. On Day 5 of each study period, subjects will receive an intravenous saline infusion, and DLco and DLno measurements will be performed before and after the procedure. On

Day 7 of each study period, the step-exercise challenge and gas transfer measurements, dyspnea assessment, measurement of NT-proBNP concentration, clinical laboratory tests, patient-reported health status (SF-36 Acute), and general safety assessments will be repeated.

All subjects will return for a Follow-up Visit approximately 2 weeks after completion of the second study period; pulmonary and safety assessments will be repeated at this visit.

The duration of participation in this study is expected to be approximately 8 weeks from Screening to the Follow-up Visit.

REVISED TEXT

4.2 Treatment Arms, Duration and Assessments

~~**Within**~~ **Approximately** 14 days (up to 25 days) ~~**of**~~ **before** the start of Period I, subjects will begin a screening evaluation to determine eligibility for entry into the study. Eligible subjects will be randomized to one of two treatment sequences. Subjects will receive either GSK2798745 **2.4 mg** or placebo once daily for a period of 7 days. After at least a 14-day washout period (preferably not greater than 30 days from last dose in Period I), subjects will participate in a second, identical 7-day study period and receive the alternate study medication.

During both study periods, the first 6 subjects will remain in the ~~**clinical research**~~ unit (**i.e. clinical research unit,** CRU) overnight for the first 2 days (Days 1 and 2) to allow for continuous safety monitoring. Subsequently, **these initial** subjects will remain in the ~~**research**~~ unit for a minimum of 4 hours after the administration of study medication (Days 3 through 7). **All** ~~**S**~~**s**ubjects will be equipped with the Preventice BodyGuardian for continuous remote monitoring of ECG, heart rate, and respiration rate (Day -1 to Day 7). **Subjects may remain in the unit for the duration of each of the 7day treatment periods for convenience.**

After the first 6 **randomized** subjects have completed the two-day, in-house safety monitoring during Periods I and II, subsequent subjects enrolled into the study will remain in the ~~**research**~~ unit for a minimum of 4 hours after the administration of study medication on all study days (Days 1 through 7). **However, subjects may remain in the unit for the duration of each of the 7 day treatment periods as determined on a case by case basis with the Investigator(s). This is not a requirement for successful completion of the study, but is mainly for purposes of subject convenience only.**

Prior to the start of each study period, all subjects will undergo an assessment of pulmonary function testing (PFTs), gas transfer measurements including DLco, DLno and Vc, breath-by-breath measurements of minute ventilation, O_2 consumption and VCO_2 production during a metered 3-minute step exercise to establish ventilatory efficiency (V_E/VCO_2), and a dyspnea assessment. Additionally, a measurement of N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) concentration, clinical laboratory tests, patient-reported health status (SF-36 Acute), and general safety assessments will be obtained. Only for subjects willing to participate in the sleep apnea sub-study, they will undergo a sleep study to ascertain whether sleep-disordered breathing patterns are apparent prior to

the first study period (Period I). Subjects with sleep-disordered breathing, defined as an apnea-hypopnea index (AHI) >5 based on polysomnography, will undergo a repeat sleep study after 6 days of treatment in each of the two study periods.

Pharmacokinetic profiles will be obtained during both study periods. On Day 4 and Day 7 of each study period, the assessments of pulmonary function will be repeated. On Day 5 of each study period, subjects will receive an intravenous saline infusion, and DLco and DLno measurements will be performed before and after the procedure. **On Day 5 of each study period, subjects will remain in the unit for a minimum of 4 hours after completion of the intravenous saline infusion.** On Day 7 of each study period, the step-exercise challenge and gas transfer measurements, dyspnea assessment, measurement of NT-proBNP concentration, clinical laboratory tests, patient-reported health status (SF-36 Acute), and general safety assessments will be repeated.

All subjects will return for a Follow-up Visit approximately 2 weeks after completion of the second study period; pulmonary and safety assessments will be repeated at this visit.

The duration of participation in this study is expected to be approximately 8 weeks from Screening to the Follow-up Visit.

PREVIOUS TEXT

4.3 Type and Number of Subjects

A sufficient number of subjects with heart failure will be enrolled so that 12 subjects complete the two study periods and critical assessments. If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigator.

After the first 12 subjects complete both study periods, an interim analysis will be performed to assess the intra-subject variability: (a) if the actual variability is less than or equal to the assumed value, the study will be completed with 12 subjects; or (b) if the actual variability is greater than the assumed value, additional subjects may need to be enrolled.

If an insufficient number of subjects participate in the sleep sub-study, additional subjects, who meet the criterion for the sleep apnea sub-study, may be enrolled to undergo the polysomnography investigation at the discretion of the Sponsor and in consultation with the Investigator.

In addition, another (separate) cohort of subjects may be enrolled to allow for the evaluation of an additional dose level at the discretion of the Sponsor in consultation with the Investigator.

REVISED TEXT

4.3 Type and Number of Subjects

A sufficient number of subjects with heart failure will be enrolled so that 12 subjects complete the two study periods and critical assessments. If subjects prematurely discontinue the study, additional replacement subjects may be randomised and assigned to the same treatment sequence at the discretion of the Sponsor in consultation with the Investigators.

After the first 12 subjects complete both study periods, an interim analysis will be performed to assess the intra-subject variability: (a) if the actual variability is less than or equal to the assumed value, the study will be completed with 12 subjects; or (b) if the actual variability is greater than the assumed value, additional subjects may need to be enrolled.

If an insufficient number of subjects participate in the sleep sub-study, additional subjects~~,~~ who meet the criterion for the sleep apnea sub-study~~,~~ may be enrolled to undergo the polysomnography investigation at the discretion of the Sponsor and in consultation with the Investigator**s**.

In addition, another (separate) cohort of subjects may be enrolled to allow for the evaluation of an additional dose level at the discretion of the Sponsor in consultation with the Investigator**s**.

PREVIOUS TEXT

4.4 Design Justification

The crossover design has been selected to provide greater statistical power with a smaller number of subjects. Each subject serves as his or her own matched control avoiding issues of comparability of groups with regard to confounding variables such as age, sex, and disease severity. The use of a placebo control allows a more rigorous assessment of the effects of the drug treatment on physiologic measures and symptom assessments. Subjects selected for this study have chronic heart failure and are stable on standard treatment to ensure that their disease status does not change significantly during the course of the study. Additionally, the limited 7-day treatment period is not expected to alter the disease status of the subjects. The 14-day washout period alleviates the possibility of carryover effects from one period to the other. Given the short duration of the study, it is expected that subjects will complete the study in order to have data in each period of the crossover for all subjects.

REVISED TEXT

4.4 Design Justification

The crossover design has been selected to provide greater statistical power with a smaller number of subjects. Each subject serves as his or her own matched control avoiding issues of comparability of groups with regard to confounding variables such as age, sex,

and disease severity. The use of a placebo control allows **for** a more rigorous assessment of the effects of the drug treatment on physiologic measures and symptom assessments. Subjects selected for this study have chronic heart failure and are stable on standard treatment to ensure that their disease status does not change significantly during the course of the study. Additionally, the limited 7-day treatment period is not expected to alter the disease status of the subjects. The 14-day washout period alleviates the possibility of carryover effects from one period to the other. Given the short duration of the study, it is expected that subjects will complete the study in order to have data in each period of the crossover for all subjects.

Section 5

PREVIOUS TEXT

5.1 Inclusion Criteria

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

AGE
13. ≥ 21 years of age at the time of signing the informed consent form

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
14. Diagnosis of heart failure (New York Heart Association Class II-IV) for a minimum of 3 months prior to screening
15. Clinically stable with no changes in optimized guidance-directed medications and no hospitalizations for heart failure for at least 1 month prior to Screening
16. NT-proBNP >1000 pg/mL measured within 6 months prior to OR at Screening
17. Average DLco measurements outside the normal range (% Predicted DLco < 80%) during the Screening Period

WEIGHT
18. Body mass index (BMI) ≥18 and ≤40 kg/m^2

SEX
19. Male or female of non-childbearing potential ➢ Male patients with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives after the last dose of study medication. h. Vasectomy with documentation of azoospermia. i. Male condom plus partner use of one of the contraceptive options below: • Contraceptive subdermal implant that meets the standard operating procedure (SOP) effectiveness criteria including a <1% rate of failure per year, as stated in the product label • Intrauterine device or intrauterine system that meets the SOP effectiveness criteria including a <1% rate of failure per year, as stated in the product label [Hatcher, 2007a] • Oral contraceptive, either combined or progestogen alone [Hatcher, 2007a] • Injectable progestogen [Hatcher, 2007a] • Contraceptive vaginal ring [Hatcher, 2007a] • Percutaneous contraceptive patches [Hatcher, 2007a] These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception. ➢ A female subject is eligible to participate if at least one of the following conditions applies: c. Non-reproductive potential defined as: • Pre-menopausal females with one of the following: • Documented tubal ligation • Documented hysteroscopic tubal occlusion procedure with follow-up confirmation of bilateral tubal occlusion • Hysterectomy • Documented bilateral oophorectomy d. Postmenopausal defined as 12 months of spontaneous amenorrhea. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment.

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

5.2 Exclusion Criteria

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

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
23. History of acute coronary syndromes including unstable angina or myocardial infarction within 6 months of screening
24. Coronary revascularization including angioplasty and stenting within 6 months of Screening
25. History of stroke or seizure disorder within 5 years of Screening
26. Diagnosis of asthma
27. Diagnosis of chronic obstructive pulmonary disease (COPD) with FEV_1 <50% of predicted measured within 4 weeks of Screening
28. History of a condition that required radiation therapy to the thorax
29. History of any type of malignancy within the past five years with the exception of successfully treated basal cell cancer of the skin
30. Active ulcer disease or gastrointestinal bleeding
31. Current or chronic history of liver disease, known hepatic impairment, or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones)
32. Alanine transaminase (ALT) >2x Upper Limit of Normal (ULN) and bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%)
33. QTc > 450 msec or QTc > 480 msec in subjects with Bundle Branch Block • The QTc is the QT interval corrected for heart rate according to Bazett's formula (QTcB), Fridericia's formula (QTcF), and/or another method, machine-read or manually over-read. • The *same* QT correction formula *must* be used for *each individual subject* throughout the study. This formula may not be changed or substituted once the subject has been enrolled. • For purposes of data analysis, QTcB, QTcF, another QT correction formula, or a composite of available values of QTc will be used as specified in the Reporting and Analysis Plan (RAP).
34. History or current evidence of any serious or clinically significant gastrointestinal, renal, endocrine, neurologic, hematologic or other condition that is uncontrolled on

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
permitted therapies or that would, in the opinion of the investigator or the GlaxoSmithKline (GSK) medical monitor, make the subject unsuitable for inclusion in this study

CONCOMITANT MEDICATIONS
35. Use of medications specified for the treatment of COPD including short- and long-acting bronchodilators (β-agonists and anticholinergics) and inhaled glucocorticoids as well as oxygen therapy 36. Use of a listed prohibited medication (Section 6.10.2) within the restricted timeframe relative to the first dose of study medication 37. Use of a strong inhibitors or inducers of cytochrome P450 (CYP) 3A or p-glycoprotein (see Section 6.10.2)

RELEVANT HABITS
38. Current smoker 39. History of drug/substance abuse within the past 2 years 40. History of alcohol abuse within 6 months of the study. Defined as an average weekly alcohol consumption of >14 drinks for men or >7 drinks for women. One drink is equivalent to 12 g of alcohol: approximately 12 ounces (360 mL) of beer, 5 ounces (150 mL) of wine, or 1.5 ounces of (45 mL) 80 proof distilled spirits

CONTRAINDICATIONS
41. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or Medical Monitor, contraindicates their participation

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
42. Presence of hepatitis B surface antigen (HBsAg), positive hepatitis C antibody test result at screening or within 3 months of screening. For potent immunosuppressive agents, subjects with presence of hepatitis B core antibody (HBcAb) should also be excluded 43. A positive pre-study drug/alcohol screen 44. Use of another investigational product in a clinical study within the following time period prior to the first administration of study medication in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer) 45. Exposure to more than 4 investigational medicinal products within 12 months prior to the first administration of study medication

REVISED TEXT

5.1 Inclusion Criteria

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

AGE
1. ≥ 21 years of age at the time of signing the informed consent form

TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2. Diagnosis of heart failure (New York Heart Association Class II-IV) for a minimum of 3 months prior to screening 3. Clinically stable with no changes in optimized guidance-directed medications and no hospitalizations for heart failure for at least 1 month prior to Screening 4. NT-proBNP >~~**1000**~~ **400** pg/mL measured within 6 months prior to OR at Screening 5. Average DLco measurements outside the normal range (% Predicted DLco < 80%) during the Screening Period

WEIGHT
6. Body mass index (BMI) ≥18 and ≤~~**40**~~ **45** kg/m^2

SEX
7. Male or female of non-childbearing potential ➢ Male patients with female partners of child bearing potential must comply with the following contraception requirements from the time of first dose of study medication until at least five half-lives of study medication OR for a cycle of spermatogenesis following five terminal half-lives after the last dose of study medication. a. Vasectomy with documentation of azoospermia. b. Male condom plus partner use of one of the contraceptive options below: • Contraceptive subdermal implant that meets the standard operating procedure (SOP) effectiveness criteria including a <1% rate of failure per year, as stated in the product label • Intrauterine device or intrauterine system that meets the SOP effectiveness criteria including a <1% rate of failure per year, as stated in the product label [Hatcher, 2007a] • Oral contraceptive, either combined or progestogen alone [Hatcher,

SEX
2007a] • Injectable progestogen [Hatcher, 2007a] • Contraceptive vaginal ring [Hatcher, 2007a] • Percutaneous contraceptive patches [Hatcher, 2007a] These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception. ➢ A female subject is eligible to participate if at least one of the following conditions applies: a. Non-reproductive potential defined as: • Pre-menopausal females with one of the following: • Documented tubal ligation • Documented hysteroscopic tubal occlusion procedure with follow-up confirmation of bilateral tubal occlusion • Hysterectomy • Documented bilateral oophorectomy b. Postmenopausal defined as 12 months of spontaneous amenorrhea. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrolment.

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

5.2 Exclusion Criteria

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

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
1. History of acute coronary syndromes including unstable angina or myocardial infarction within 6 months of screening 2. Coronary revascularization including angioplasty and stenting within 6 months of Screening

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
3. History of stroke or seizure disorder within 5 years of Screening 4. Diagnosis of asthma 5. Diagnosis of chronic obstructive pulmonary disease (COPD) with FEV_1 <50% of predicted measured within 4 weeks of Screening 6. History of a condition that required radiation therapy to the thorax 7. History of any type of malignancy within the past five years with the exception of successfully treated basal cell cancer of the skin 8. Active ulcer disease or gastrointestinal bleeding at **the time of screening** 9. Current or chronic history of liver disease, known hepatic impairment, or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones) 10. Alanine transaminase (ALT) >2x Upper Limit of Normal (ULN) and bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%) 11. QTc > 450 msec or QTc > 480 msec in subjects with Bundle Branch Block • The QTc is the QT interval corrected for heart rate according to Bazett's formula (QTcB), Fridericia's formula (QTcF), and/or another method, machine-read or manually over-read. • The *same* QT correction formula *must* be used for *each individual subject* throughout the study. This formula may not be changed or substituted once the subject has been enrolled. • For purposes of data analysis, QTcB, QTcF, another QT correction formula, or a composite of available values of QTc will be used as specified in the Reporting and Analysis Plan (RAP). 12. History or current evidence of any serious or clinically significant gastrointestinal, renal, endocrine, neurologic, hematologic or other condition that is uncontrolled on permitted therapies or that would, in the opinion of the investigator or the GlaxoSmithKline (GSK) medical monitor, make the subject unsuitable for inclusion in this study

CONCOMITANT MEDICATIONS
13. Use of medications specified for the treatment of COPD including short- and long-acting bronchodilators (β-agonists and anticholinergics) and inhaled glucocorticoids as well as oxygen therapy 14. Use of a listed prohibited medication (Section 6.10.2) within the restricted timeframe relative to the first dose of study medication 15. Use of a strong inhibitors or inducers of cytochrome P450 (CYP) 3A or p-

glycoprotein (see Section 6.10.2)

RELEVANT HABITS
16. Current smoker **~~17. History of drug/substance abuse within the past 2 years~~** 17. History of alcohol abuse within 6 months of the study **in the opinion of the investigator(s)**. **~~Defined as an average weekly alcohol consumption of >14 drinks for men or >7 drinks for women. One drink is equivalent to 12 g of alcohol: approximately 12 ounces (360 mL) of beer, 5 ounces (150 mL) of wine, or 1.5 ounces of (45 mL) 80 proof distilled spirits~~**

CONTRAINDICATIONS
18. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or Medical Monitor, contraindicates their participation

DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
19. Presence of hepatitis B surface antigen (HBsAg), positive hepatitis C antibody test result at screening or within 3 months of screening. For potent immunosuppressive agents, subjects with presence of hepatitis B core antibody (HBcAb) should also be excluded 20. A positive pre-study drug/alcohol screen **(excluding prescribed medications)** 21. Use of another investigational product in a clinical study within the following time period prior to the first administration of study medication in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer) 22. Exposure to more than 4 investigational medicinal products within 12 months prior to the first administration of study medication

PREVIOUS TEXT

5.3 Eligibility for Optional Sleep Apnea Sub-Study

Subjects eligible for the main study will be eligible for the sleep apnea sub-study, if the following inclusion criterion is met:

REVISED TEXT

5.3 Eligibility for Optional Sleep Apnea Sub-Study

Subjects eligible for the main study will be eligible for the sleep apnea sub-study, if the following inclusion criteri**~~ona~~** **~~is~~** **are** met:

Section 6

PREVIOUS TEXT

6.2 Treatment Assignment

Subjects will be assigned to study treatment in accordance with the randomization schedule generated in RandAll NG by Clinical Statistics prior to the start of the study, using validated internal software.

Subjects will be randomized into the study by means of an interactive voice response system (IVRS) i.e. RAMOS NG, to receive one the two treatment regimens shown below in Table 1.

REVISED TEXT

6.2 Treatment Assignment

Subjects will be assigned to study treatment in accordance with the randomization schedule generated in RandAll NG by Clinical Statistics prior to the start of the study, using validated internal software.

Subjects will be randomized into the study by means of an interactive ~~**voice**~~ **web** response system (I~~V~~**W**RS) i.e. RAMOS NG, to receive one the two treatment regimens shown below in Table 1.

PREVIOUS TEXT

6.3 Blinding

This will be a double-blind study where the sponsor and clinical pharmacist are un-blinded and the following will apply.

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

- A subject may continue in the study if that subject's treatment assignment is unblinded by the discretion of the Investigator in consultation with the Medical Monitor.
- GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any subject with an Serious Adverse Event (SAE) . If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the subject's treatment assignment, may be sent to investigators in accordance with local regulations and/or GSK policy.

REVISED TEXT

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This will be a double-blind study where the sponsor and clinical pharmacist are un-blinded and the following will apply.

- The investigator or treating physician may unblind a subject's treatment assignment **only in the case of an emergency** OR in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject as judged by the investigator.
- Investigators have direct access to the subject's individual study treatment **via the IWRS (RAMOS NG).**
- It is preferred (but not required) that the investigator first contacts the Medical Monitor or appropriate GSK study personnel to discuss options **before** unblinding the subject's treatment assignment.
- If GSK personnel are not contacted before the unblinding, the investigator must notify GSK as soon as possible after unblinding, but without revealing the treatment assignment of the unblinded subject, unless that information is important for the safety of subjects currently in the study.
- The date and reason for the unblinding must be fully documented in the CRF
- A subject may continue in the study if that subject's treatment assignment is unblinded by the discretion of the Investigator in consultation with the Medical Monitor.
- GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any subject with a~~n~~ Serious Adverse Event (SAE)~~-~~. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the subject's treatment assignment, may be sent to investigators in accordance with local regulations and/or GSK policy.

PREVIOUS TEXT

6.7 Treatment of Study Treatment Overdose

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

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

5. Contact the Medical Monitor immediately
6. Closely monitor the subject for adverse events (AEs)/serious adverse events (SAEs) and laboratory abnormalities until GSK2798745 can no longer be detected systemically (at least 5 days for GSK2798745)
7. Obtain a plasma sample for pharmacokinetic (PK) analysis within 1 days from the date of the last dose of study treatment if requested by the Medical Monitor (determined on a case-by-case basis)
8. Document the quantity of the excess dose as well as the duration of the overdosing in the CRF.

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

REVISED TEXT

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

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

1. Contact the Medical Monitor immediately.
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3. Obtain a plasma sample for pharmacokinetic (PK) analysis within 1 days from the date of the last dose of study treatment if requested by the Medical Monitor (determined on a case-by-case basis).
4. Document the quantity of the excess dose as well as the duration of the overdosing in the CRF.

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

PREVIOUS TEXT

6.9.1 Monitoring in the Clinical Research Unit

The first six subjects who participate in the study will be admitted to the Clinical Research Unit to be monitored for the first two days (Day 1 and Day 2) following the first administration of study medication in both study periods. Subsequently, these subjects will remain in the unit for a minimum of 4 hours after the administration of study medication on each of the remaining 5 days (Days 3 through 7) of each study period. On Day 5, subjects will remain in the unit for a minimum of 4 hours after the completion of the saline infusion.

For the remainder of subjects participating in the study, they will remain in the unit for a minimum of 4 hours after the administration of study medication on each of the 7 days of each study period.

Following the completion of all assessments on each study day, subjects may return home. For subjects who cannot return home each evening for multiple reasons including but not limited to distance from clinical site or transportation issues, housing will be provided. All subjects will be continuously monitored remotely (see Section 7.4.7).

REVISED TEXT

6.9.1 Monitoring in the ~~Clinical Research~~ Unit

The first six subjects who participate in the study will be admitted to the ~~**Clinical Research Uu**~~nit to be monitored for the first two days (Day 1 and Day 2) following the first administration of study medication in both study periods. Subsequently, these subjects will remain in the unit for a minimum of 4 hours after the administration of study medication on each of the remaining 5 days (Days 3 through 7) of each study period. On Day 5, subjects will remain in the unit for a minimum of 4 hours after the completion of the saline infusion.

For the remainder of subjects participating in the study, they will remain in the unit for a minimum of 4 hours after the administration of study medication on each of the 7 days of each study period.

Following the completion of all assessments on each study day, subjects may return home. For subjects who cannot return home each evening for multiple reasons including but not limited to distance from clinical site or transportation issues, housing will be provided **or they may opt to remain in the unit for the duration of the study period. Housing should be consistent in both study periods.** All subjects will be continuously monitored remotely (see Section 7.4.7).

PREVIOUS TEXT

6.9.2 Meal and Dietary Restrictions

Subjects are not permitted to consume red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days prior to the first dose of study medication until their discharge from the unit after their last dose of study medication in each period.

Three meals per day will be provided by dietary department either in the clinical research unit or as a boxed meal(s) to go.

No meal restrictions will be placed with regard to drug administration. Subjects will be allowed to consume a standard breakfast prior to drug administration.

REVISED TEXT

6.9.2 Meal and Dietary Restrictions

Subjects are not permitted to consume red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 7 days prior to the first dose of study medication until their discharge from the unit after their last dose of study medication in each period.

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No meal restrictions will be placed with regard to drug administration. Subjects will be allowed to consume a standard breakfast prior to drug administration.

PREVIOUS TEXT

6.9.3 Caffeine and Alcohol

During each study period, subjects will abstain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate) for 24 hours prior to the start of dosing until completion of all assessments.

During each study period, subjects will abstain from alcohol for 24 hours prior to the start of dosing until completion of all assessments.

REVISED TEXT

6.9.3 Caffeine and Alcohol

During each study period, **it is recommended that** subjects will ~~**abstain from ingesting**~~ **minimize ingestion of caffeine**- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate) for 24 hours prior to the start of dosing until completion of all assessments.

During each study period, subjects will abstain from alcohol for 24 hours prior to the start of dosing until completion of all assessments.

PREVIOUS TEXT

6.10.2. Prohibited Medications and Non-Drug Therapies

Except for the permitted medications noted above (Section 6.10.1), subjects must abstain from taking non-prescription drugs (including vitamins and dietary or herbal supplements), within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose of study medication until completion of the follow-up visit, unless in the opinion of the Investigator and sponsor the medication will not interfere with the study.

Subjects must provide to the investigator information/list of all medications they are currently taking.

GSK2798745 has weak CYP3A4 inhibition potential. There is a likelihood that the concentrations of drugs that are substrates of CYP3A4 may be increased. HMG-CoA reductase inhibitors such as atorvastatin and simvastatin are examples of CYP3A4 substrates that are likely to be taken by the eligible patients as part of their medications. The concentrations of these statins will be monitored during the study. The investigators may also consider substitutions of these medications.
Subjects should avoid using drugs that are strong inhibitors or inducers of Cytochrome P450 (CYP) 3A4 or p-glycoprotein (P-gp) because they may alter GSK2798745 concentrations. The list of background therapy/drugs may be modified based on emerging data. These include, but are not limited to, those listed in Table 2 consider therapeutic substitutions for these medications.

GSK2798745 systemic concentrations will be monitored to detect any drug interactions with moderate CYP3A4 inhibitors or P-gP inhibitors (see SRM for a detailed list).

It is strongly recommended that patients avoid using drugs that are sensitive substrates of Cytochrome P450 (CYP) 3A4 and/or P-gp or that have a low therapeutic index because concentrations of these substrates may be increased by GSK2798745. If co-administration of medications with interaction potential with GSK2798745 is necessary, investigators should monitor subjects for loss of efficacy or consider substitutions of these medications. See SRM for a detailed list. The list may be modified based on emerging data. All concomitant medications should be reviewed by the Medical Monitor and will be to the discretion of the Investigator and Medical Monitor whether the medication can be continued and/or the subject can participate in the study.

REVISED TEXT

6.10.2. Prohibited Medications and Non-Drug Therapies

Except for the permitted medications noted above (Section 6.10.1), subjects must abstain from taking non-prescription drugs (including vitamins and dietary or herbal

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Subjects must provide to the investigator information/list of all medications they are currently taking.

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It is strongly recommended that patients avoid using drugs that are sensitive substrates of Cytochrome P450 (CYP) 3A4 and/or P-gp or that have a low therapeutic index because concentrations of these substrates may be increased by GSK2798745. If co-administration of medications with interaction potential with GSK2798745 is necessary, investigators should monitor subjects for loss of efficacy or consider substitutions of these medications. See SRM for a detailed list. The list may be modified based on emerging data. All concomitant medications ~~**should**~~ **may** be reviewed by the Medical Monitor and **it** will be **up** to the discretion of the Investigator and **if necessary the** Medical Monitor**,** whether the medication can be continued and/or the subject can participate in the study.

PREVIOUS TEXT

7.1. Time and Events Table

Procedure	Screening Period	Treatment Periods I and II (Day)										Follow-up
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	8	9	14 ± 4 days after last dose of study medication
Informed consent	X											
Inclusion and exclusion criteria	X											
Demography	X											
Full physical examination including height & weight	X											
Brief physical exam including weight		X				X			X			X
Medical history/Medication history	X											
Hepatitis B & C screen	X											
Echocardiogram	X											
Urine drug and alcohol breath test	X	X										
Clinical lab assessments[14]	X	X				X			X			X
NT-proBNP	X	X							X			
Serum CTX[11]		X							X			
Fecal Occult Blood Test[12]	X								X			
Pharmacokinetic sample[1]		X	X	X	X	X	X	X	X	X	X	
Digoxin sample[2]		X	X	X	X	X	X	X	X			X
Troponin	X	X		X		X			X			X
DLco/DLno[10]	X	X				X	X		X			X

Procedure	**Screening Period**	**Treatment Periods I and II (Day)**										**Follow-up**
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	8	9	14 ± 4 days after last dose of study medication
12-lead ECG/ time & frequency domains[3]	X[9]	X[9]				X			X			X
Vital signs	X	X	X	X	X	X	X	X	X			X
Weight			X	X	X		X	X				
Genetic sample		For subjects who provide consent, sample collected after start of treatment										
Study treatment[4]			X	X	X	X	X	X	X			
AE/SAE review		X	←==========→									X
Concomitant med review		X	←==========→									X
Pulmonary function tests		X				X			X			X
Step exercise test/breath-by-breath gas exchange		X							X			X
Dyspnea assessment		X					X		X			X
Orthopnea							X					
SF-36 Acute		X							X			X
Polysomnography including oximetry (Sleep Apnea Clinic)[5,13]		←==========→						←==========→				
Plasma and 8-hour urine catecholamines[6]		X						X				
Saline infusion							X					
Admission to CRU[7]			X									
Discharge from CRU[7]					X							
Meals[8]		X	X	X	X	X	X	X	X			
Appetite Assessment (SNAQ)		X				X			X			X
CSSRS (Suicidality)		X							X			X
Audiometry		X[13]							X			X

Procedure	Screening Period	Treatment Periods I and II (Day)										Follow-up
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	8	9	14 ± 4 days after last dose of study medication
BodyGuardian Monitor (Preventice)		←==========Continuous==========→										

1. Pharmacokinetic samples to determine GSK2798745 and any metabolite(s) concentrations will be obtained at the following times on Day 1 of each treatment period: Predose, 0.5, 1, 1.5, 2, 3, 5, 8 and 12 hours and at the following times on Day 7 of each treatment period: Predose, 0.5, 1, 1.5, 2, 3, 5 and 10 hours. On Day 2 of each treatment period, samples will be collected predose and at 12 hours post dose. On Days 3 through 6, PK samples will be collected predose. On Days 8 and 9, samples will be collected at 24 and 48 hours, respectively, after the last dose of study medication administered on Day 7. The time points may be modified based on emerging data. Some of these timepoints may also be utilized to collect samples to determine concentrations of other medications (e.g., atorvastatin and/or simvastatin). See the SRM for detailed information.
2. Digoxin concentrations will be monitored only in those subjects who are taking digoxin
3. Time and frequency domains assessed by ECG obtained on Day -1 and Day 7
4. Subjects will remain in the CRU for a minimum of 4 hours after each administration of study medication. [Note: The first 6 subjects enrolled will remain in the CRU for 52 hours after the first dose of study medication in each period]
5. Only for subjects participating in the sub-study: At Day -1 of the first study period only, subjects will undergo an overnight sleep study. Only those subjects who are participating in the sub-study with sleep disordered breathing will undergo additional sleep studies on Day 6 of both study Periods I and II.
6. Only for subjects participating in the sub-study: Urinary norepinephrine (NE) and epinephrine (E) concentrations will be determined from 8-hour collections obtained during the overnight sleep study; urinary creatinine will also be measured to normalize the NE and E concentrations. A blood sample to determine plasma concentrations of NE and E will be obtained the morning after the sleep study between the hours of 9:00 am and 10:00 am after the subject has been supine for 20 minutes in a quiet room.
7. The first 6 subjects enrolled will remain in the CRU for two days (Day 1 and Day 2) after the first dose of study medication in each period.
8. Three meals per day will be provided by the dietary department either in the CRU or as a boxed meal(s) to take home.
9. ECGs to be performed in triplicate
10. On the days where exercise or the saline infusion is performed, DLco/DLno will be completed just prior to and after both exercise and the saline infusion.
11. Blood sample for analysis of Serum CTX must be collected when the subject is fasting. See Section 7.4.9.1

12. Fecal Occult Blood Test (FOBT) cards will be provided to subjects at the end of the screening visit and must be completed and sent back to the laboratory prior to the baseline visit according to the laboratory's standard collection procedures. Similarly, subjects will be given FOBT cards at the end of the Day 7 visit of each study period with completion instructions. See Section 7.4.9.2
13. Day -1 audiometry and sleep study assessments may be completed 7 days prior to Day -1.
14. Clinical laboratory assessments will be collected as a fasting sample.

REVISED TEXT

7.1. Time and Events Table

Procedure	Screening Period	Treatment Periods I and II (Day)										Follow-up
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	8	9	14 ± 4 days after last dose of study medication
Informed consent	X											
Inclusion and exclusion criteria	X											
Demography	X											
Full physical examination including height & weight	X											
Brief physical exam including weight		X				X			X			X
Medical history/Medication history	X											
Hepatitis B & C screen	X											
Echocardiogram	X											
Urine drug and alcohol ~~breath~~ test	X	X										
Clinical lab assessments[14]	X	X				X			X			X
NT-proBNP	X	X							X			
Serum CTX[11]		X							X			

Procedure	**Screening Period**	**Treatment Periods I and II (Day)**										**Follow-up**
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	8	9	14 ± 4 days after last dose of study medication
Fecal Occult Blood Test[12]	X								X			
Pharmacokinetic sample[1]		~~X~~	X	X	X	X	X	X	X	X	X	
Digoxin sample[2]		X	X	X	X	X	X	X	X			X
Troponin	X	X		X		X			X			X
DLco/DLno[10]	X[15]	X				X	X		X			X
12-lead ECG/ time & frequency domains[3]	X[9]	X[9]				X			X			X
Vital signs	X	X	X	X	X	X	X	X	X			X
Weight			X	X	X		X	X				
Genetic sample		For subjects who provide consent, sample collected after start of treatment										
Study treatment[4]			X	X	X	X	X	X	X			
AE/SAE review		X	←==========→									X
Concomitant med review		X	←==========→									X
Pulmonary function tests		X				X			X			X
Step exercise test/breath-by-breath gas exchange		X							X			X
Dyspnea assessment		X					X		X			X
Orthopnea							X					
SF-36 Acute		X							X			X
Polysomnography including oximetry (Sleep Apnea Clinic)[5,13]		←==========→						←==========→				
Plasma and 8-hour urine catecholamines[6]		X						X				
Saline infusion							X					
Admission to ~~CRU~~ unit[7]			X									

Procedure	Screening Period	Treatment Periods I and II (Day)										Follow-up
	Day -25 to Day -1 prior to Period I	-1	1	2	3	4	5	6	7	8	9	14 ± 4 days after last dose of study medication
Discharge from ~~CRU~~ unit[7]					X							
Meals[8]		X	X	X	X	X	X	X	X			
Appetite Assessment (SNAQ)		X				X			X			X
CSSRS (Suicidality)		X							X			X
Audiometry		X[13]							X[17]			X
BodyGuardian Monitor (Preventice)		←==========Continuous==========→										

1. Pharmacokinetic samples to determine GSK2798745 and any metabolite(s) concentrations will be obtained at the following times on Day 1 of each treatment period: Predose, 0.5, 1, 1.5, 2, 3, 5, 8 and 12 hours and at the following times on Day 7 of each treatment period: Predose, 0.5, 1, 1.5, 2, 3, 5 and 10 hours. On Day 2 of each treatment period, samples will be collected predose and at 12 hours post dose. On Days 3 through 6, PK samples will be collected predose. On Days 8 and 9, samples will be collected at 24 and 48 hours, respectively, after the last dose of study medication administered on Day 7. The time points may be modified based on emerging data. Some of these timepoints ~~**may**~~ **will-**also be utilized to collect samples to determine concentrations of other medications (e.g., atorvastatin and/or simvastatin). See the SRM for detailed information.
2. Digoxin concentrations will be monitored only in those subjects who are taking digoxin
3. Time and frequency domains assessed by ECG obtained on Day -1 and Day 7
4. Subjects will remain in the ~~**CRU**~~ **unit** for a minimum of 4 hours after each administration of study medication. [Note: The first 6 subjects enrolled will remain in the ~~**CRU**~~ **unit** for 52 hours after the first dose of study medication in each period]
5. Only for subjects participating in the sub-study: At Day -1 of the first study period only, subjects will undergo an overnight sleep study. Only those subjects who are participating in the sub-study with sleep disordered breathing will undergo additional sleep studies on Day 6 of both study Periods I and II.
6. Only for subjects participating in the sub-study: Urinary norepinephrine (NE) and epinephrine (E) concentrations will be determined from 8-hour collections obtained during the overnight sleep study; urinary creatinine will also be measured to normalize the NE and E concentrations. A blood sample to determine plasma concentrations of NE and E will be obtained the morning after the sleep study between the hours of 9:00 am and 10:00 am after the subject has been supine for 20 minutes in a quiet room.

7. The first 6 subjects enrolled will remain in the ~~**CRU**~~ **unit** for two days (Day 1 and Day 2) after the first dose of study medication in each period. **Subjects may remain in the unit for the duration of each of the 7 day treatment periods for convenience. Housing should be consistent in both study periods.**
8. Three meals per day will be provided by the dietary department either in the ~~**CRU**~~ **unit** or as a boxed meal(s) to take home.
9. ECGs to be performed in triplicate
10. On the days ~~**where**~~ **when** exercise or the saline infusion is performed, DLco/DLno will be completed just prior to and after both exercise and the saline infusion.
11. Blood sample for analysis of Serum CTX must be collected when the subject is fasting. See Section 7.4.9.1
12. Fecal Occult Blood Test (FOBT) cards will be provided to subjects at the end of the screening visit and must be completed and sent back to the laboratory prior to the baseline visit according to the laboratory's standard collection procedures. Similarly, subjects will be given FOBT cards at the end of the Day 7 visit of each study period with completion instructions. See Section 7.4.9.2
13. Day -1 audiometry and sleep study assessments may be completed 7 days prior to Day -1.
14. Clinical laboratory assessments will be collected as a fasting sample.
15. **The screening visit will only collect %Predicted DLco (DLco single breath)**
16. **If a subject has had an echocardiogram completed within the last 6 months prior to screening, it does not need to be repeated.**
17. **Day 7 Audiometry may be completed with a window of ± 2 day**

PREVIOUS TEXT

7.2 Screening and Critical Baseline Assessments

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

Medical/medication/alcohol/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5. Cardiovascular medical history/risk factors will also be assessed at screening, including the following items pertaining to medical and medication history:

- Onset and type of symptoms
- Years since diagnosis of HF
- New York Heart Association (NYHA) class
- LVEF (%)
- Years since diagnosis of sleep apnea (if applicable)
- Degree of exercise intolerance: distance/ stairs/time prior to breathlessness
- Presence of orthopnea and/or paroxysmal nocturnal dyspnea
- Peripheral edema: presence and height beyond ankle; recent weight gains, level of peripheral pitting edema
- Significant past medical history including onset, etiology, and results of any recent relevant investigations of heart failure, as applicable
- Medication history

Procedures conducted as part of the subject's routine clinical management and obtained prior to signing of informed consent may be utilized for Screening or baseline purposes provided the procedure meets the protocol-defined criteria and has been performed in the timeframe of the study.

During the Screening Period prior to the first study period (Period I), subjects will have the following assessments performed to determine eligibility for enrollment:

- NT-proBNP
- DLco/DLno
- Polysomnography (eligibility into sub-study)

REVISED TEXT

7.2 Screening and Critical Baseline Assessments

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

Medical/medication/alcohol/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5. Cardiovascular medical history/risk factors will also be assessed at screening, including the following items pertaining to medical and medication history:

- Onset and type of symptoms
- Years since diagnosis of HF
- New York Heart Association (NYHA) class
- LVEF (%)
- Years since diagnosis of sleep apnea (if applicable)
- Degree of exercise intolerance: distance/ stairs/time prior to breathlessness
- Presence of orthopnea and/or paroxysmal nocturnal dyspnea
- Peripheral edema: presence and height beyond ankle; recent weight gains, level of peripheral pitting edema
- Significant past medical history including onset, etiology, and results of any recent relevant investigations of heart failure, as applicable
- Medication history

Procedures conducted as part of the subject's routine clinical management and obtained prior to signing of informed consent may be utilized for Screening or baseline purposes provided the procedure meets the protocol-defined criteria and has been performed in the timeframe of the study.

During the Screening Period prior to the first study period (Period I), subjects will have the following assessments performed to determine eligibility for enrollment:

- NT-proBNP
- ~~**DLco/DLno**~~ **% Predicted DLco**
- Polysomnography (eligibility into sub-study)

PREVIOUS TEXT

7.3.1. DLco and DLno

The diffusing capacity of the lung to carbon monoxide (DLco) or nitric oxide (DLno) is a measure of the ability of a gas to transfer from the alveoli across the alveolar epithelium and the capillary endothelium to the red blood cells. Changes in DLco and DLno reflect the alveolar-capillary membrane conductance (D_M). Since nitric oxide has a greater affinity for hemoglobin, the diffusion of nitric oxide is mainly limited by the transfer of gas across the alveolar capillary membrane. Measurements are made simultaneously. Acute pulmonary congestion causes a reduction in DLco. In patients with heart failure, DLco is decreased and serves as a predictor of disease progression. An impairment in the

diffusing capacity of the lung may be related to the symptoms and exercise intolerance associated with heart failure.

DLco will be measured during the Screening Period to determine eligibility for enrollment into the study. Subjects must have a % Predicted DLco <80%.

During each study period, DLco and DLno will be measured just prior to and after the 3-minute step test on Days -1, and 7 and at the Follow-up Visit. Measurements will also be made just prior to and after an intravenous saline infusion on Day 6.

Details of the pulmonary testing methodology including diffusing capacity and measured components are included in the SRM. Refer to the instruction manual(s) for the equipment for further details.

REVISED TEXT

7.3.1. DLco and DLno

The diffusing capacity of the lung to carbon monoxide (DLco) or nitric oxide (DLno) is a measure of the ability of a gas to transfer from the alveoli across the alveolar epithelium and the capillary endothelium to the red blood cells. Changes in DLco and DLno reflect the alveolar-capillary membrane conductance (D_M). Since nitric oxide has a greater affinity for hemoglobin, the diffusion of nitric oxide is mainly limited by the transfer of gas across the alveolar capillary membrane. Measurements are made simultaneously. Acute pulmonary congestion causes a reduction in DLco. In patients with heart failure, DLco is decreased and serves as a predictor of disease progression. An impairment in the diffusing capacity of the lung may be related to the symptoms and exercise intolerance associated with heart failure.

DLco will be measured during the Screening Period to determine eligibility for enrollment into the study. Subjects must have a % Predicted DLco <80%.

During each study period, DLco and DLno will be measured just prior to and after the 3-minute step test on Days -1, and 7 and at the Follow-up Visit. Measurements will also be made just prior to and after an intravenous saline infusion on Day ~~6~~ **5**.

Details of the pulmonary testing methodology including diffusing capacity and measured components are included in the SRM. Refer to the instruction manual(s) for the equipment for further details.

PREVIOUS TEXT

7.3.2. Submaximal Exercise Test

Blockade of the TRPV4 channel with GSK2798745 may decrease pulmonary edema resulting in an increase in exercise capacity and/or oxygen uptake.

Subjects will be asked to participate in a submaximal exercise test that consists of 3 parts: a 2-minute resting baseline, a 3-minute step exercise, and a 1-minute recovery period

[Woods, 2011]. Throughout the test, breathing pattern, gas exchange, and heart rate will be monitored using a simplified gas analysis system (SHAPE Medical Systems, Inc). Respiratory exchange ratio (RER) and the Borg Rating of Perceived Exertion (RPE) measures will be utilized to ensure subjects perform progressive exercise while maintaining a submaximal level throughout the exercise period. Minute ventilation, breath frequency, tidal volume, oxygen consumption, carbon dioxide production, RER and end tidal CO_2 will be obtained from the breath-by-breath gas measurements. The ventilation/carbon dioxide production (VE/VCO_2) slope and other variables will be derived from this data.

The exercise protocol will be completed on Day -1 and Day 7 of each study period. DLco/DLno measurements will be obtained before and after the exercise challenge. Additional details of the exercise protocol for the 3-minute step test are provided in the SRM.

REVISED TEXT

7.3.2. Submaximal Exercise Test

Blockade of the TRPV4 channel with GSK2798745 may decrease pulmonary edema resulting in an increase in exercise capacity and/or oxygen uptake.

Subjects will be asked to participate in a submaximal exercise test that consists of 3 parts: a 2-minute resting baseline, a 3-minute step exercise, and a 1-minute recovery period [Woods, 2011]. Throughout the test, breathing pattern, gas exchange, and heart rate will be monitored using a simplified gas analysis system (**i.e.** SHAPE Medical Systems, Inc). Respiratory exchange ratio (RER) and the Borg Rating of Perceived Exertion (RPE) measures will be utilized to ensure subjects perform progressive exercise while maintaining a submaximal level throughout the exercise period. Minute ventilation, breath frequency, tidal volume, oxygen consumption, carbon dioxide production, RER and end tidal CO_2 will be obtained from the breath-by-breath gas measurements. The ventilation/carbon dioxide production (VE/VCO_2) slope and other variables will be derived from this data.

The exercise protocol will be completed on Day -1 and Day 7 of each study period. DLco/DLno measurements will be obtained before and after the exercise challenge. Additional details of the exercise protocol for the 3-minute step test are provided in the SRM.

PREVIOUS TEXT

7.3.3. Intravenous Saline Infusion

In patients with chronic HF, intravenously infused saline elicited a reduction of D_M [Puri, 1999] suggesting that the abnormal pulmonary diffusion in this population may have a variable component that could be amenable to therapeutic intervention. Similarly, an infusion of saline into the pulmonary artery significantly reduced both DLco and D_M in patients with chronic HF but not in control subjects [Guazzi, 1999]; the changes in DM were inversely related to VE/VCO2 [Guazzi,2001]. The hemodynamic effects of volume

expansion with saline loading has been shown to be associated with increases in left and right heart filling pressures and pulmonary artery pressures in patients with HF [Andersen, 2015]. In this latter study, no patient developed dyspnea or any evidence of pulmonary congestion despite increase in cardiac filling pressures.

All subjects will undergo an intravenous 0.9% NaCl infusion on Day 5 of both study periods in the presence of a cardiologist specializing in heart failure. The procedure will be conducted only if the subject is without any signs of obvious congestion as determined by the Investigator. Subjects will be carefully monitored both during and after the infusion and will be provided intravenous diuretics if necessary. Details of this procedure are described in Appendix 9.

DLco and DLno measurements will be obtained prior to the initiation of the infusion and after the completion of the infusion.

REVISED TEXT

7.3.3. Intravenous Saline Infusion

In patients with chronic HF, intravenously infused saline elicited a reduction of D_M [Puri, 1999] suggesting that the abnormal pulmonary diffusion in this population may have a variable component that could be amenable to therapeutic intervention. Similarly, an infusion of saline into the pulmonary artery significantly reduced both DLco and D_M in patients with chronic HF but not in control subjects [Guazzi, 1999]; the changes in DM were inversely related to VE/VCO2 [Guazzi, 2001]. The hemodynamic effects of volume expansion with saline loading has been shown to be associated with increases in left and right heart filling pressures and pulmonary artery pressures in patients with HF [Andersen, 2015]. In this latter study, no patient developed dyspnea or any evidence of pulmonary congestion despite increase in cardiac filling pressures.

All subjects will undergo an intravenous 0.9% NaCl infusion on Day 5 of both study periods in the presence of a cardiologist ~~**specializing in heart failure**~~. The procedure will be conducted only if the subject is without any signs of obvious congestion as determined by the Investigator. Subjects will be carefully monitored both during and after the infusion and will be provided intravenous diuretics if necessary. Details of this procedure are described in Appendix 9.

DLco and DLno measurements will be obtained prior to the initiation of the infusion and after the completion of the infusion. **Additional details are included in the SRM.**

PREVIOUS TEXT

7.3.4. Pulmonary Function Tests

Measures of pulmonary function have been shown to predict prognosis in patients with HF [Olson, 2013].

Sprirometry measures will include forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) as well as forced expiratory flows, FEF_{25-75} FEF_{50}, and FEF_{75}

Functional residual capacity (FRC) and end-expiratory lung volume (EELV) measured by body plethysmography.

REVISED TEXT

7.3.4. Pulmonary Function Tests

Measures of pulmonary function have been shown to predict prognosis in patients with HF [Olson, 2013].

Sprirometry measures will include forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) as well as forced expiratory flows, FEF_{25-75} FEF_{50}, and FEF_{75}

Functional residual capacity (FRC) and end-expiratory lung volume (EELV) **will be** measured by body plethysmography.

PREVIOUS TEXT

7.3.6. Polysomnography

Subjects only participating in the sleep apnea substudy will undergo overnight polysomnography during the Screening Period. Consenting subjects who meet all the eligibility criteria for the main study and have an apnea/hypopnea index (AHI) ≥5 events/hour based on polysomnography will participate in subsequent sleep studies on Day 6 of both study periods.

Multiple recordings will be monitored including electroencephlogram (EEG), electrooculogram (EOG), electromyogram (EMG), electrocardiogram (ECG), and oximetry. Sleep stages and arousals, respiratory rate, and O_2 saturation will be measured. Disordered breathing events will be classified as apneas or hypopneas and as either obstructive or central.

Additional details outlining the polysomnography procedure are detailed in the SRM.

REVISED TEXT

7.3.6. Polysomnography

Only S~~s~~ubjects ~~**only**~~ participating in the sleep apnea substudy will undergo overnight polysomnography during the Screening Period. Consenting subjects who meet all the eligibility criteria for the main study and have an apnea/hypopnea index (AHI) ≥5 events/hour based on polysomnography will participate in subsequent sleep studies on Day 6 of both study periods.

Multiple recordings will be monitored including electroencephlogram (EEG), electrooculogram (EOG), electromyogram (EMG), electrocardiogram (ECG), and oximetry. Sleep stages and arousals, respiratory rate, and O_2 saturation will be measured.

Disordered breathing events will be classified as apneas or hypopneas and as either obstructive or central.

Additional details outlining the polysomnography procedure are detailed in the SRM.

PREVIOUS TEXT

7.4.5. Electrocardiogram (ECG)

- Triplicate 12-Lead ECGs will be obtained during the Screening Period and at baseline and single 12-lead ECGs will be obtained on Days -1, 4 and 7 and the Follow-up Visit using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF or QTcB intervals.
- Refer to Section 5.5.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- Resting and stationary measures will also be collected to determine heart rate variability and time domains

REVISED TEXT

7.4.5. Electrocardiogram (ECG)

- Triplicate 12-Lead ECGs will be obtained during the Screening Period and at baseline **(Day -1)** and single 12-lead ECGs will be obtained on Days ~~-1~~, 4 and 7 and the Follow-up Visit using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF or QTcB intervals.
- Refer to Section 5.5.2 for QTc withdrawal criteria and additional QTc readings that may be necessary.
- Resting and stationary measures will also be collected to determine heart rate variability and time domains

PREVIOUS TEXT

7.4.6. Echocardiogram

A transthoracic echocardiogram will be performed during the Screening Period. Images will be obtained in standard views (e.g., long axis parasternal, short axis parasternal, and apical 2, 3, 4 and 5 chamber). The time to acquire images should not exceed 30 minutes.

REVISED TEXT

7.4.6. Echocardiogram

A transthoracic echocardiogram will be performed during the Screening Period, **unless the subject has had an echocardiogram completed within the 6 months prior to screening**. Images will be obtained in standard views (e.g., long axis parasternal, short axis parasternal, and apical 2, 3, 4 and 5 chamber). The time to acquire images should not exceed 30 minutes.

PREVIOUS TEXT

Table 3 Clinical Laboratory Assessments

Laboratory Assessments	**Parameters**			
Hematology	Platelet Count	*RBC Indices:*	*WBC count with Differential:*	
	RBC Count	MCV	Neutrophils	
	Hemoglobin	MCH	Lymphocytes	
	Hematocrit	MCHC	Monocytes	
	WBC Count (absolute)		Eosinophils	
	Reticulocyte count		Basophils	
Clinical Chemistry	BUN	Potassium	AST (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	ALT (SGPT)	Total Protein
	Glucose, fasting	Calcium	Alkaline phosphatise	Albumin
	CPK	Uric Acid	GGT	Troponin
	Chloride			
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal) • Urinary norepinephrine and epinephrine (during sleep study)			
Other Screening Tests	• Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Digoxin concentrations (only in subjects treated with Digoxin) • Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • HMG CoA Reductase Inhibitor concentration (only in subjects taking medication)			
Biomarker(s)/ Other Assessments	• N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) • Norepinephrine and Epinephrine • Serum Collagen Type I C-Telopeptide (CTX), fasting • Fecal Occult Blood Test (FOBT)			
NOTES : Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 5.5.1 and Appendix 2				

REVISED TEXT

Table 3 Clinical Laboratory Assessments

Laboratory Assessments	**Parameters**			
Hematology	Platelet Count	*RBC Indices*:	*WBC count with Differential*:	
	RBC Count	MCV	Neutrophils	
	Hemoglobin	MCH	Lymphocytes	
	Hematocrit	MCHC	Monocytes	
	WBC Count (absolute)		Eosinophils	
	Reticulocyte count		Basophils	
Clinical Chemistry	BUN	Potassium	AST (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	ALT (SGPT)	Total Protein
	Glucose, fasting	Calcium	Alkaline phosphatise	Albumin
	CPK	Uric Acid	GGT	~~**Troponin**~~
	Chloride			
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood and ketones by dipstick • Microscopic examination (if blood or protein is abnormal) • Urinary norepinephrine and epinephrine (during sleep study)			
Other Screening Tests	• Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • Digoxin concentrations (only in subjects treated with Digoxin) • Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • HMG CoA Reductase Inhibitor concentration (only in subjects taking medication)			
Biomarker(s)/ Other Assessments	• N-terminal pro-B-type Natriuretic Peptide (NT-proBNP) • Norepinephrine and Epinephrine **(substudy only)** • Serum Collagen Type I C-Telopeptide (CTX), fasting • Fecal Occult Blood Test (FOBT) • **Troponin**			
NOTES : Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 5.5.1 and Appendix 2				

PREVIOUS TEXT

7.4.9.2 Fecal Occult Blood Test (FOBT)

Based on the gastric erosions found in a preclinical model (dogs at ≥3 mg/kg), FOBT will be performed to assess any possible drug-related GI blood loss. Due to concomitant anti-platelet therapy that many patients with heart failure receive, along with the common incidence of intestinal mucosal edema and associated diarrhea, findings of fecal blood would not be unexpected in this population. Subjects will be tested for the presence of fecal occult blood prior to the start of study period I (between Screening and Baseline) and after each of the two study periods.

Once subjects have signed the consent form and have been enrolled into the study based on meeting the eligibility criteria, they will be provided 3 FOBT cards at the end of the screening visit. Study staff will provide the subjects with instructions for completing the tests and subsequent shipment of the tests back to the laboratory. This assessment will be completed between Screening and Baseline (Day -1). Additionally, subjects will be provided 3 FOBT cards on Day 7 of each study period with the same instructions for completion and shipment for laboratory analysis.

REVISED TEXT

7.4.9.2 Fecal Occult Blood Test (FOBT)

Based on the gastric erosions found in a preclinical model (dogs at ≥3 mg/kg), FOBT will be performed to assess any possible drug-related GI blood loss. Due to concomitant anti-platelet therapy that many patients with heart failure receive, along with the common incidence of intestinal mucosal edema and associated diarrhea, findings of fecal blood would not be unexpected in this population. Subjects will be tested for the presence of fecal occult blood prior to the start of ~~study~~ **treatment** period I (between Screening and Baseline) and after each of the two ~~study~~ **treatment** periods.

Once subjects have signed the consent form and have been enrolled into the study based on meeting the eligibility criteria, they will be provided 3 FOBT cards at the end of the screening visit. Study staff will provide the subjects with instructions for completing the tests and subsequent shipment of the tests back to the laboratory. This assessment will be completed between Screening and Baseline (Day -1). Additionally, subjects will be provided 3 FOBT cards on Day 7 of each ~~study~~ **treatment** period with the same instructions for completion and shipment for laboratory analysis.

PREVIOUS TEXT

7.5.1. Blood Sample Collection

Blood samples for pharmacokinetic analysis of GSK2798745 will be collected at the time points indicated in Section 7.1 Time and Events Table.

Approximately 2 mL of blood will be collected in EDTA tubes. The actual date and time of each blood sample collection will be recorded. The timing and volume of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure

adequate PK monitoring of GSK2798745 and, if possible, any relevant GSK2798745 metabolites.

Where possible, PK monitoring of relevant co-administered drugs and their metabolites may also be undertaken (e.g. atorvastatin and/or simvastatin). Approximately 2 mL of blood will be collected into sodium heparin tubes. The actual date and time of each blood sample collection will be recorded. The volume of such samples may be altered and additional time points may be added to ensure adequate statin PK monitoring.

Additional collection, processing, storage and shipping procedures are provided in the Study Reference Manual (SRM).

REVISED TEXT

7.5.1. Blood Sample Collection

Blood samples for pharmacokinetic analysis of GSK2798745 will be collected at the time points indicated in Section 7.1 Time and Events Table.

Approximately 2 mL of blood will be collected in EDTA tubes. The actual date and time of each blood sample collection will be recorded. The timing and volume of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure adequate PK monitoring of GSK2798745 and, if possible, any relevant GSK2798745 metabolites.

Where possible, PK monitoring of relevant co-administered drugs and their metabolites ~~**may**~~ **will** also be undertaken (~~**e.g.**~~ atorvastatin and~~**/or**~~ simvastatin). Approximately 2 mL of blood will be collected into sodium heparin tubes. The actual date and time of each blood sample collection will be recorded. The volume of such samples may be altered and additional time points may be added to ensure adequate statin PK monitoring.

Additional collection, processing, storage and shipping procedures are provided in the Study Reference Manual (SRM).

PREVIOUS TEXT

7.5.2. Sample Analysis

Plasma analysis will be performed under the control of Platform Technologies and Science-Drug Metabolism and Pharmacokinetics (PTS-DMPK)/ GlaxoSmithKline, the details of which will be included in the Study Reference Manual (SRM). Concentrations of GSK2798745, and co-administered statins and corresponding metabolites (e.g., atorvastatin and metabolites, or simvastatin and metabolites) will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Plasma samples may be analyzed for metabolite M1 dependent upon the availability of an assay. Until this time, GSK will store the remaining plasma from the PK plasma samples for future possible metabolite analysis.

REVISED TEXT

7.5.2. Sample Analysis

Plasma analysis will be performed under the control of Platform Technologies and Science-Drug Metabolism and Pharmacokinetics (PTS-DMPK)/ GlaxoSmithKline, the details of which will be included in the Study Reference Manual (SRM). Concentrations of GSK2798745, and co-administered statins and corresponding metabolites (~~**e.g.,**~~ atorvastatin and metabolites, ~~**or**~~ **and** simvastatin and metabolites)will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Plasma samples may be analyzed for metabolite M1 dependent upon the availability of an assay. Until this time, GSK will store the remaining plasma from the PK plasma samples for future possible metabolite analysis.

PREVIOUS TEXT

9.3.1 Analysis Populations

All Subjects Population

The 'All Subjects Population' is defined as all randomized subjects who receive at least one dose of study medication.

Per Protocol Population

The 'Per Protocol Population' is defined as subjects in the 'All Subjects' population having baseline and post-baseline assessments of the endpoint of interest for both periods.

REVISED TEXT

9.3.1. Analysis Populations

All Subjects Population

The 'All Subjects Population' is defined as all randomized subjects who receive at least one dose of study medication.

Per Protocol Population

~~**The 'Per Protocol Population' is defined as subjects in the 'All Subjects' population having baseline and post-baseline assessments of the endpoint of interest for both periods.**~~ **The 'Per Protocol Population' will consist of any Analysis Population subjects who are compliant with protocol-specific criteria. Subjects with specified protocol deviations will be excluded.**

PREVIOUS TEXT

9.4.1. Primary Analyses

Profiles for DLco will be summarized and presented graphically by treatment group as appropriate for the data. The change from baseline in DLco at Day 4, Day 6 and Day 7 will be calculated and summarized in tabular format and/or graphically by treatment. For the change from baseline in DLco, a statistical analysis will be performed using a mixed effect model with repeated measures with a fixed effect term for treatment, period and day, with a random effect for subject, day in the repeated statement with subject being subject by period, and baseline as a covariate, if data permit. Point estimates and associated 95% confidence intervals will be constructed for the differences between the active treatment and placebo.

Full details of analysis will be specified in the RAP.

REVISED TEXT

9.4.1. Primary Analyses

Profiles for DLco will be summarized and presented graphically by treatment group as appropriate for the data. The change from baseline in DLco at Day 4, Day ~~6~~ **5** and Day 7 will be calculated and summarized in tabular format and/or graphically by treatment. For the change from baseline in DLco, a statistical analysis will be performed using a mixed effect model with repeated measures with a fixed effect term for treatment, period and day, with a random effect for subject, day in the repeated statement with subject being subject by period, and baseline as a covariate, if data permit. Point estimates and associated 95% confidence intervals will be constructed for the differences between the active treatment and placebo.

Full details of analysis will be specified in the RAP.

PREVIOUS TEXT

9.4.2. Secondary Analyses

For other diffusing capacity pharmacodynamic (PD) endpoints such as DLno, D_M and Vc, similar analyses as in Section 9.4.1 will be provided, as data permit. For these PD endpoints, which may be $\log_e$ transformed prior to analysis to satisfy model assumptions, point estimates and their associated 95% confidence intervals will be back-transformed to provide point estimates and 95% confidence intervals for the ratios.

For pulmonary function measurement endpoints such as FVC, FEV1, FEF_{25-75} FEF_{50}, and FEF_{75}, FRC and EELV, similar analysis as in Section 9.4.1 will be provided, as data permit. For these PD endpoints, which may be $\log_e$ transformed prior to analysis to satisfy model assumptions, point estimates and their associated 95% confidence intervals will be back-transformed to provide point estimates and 95% confidence intervals for the ratios.

For respiratory rate measurement and dyspnea score, similar analysis as in Section 9.4.1 will be provided, as data permit.

In terms of DLco and DLno changes from baseline after saline challenge on Day 6 and after exercise on Day 7, the change from baseline for the challenge (after-before) will be calculated and summarized in tabular format by treatment. A statistical analysis will be performed using a mixed effect model with a fixed effect term for treatment, period and sequence, random effect term for subject-within-sequence, and baseline as a covariate, if data permit. Point estimates and associated 95% confidence intervals will be constructed for the differences between the active treatment and placebo.

In terms of ventilatory efficiency, scatter plots for Ve/VCO2 at Baseline and Day 7 will be performed and the slope or correlation of Ve/VO2 provided. Summary statistics for Ve and VCO2 and change from baseline measurement will be provided.

Details on the analysis of other endpoints will be provided in RAP.

Pharmacokinetic analyses will be the responsibility of the Clinical Pharmacology Modeling & Simulation Department within GlaxoSmithKline. Plasma GSK2798745 concentration-time data will be analyzed by non-compartmental methods with the latest available version of WinNonlin software. Calculations will be based on the actual sampling times recorded during the study. From the plasma concentration-time data, the pharmacokinetic parameters such as Cmax and AUC may be determined, as data permit.

The details of the PK analysis will be listed in the RAP.

REVISED TEXT

9.4.2. Secondary Analyses

For other diffusing capacity pharmacodynamic (PD) endpoints such as DLno, D_M and Vc, similar analyses as in Section 9.4.1 will be provided, as data permit. For these PD endpoints, which may be $\log_e$ transformed prior to analysis to satisfy model assumptions, point estimates and their associated 95% confidence intervals will be back-transformed to provide point estimates and 95% confidence intervals for the ratios.

For pulmonary function measurement endpoints such as FVC, FEV1, FEF_{25-75} FEF_{50}, and FEF_{75}, FRC and EELV, similar analysis as in Section 9.4.1 will be provided, as data permit. For these PD endpoints, which may be $\log_e$ transformed prior to analysis to satisfy model assumptions, point estimates and their associated 95% confidence intervals will be back-transformed to provide point estimates and 95% confidence intervals for the ratios.

For respiratory rate measurement and dyspnea score, similar analysis as in Section 9.4.1 will be provided, as data permit.

In terms of DLco and DLno changes from baseline after saline challenge on Day ~~6~~**5** and after exercise on Day 7, the change from baseline for the challenge (after-before) will be calculated and summarized in tabular format by treatment. A statistical analysis will be performed using a mixed effect model with a fixed effect term for treatment, period and sequence, random effect term for subject-within-sequence, and baseline as a covariate, if

data permit. Point estimates and associated 95% confidence intervals will be constructed for the differences between the active treatment and placebo.

In terms of ventilatory efficiency, scatter plots for Ve/VCO2 at Baseline and Day 7 will be performed and the slope or correlation of Ve/VO2 provided. Summary statistics for Ve and VCO2 and change from baseline measurement will be provided.

Details on the analysis of other endpoints will be provided in RAP.

Pharmacokinetic analyses will be the responsibility of the Clinical Pharmacology Modeling & Simulation Department within GlaxoSmithKline. Plasma GSK2798745 concentration-time data will be analyzed by non-compartmental methods with the latest available version of WinNonlin software. Calculations will be based on the actual sampling times recorded during the study. From the plasma concentration-time data, the pharmacokinetic parameters such as Cmax and AUC may be determined, as data permit.

The details of the PK analysis will be listed in the RAP.

PREVIOUS TEXT

9.4.4. Exploratory Analyses

Pharmacokinetic analyses will be the responsibility of the Clinical Pharmacokinetics Modeling & Simulation Department within GlaxoSmithKline. Calculations will be based on the actual sampling times recorded during the study. The systemic concentrations of any HMG-CoA reductase inhibitors (e.g., simvastatin, atorvastatin) and their metabolites will be summarized, as data permit. The details of the PK analysis will be listed in the RAP.

Data from the polysomnography sub-study will be presented in tablular and/or graphical format and summarized descriptively.

REVISED TEXT

9.4.4. Exploratory Analyses

Pharmacokinetic analyses **for the statins and any statin metabolites** will be the responsibility of the Clinical Pharmacokinetics Modeling & Simulation Department within GlaxoSmithKline. Calculations will be based on the actual sampling times recorded during the study. The systemic concentrations of any HMG-CoA reductase inhibitors (e.g., simvastatin, atorvastatin) and their metabolites will be summarized, as data permit. The details of the PK analysis will be listed in the RAP.

Data from the polysomnography sub-study will be presented in tablular and/or graphical format and summarized descriptively.

PREVIOUS TEXT

Appendix 1: Abbreviations and Trademarks

Abbreviations

ADHF	Acute decompensated heart failure
AE	Adverse Event
AHI	Apnea-hypopnea index
ALT	Alanine transaminase
AST	Aspartate aminotransferase
AUC	Area under the curve
BMI	Body mass index
Ca^{2+}	Calcium
C-CASA	Columbia Classification Algorithm for Suicide Assessment
CHF	Chronic heart failure
CI	Confidence intervals
Cmax	Maximum concentration
CMT2C	Charcot-Marie-Tooth disease Type 2C
CNS	Central nervous system
CO_2	Carbon dioxide
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disease
CPK	Creatine phosphokinase
CRF	Case report form
CRU	Clinical research unit
CSA	Central sleep apnea
CSR	Cheyne-Strokes respiration
C-SSRS	Columbia Suicide Severity Rating Scale
cTn	Troponin (cardiac)
CTX	Collagen Type I Telopeptide
CV	Cardiovacsular
CYP	Cytochrome P450 enzyme
DLco	Diffusing capacity of the lung for carbon monoxide
DLno	Diffusing capacity of the lung for nitric oxide
D_M	Alveolar-caplillary membrane conductance
DMPK	Drug Metabolism and Pharmacokinetics
DNA	Deoxyribonucleic acid
DPD	Deoxypyridinoline
E	Epinephrine
ECG	Electrocardiogram
EEG	Electroencephalography
EELV	End expiratory lung volume
EMG	Electromyogram
EOG	Electrooculogram
ER	Emergency Room
FDA	Food and Drug Administration

FEF	Forced expiratory flows
FEV_1	Forced expiratory volume in 1 second
FOBT	Fecal Occult Blood Test
FRC	Forced residual capacity
FSH	Follicle stimulating hormone
FVC	Forced vital capacity
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilence
GSK	GlaxoSmithKline
HBcAb	Hepatitis B core antibody
HbsAg	Hepatitis B surface antigen
HEK	Human embryonic kidney cells
HF	Heart failure
HR	Heart rate
HRT	Hormone Replacement Therapy
IDSL	Integrated Data Standards Library
IEC	Independent ethics committee
IgM	Immunoglobulin
INR	International normalized ratio
IP	Investigational Product
IRB	Institutional review board
IVRS	Interactive voice response system
Kg	Kilogram
KO	Knockout
LDH	Lactate dehydrogenase
LFT	Liver function tests
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
MSDS	Material Safety Data Sheet
Na	Sodium
NaCl	Sodium chloride
NE	Norepinephrine
nM	Nanomolar
NO	Nitric oxide
NOAEL	No observed adverse event level
NYHA	New York Heart Association
O_2	Oxygen
OSA	Obstructive sleep apnea
P1NP	Procollagen type 1 amino-terminal propeptide
PD	Pharmacodynamic
PFT	Pulmonary function tests
P-gp	P-glycoprotein
PGx	Pharmacogenomics
PK	Pharmacokinetics
PTH	Parathyroid hormone

PTS-DMPK	Platform Technologies and Sciences-Drug Metabolism and Pharmacokinetics
QTc	QT interval corrected
QTcB	QT interval corrected according to Bazett's formula
QTcF	QT interval corrected according to Fridericia's formular
RAP	Reporting and analysis plan
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
SAE	Serious Adverse Event
SDw	Within-subject variability
SOP	Standard Operating Procedure
SRM	Study reference manual
t½	Half-life
tmax	Time to maximum observed plasma concentration
TRPV4	Transient receptor potential vanilloid 4
µg	Micrograms
ULN	Upper limit of normal
VCO_2	Volume of carbon dioxide production
Vc	Capillary blood volume
VE	Ventilation
VO_2	Volume of oxygen
WBC	White blood cells

REVISED TEXT

Appendix 1: Abbreviations and Trademarks

Abbreviations

ADHF	Acute decompensated heart failure
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AHI	Apnea-hypopnea index
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Cmax	Maximum concentration
CMT2C	Charcot-Marie-Tooth disease Type 2C
CNS	Central nervous system
CO_2	Carbon dioxide
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disease

CPK	Creatine phosphokinase
CRF	Case report form
CRU	Clinical research unit
CSA	Central sleep apnea
CSR	Cheyne-Strokes respiration
C-SSRS	Columbia Suicide Severity Rating Scale
cTn	Troponin (cardiac)
CTX	Collagen Type I Telopeptide
CV	Cardiovacsular
CYP	Cytochrome P450 enzyme
DLco	Diffusing capacity of the lung for carbon monoxide
DLno	Diffusing capacity of the lung for nitric oxide
D_M	Alveolar-caplillary membrane conductance
DMPK	Drug Metabolism and Pharmacokinetics
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DPD	Deoxypyridinoline
E	Epinephrine
ECG	Electrocardiogram
EEG	Electroencephalography
EELV	End expiratory lung volume
EMG	Electromyogram
EOG	Electrooculogram
ER	Emergency Room
FDA	Food and Drug Administration
FEF	Forced expiratory flows
FEV_1	Forced expiratory volume in 1 second
FOBT	Fecal Occult Blood Test
FRC	Forced residual capacity
FSH	Follicle stimulating hormone
FVC	Forced vital capacity
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilence
GSK	GlaxoSmithKline
HBcAb	Hepatitis B core antibody
HbsAg	Hepatitis B surface antigen
HEK	Human embryonic kidney cells
HF	Heart failure
HR	Heart rate
HRT	Hormone Replacement Therapy
IDSL	Integrated Data Standards Library
IEC	Independent ethics committee
IgM	Immunoglobulin
INR	International normalized ratio
IP	Investigational Product
IRB	Institutional review board
I~~V~~WRS	Interactive ~~voice~~ **web** response system

Kg	Kilogram
KO	Knockout
LDH	Lactate dehydrogenase
LFT	Liver function tests
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
MSDS	Material Safety Data Sheet
Na	Sodium
NaCl	Sodium chloride
NE	Norepinephrine
nM	Nanomolar
NO	Nitric oxide
NOAEL	No observed adverse event level
NYHA	New York Heart Association
O_2	Oxygen
OSA	Obstructive sleep apnea
P1NP	Procollagen type 1 amino-terminal propeptide
PD	Pharmacodynamic
PFT	Pulmonary function tests
P-gp	P-glycoprotein
PGx	Pharmacogenomics
PK	Pharmacokinetics
PTH	Parathyroid hormone
PTS-DMPK	Platform Technologies and Sciences-Drug Metabolism and Pharmacokinetics
QTc	QT interval corrected
QTcB	QT interval corrected according to Bazett's formula
QTcF	QT interval corrected according to Fridericia's formular
RAP	Reporting and analysis plan
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
SAE	Serious Adverse Event
SDw	Within-subject variability
SOP	Standard Operating Procedure
SRM	Study reference manual
t½	Half-life
tmax	Time to maximum observed plasma concentration
TRPV4	Transient receptor potential vanilloid 4
µg	Micrograms
ULN	Upper limit of normal
VCO_2	Volume of carbon dioxide production
Vc	Capillary blood volume
VE	Ventilation
VO_2	Volume of oxygen
WBC	White blood cells

PREVIOUS TEXT

Appendix 9: Intravenous Saline Infusion

Rapid intravenous saline infusion has been employed to elicit fluid extravasation from the vasculature to the pulmonary interstitium. This methodology has been previously utilized in studies with healthy volunteers [Synder, 2006] and patients with heart failure [Fujimoto, 2013; Robbins, 2014; Andersen, 2015].

Subject Evaluation Prior to Infusion

On Day 5 of both study periods, the subject will be admitted to the clinical research unit (CRU) and undergo routine weight measurement and vital sign assessments including temperature, systolic and diastolic blood pressures, heart rate, respiratory rate, and pulse oximetry to assess O_2 saturation. The subject will also undergo a pulmonary examination.

While in a sitting position, a dyspnea assessment, utilizing a standardized 5-point Likert scale, will be obtained to document the subject's current status. A subject, who is not severely or very severely short of breath, will undergo an orthopnea test (placed supine with head $\leq$ 20 degrees relative to horizontal). After an equilibration period of 2 minutes, the 5-point scale will be repeated. A subject who reports a worse dyspnea score when supine compared to sitting will be categorized as having orthopnea. Subjects categorized with having orthopnea will not be eligible to participate in the saline infusion.

Study treatment will be administered after all planned pre-dose assessments for Day 5 are completed.

Saline Infusion Procedure

A subject, who is judged by the Investigator to be in a stable condition and without obvious congestion, will then undergo the intravenous saline infusion procedure. This procedure will be conducted in the clinical research unit, which is adjacent to the catheterization laboratory, in the presence of a cardiologist and monitoring personnel. A crash cart will be accessible, and intravenous diuretic will be available, if necessary.

An 18-gauge venous catheter will be inserted into a prominent antecubital vein while the subject is semi-recumbent (approximately 45°). The subject will remain in a semi-recumbent position throughout the procedure.

DLco and DLno measurements and simple spirometry assessments will be obtained prior to the initiation of the infusion.

Prewarmed 0.9% normal saline will be infused intravenously at a rate of 150 mL/min to a total volume of 500 mL. During the saline infusion, blood pressure, heart rate, respiratory rate, and O_2 saturation, will be continuously monitored, and any physical symptoms recorded.

DLco and DLno measurements and spirometry assessments will be obtained after the completion of the infusion.

Post-infusion Monitoring

Following the completion of the saline infusion procedure, the subject will remain in the clinical research unit for the next 4 hours. Blood pressure, heart rate, respiratory rate, and O_2 saturation, will be continuously monitored, and any physical symptoms recorded. The subject will also be assessed for dyspnea and orthopnea, as described above, at hourly intervals.

After the 4-hour period, the subject will be eligible to leave the CRU if heart rate and respiratory rate are within 20% of the baseline value, O_2 saturation is within 2% of the baseline value, and if the dyspnea score is the same as the baseline score.

The subject will continue to be monitored by the Preventice BodyGuardian Remote Monitoring System as described in Section 7.4.7 of the protocol; ECG, heart rate, respiratory rate and activity level will be measured continuously.

Any worsening of heart failure that requires treatment in the 48 hours subsequent to the saline infusion will be reported as a serious adverse event.

Individual Stopping Criteria During the Saline Infusion Procedure

If the subject demonstrates one of the following changes during the saline infusion, the infusion should be discontinued:

- Decrease in O2 saturation of >4% compared to baseline
- Increase in respiratory rate of >20% compared to baseline
- Any increase in dyspnea perceived by the patient

If the subject has met one of these criteria during or immediately after the infusion, the subject should be carefully monitored and given intravenous diuretic treatment, intravenous or sublingual nitrates, or other treatment as required.

The subject may continue in the study. However, if this response occurred in the first study period, the subject should not undergo a second saline infusion in the subsequent study period.

Discontinuation of Saline Infusion Procedure

If during the conduct of the study, **two** subjects meet the individual stopping criteria during the infusion or demonstrate a worsening of heart failure in the 48 hours after completion of the infusion, this challenge procedure will be removed from the study design. No other subjects will undergo this assessment.

Reporting of Adverse Outcomes Associated with the Saline Infusion

Any of the following outcomes must be reported to the sponsor as a serious adverse event:

- Treatment with intravenous diuretic subsequent to the saline infusion
- Need for continued monitoring in the CRU beyond the 4-hour mandated observation period
- Any worsening of heart failure that requires treatment during the 48-hour monitoring period after the saline infusion procedure

These events will be reported by the investigator as serious adverse events in the InForm electronic data collection tool. They will be reported by the sponsor to the FDA as an Expedited Report via electronic submission in eCTD format to the IND, as a 7-day or 15-day IND safety report, as applicable per timeframe specified in 21 CFR 312.32(c)(1). The completed report will also be provided to the IRB.

REVISED TEXT

Appendix 9: Intravenous Saline Infusion

Rapid intravenous saline infusion has been employed to elicit fluid extravasation from the vasculature to the pulmonary interstitium. This methodology has been previously utilized in studies with healthy volunteers [Synder, 2006] and patients with heart failure [Fujimoto, 2013; Robbins, 2014; Anderson, 2015].

Subject Evaluation Prior to Infusion

On Day 5 of both study periods, the subject will be admitted to the ~~**clinical research**~~ unit ~~**(CRU)**~~ and undergo routine weight measurement and vital sign assessments including temperature, systolic and diastolic blood pressures, heart rate, respiratory rate, and pulse oximetry to assess O_2 saturation. The subject will also undergo a pulmonary examination.

While in a sitting position, a dyspnea assessment, utilizing a standardized 5-point Likert scale, will be obtained to document the subject's current status. A subject~~s~~ who is not severely or very severely short of breath~~s~~ will undergo an orthopnea test (placed supine with head $\leq$ 20 degrees relative to horizontal). After an equilibration period of 2 minutes, the 5-point scale will be repeated. A subject who reports a worse dyspnea score when supine compared to sitting will be categorized as having orthopnea. Subjects categorized ~~**with**~~ **as** having orthopnea will not be eligible to participate in the saline infusion.

Study treatment will be administered after all planned pre-dose assessments for Day 5 are completed.

Saline Infusion Procedure

A subject~~s~~ who is judged by the Investigator to be in a stable condition and without obvious congestion~~s~~ will then undergo the intravenous saline infusion procedure. This

procedure will be conducted in the ~~**clinical research**~~ unit, ~~**which is adjacent to the catheterization laboratory**~~, in the presence of a cardiologist and monitoring personnel. A crash cart will be accessible, and intravenous diuretic will be available, if necessary.

A~~**n 18-gauge**~~ venous catheter will be inserted ~~**into a prominent antecubital vein**~~ while the subject is semi-recumbent (approximately 45°). The subject will remain in a semi-recumbent position throughout the procedure.

DLco and DLno measurements and simple spirometry assessments will be obtained prior to the initiation of the infusion.

~~**Prewarmed**~~ 0.9% normal saline will be infused intravenously at a rate of **approximately** 150 mL/min to a total volume of 500 mL. During the saline infusion, blood pressure, heart rate, respiratory rate, and O_2 saturation**,** will be continuously monitored, and any physical symptoms recorded.

DLco and DLno measurements and spirometry assessments will be obtained after the completion of the infusion. **Additional details are provided in the SRM.**

Post-infusion Monitoring

Following the completion of the saline infusion procedure, the subject will remain in the ~~**clinical research**~~ unit for the next 4 hours. Blood pressure, heart rate, respiratory rate, and O_2 saturation~~,~~ will be continuously monitored, and any physical symptoms recorded. The subject will also be assessed for dyspnea and orthopnea, as described above, at hourly intervals.

After the 4-hour period, the subject will be eligible to leave the ~~**CRU**~~ **unit** if heart rate and respiratory rate are within 20% of the baseline value, O_2 saturation is within 2% of the baseline value, and if the dyspnea score is the same as the baseline score.

The subject will continue to be monitored by the Preventice BodyGuardian Remote Monitoring System as described in Section 7.4.7 of the protocol; ECG, heart rate, respiratory rate and activity level will be measured continuously.

Any worsening of heart failure that requires treatment in the 48 hours subsequent to the saline infusion will be reported as a serious adverse event.

Individual Stopping Criteria During the Saline Infusion Procedure

If the subject demonstrates one of the following changes during the saline infusion, the infusion should be discontinued:

- Decrease in O2 saturation of >4% compared to baseline
- Increase in respiratory rate of >20% compared to baseline
- Any increase in dyspnea perceived by the patient

If the subject has met one of these criteria during or immediately after the infusion, the subject should be carefully monitored and given intravenous diuretic treatment, intravenous or sublingual nitrates, or other treatment as required.

The subject may continue in the study. However, if this response occurred in the first study period, the subject should not undergo a second saline infusion in the subsequent study period.

Discontinuation of Saline Infusion Procedure

If during the conduct of the study, **two** subjects meet the individual stopping criteria during the infusion or demonstrate a worsening of heart failure in the 48 hours after completion of the infusion, this challenge procedure will be removed from the study design. No other subjects will undergo this assessment.

Reporting of Adverse Outcomes Associated with the Saline Infusion

Any of the following outcomes must be reported to the sponsor as a serious adverse event:

- Treatment with intravenous diuretic subsequent to the saline infusion
- Need for continued monitoring in the ~~**CRU**~~ **unit** beyond the 4-hour mandated observation period
- Any worsening of heart failure that requires treatment during the 48-hour monitoring period after the saline infusion procedure

These events will be reported by the investigator as serious adverse events in the InForm electronic data collection tool. They will be reported by the sponsor to the FDA as an Expedited Report via electronic submission in eCTD format to the IND, as a 7-day or 15-day IND safety report, as applicable per timeframe specified in 21 CFR 312.32(c)(1). The completed report will also be provided to the IRB.

12.11. Appendix 11: Country Specific Requirements

No country-specific requirements exist.