TITLE PAGE

Protocol Title: A randomized, repeat dose, open label, parallel group, multi-center study to evaluate the effect of daprodustat compared to darbepoetin alfa on forearm blood flow in participants with anemia of chronic kidney disease that are not dialysis dependent

Protocol Number: 205767/Amendment 02

Short Title: **A**nemia **S**tudies in **C**KD: **E**rythropoiesis via a **N**ovel PHI **D**aprodustat – **F**orearm **B**lood **F**low (**ASCEND-FBF**)

Compound Number: GSK1278863

Sponsor Name and Legal Registered Address:

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Regulatory Agency Identifying Number(s):

IND Number 101,291

EudraCT number: 2017-002268-42

Approval Date: 07-AUG-2019

SPONSOR SIGNATORY:

PPD

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7 August 2019
Date

PPD

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Amendment 2	07-Aug-2019
Amendment 1	29-Jan-2018
Original Protocol	17-Nov-2017

Amendment 2 07-AUG-2019

Overall Rationale for the Amendment: Due to a challenge agent suddenly becoming commercially unavailable with no immediate ability to reobtain this, the study has been modified to allow flexibility for the forearm blood flow testing to be performed with this agent if it becomes available again, and to be performed without it if not.

Section # and Name	Description of Change	Brief Rationale
Short Title	The short title of the study was changed.	All global studies for this asset have a similar nomenclature; this change is to align globally.
1 Synopsis	The short title was changed.	See above
1 Synopsis	"Citrate" was changed to "acetate" for L-NG-monomethyl arginine acetate (L-NMMA) throughout the document.	For correction.
1 Synopsis	A note was added at the end.	For clarity of how the challenge agents are to be used in the study.
1 and 4 Objectives and Endpoints	The word "ratio" was removed from several endpoints.	For clarity of reading only; there is no change in how the endpoints are characterized nor calculated.
1 and 4 Objectives and Endpoints	A Safety section was added. The existing safety endpoints were then moved to this section. No additions or deletions in the endpoint text.	To better characterize the nature of the endpoint.
1 and 4 Objectives and Endpoints	The last exploratory endpoint was removed.	No further models have been deemed necessary to explore as the existing exploratory endpoint is sufficient.
2 Schedule of Activities	A central lab hematology check was added to on study visits.	For safety, to compare with HemoCue. This is also consistent with the other daprodustat protocols.

Section # and Name	Description of Change	Brief Rationale
2 Schedule of Activities	The potential repeat FBF visits were added to the schedule of activities table.	For clarity on the procedures to be performed at the repeat FBF visits.
3.1 Study Rationale	The term “if available” was added.	To allow for alteration in assessments depending on the availability of the challenge agent.
5.5 Dose Justification	“Clinical judgement” was added to the decision making regarding darbepoetin dosing.	For safety of the participants; this allows the PI to exercise clinical judgement and management when dosing rhEPO to study participants.
6.1 Inclusion Criteria and 6.2 Exclusion Criteria	Minor additions to the lab reporting parameters were added to reflect both conventional and SI units.	For ease of identifying study criteria using both units.
6.2 Exclusion Criteria	Ferritin exclusion criteria was lowered.	To facilitate enrolment.
6.2 Exclusion Criteria	Updated the language in the exclusion criterion #17 regarding blood pressure.	Changed language to align with updates in benefit/risk profile and DCSI.
6.2 Exclusion Criteria	Minor update to the platelet count reporting.	For correction of reporting units..
7.1.1.2 Darbepoetin Alfa Dosing Information	“Clinical judgement” was added to the decision making regarding darbepoetin dosing. Additionally, the option to hold or delay a dose was included.	For safety of the participants; this allows the PI to exercise clinical judgement and management when dosing rhEPO to study participants.
7.1.2 Challenge Agents	A note was added at the beginning of the section.	For clarity of how the challenge agents are to be used in the study as well as described in the protocol.
7.2 Dose Modification	Added rows to Table 1 to allow subjects with a rapid rise in Hgb to have their treatment dose decreased.	To be consistent with management in other daprodustat studies as well as prevent unnecessary subject withdrawal.
7.7.2 Prohibited Medications	High dose clopidogrel (300mg) was added.	An additional example of a strong CYP2C8 inhibitor was added.
8.1 Discontinuation Criteria	A statement was added at the end for the PI to be able to withdraw the participant at his/her discretion.	For safety of the participants; this allows the PI to exercise clinical judgement and management.
8.1.2 Hgb Stopping Criteria	The structure of Table 2 was modified.	For clear distinction between assessment of the Hgb and resultant action(s).
8.1.2 Hgb Stopping Criteria	The allowable increase of Hgb over a 2 week period was changed.	To be consistent with management in other similar studies as well as prevent unnecessary subject withdrawal.
8.1.2 Hgb Stopping Criteria	A stopping criterion was added of Hgb increase over a 4 week period	For patient safety.
8.1.2 Hgb Stopping Criteria	Removed criterion for mandatory withdrawal of subjects who show rapid rise in Hgb. Subjects exhibiting a rapid rise in	To be consistent with management in other daprodustat studies as well as prevent unnecessary subject withdrawal.

Section # and Name	Description of Change	Brief Rationale
	Hgb over a 2- or 4-week period may now have their treatment dose decreased at Day 28 instead (see change made to Section 7.2, above).	
9.2.5 Adverse Events of Special Interest	An AESI was added to this section.	A new AESI of 'worsening of hypertension' has been identified (see risk table in Appendix 7).
9.2.8 Pregnancy	Text was updated to reflect guidance given in Appendix 5.	For correction.
9.6.1 Venous Occlusion…..	Description of the procedure to be followed if one or both of the secondary challenge agents are unavailable.	For clarity.
9.6.1.2 Participants Unable to….	Provision was made in the protocol for a potential repeat of the FBF procedures on Day 1 if the participant agrees.	For retention of participants in the study.
9.6.1.3 FBF Quality Control Criteria	An update was made as to where the QC criteria can be found.	For correction.
9.6.1.4 Delayed or Repeat FBF Procedures	This was revised to describe the study procedures to be followed in the event of delays or repeats to FBF procedures.	For clarity of guidance to the investigators.
10.1 Sample Size Determination	Additional language was provided to further describe the procedures for sample size; no changes made to the intent of this analysis.	For clarification of maximum number of subjects if sample size re-estimation suggests a different sample size is needed.
10.1.2 Sample Size Re-estimation	See above (10.1).	See above (10.1).
10.2.1.2 Secondary Comparisons and 10.3.3 Other Analyses	This analysis was updated to reflect the potential for decreased data due to unavailability of challenge agents.	For clarification of procedures in this instance.
10.3.4 Interim Analysis	Text was added to this section.	To better explain the purpose of the potential interim analysis, as well as add more details around the operating characteristics and decision thresholds.
12.2 Clinical Laboratory Tests	Provision was made for the new hematology lab test to be performed locally if the central lab is unavailable.	For better flow of study and promote recruitment and retention.
12.7 Appendix 7: Risk Assessment	Several updates were made to the risk assessment table.	To reflect current information regarding risk and to align with changes made to the risk table across the global asset program.
12.8.2 (Sub Study) Objectives	The word "ratio" was removed from the endpoints.	For consistency with the main endpoints; see above.

Section # and Name	**Description of Change**	**Brief Rationale**
and Endpoints		
12.8.3 (Sub Study) Study Design	Update was made to the selection of participants from each treatment group.	To facilitate recruitment and ease of site management.
12.8.5 (Sub Study) Study Assessments	Minor updates made to the description of the devices used.	For clarification and correction.

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1. SYNOPSIS

Protocol Title: A randomized, repeat dose, open label, parallel group, multi-center study to evaluate the effect of daprodustat compared to darbepoetin alfa on forearm blood flow in participants with anemia of chronic kidney disease that are not dialysis dependent

Short Title: **A**nemia **S**tudies in **C**KD: **E**rythropoiesis via a **N**ovel PHI **D**aprodustat – **F**orearm **B**lood **F**low (**ASCEND-FBF**)

Rationale: Several recent studies have demonstrated that the treatment of patients with anemia of CKD that returns them to normal - as compared to lower - hemoglobin (Hgb) levels by using recombinant human erythropoietins (rhEPOs) is associated with a higher mortality rate, an increased risk for a composite endpoint of death, myocardial infarction (MI), hospitalization for congestive heart failure and stroke, and an increased risk of death and major cardiovascular events (CV) (i.e. myocardial infarction, stroke, heart failure, myocardial ischemia). The reason for the increased risk for mortality and CV events is not known, but has been hypothesized to be due to the dose of epoetin alfa administered rather than the level of Hgb achieved, with higher doses associated with increased risk.

It is known that endothelial dysfunction may be predictive of CV events. In this regard, it has been shown that endothelial dysfunction, as assessed by flow-mediated dilation of the brachial artery, is an independent predictor of major cardiovascular events in peritoneal dialysis patients; however, in this study it was not clear whether these patients received rhEPO. It has increasingly become apparent that the vascular endothelium plays a critical role in normal vascular homeostasis through several mechanisms, in particular release of endothelium-derived relaxing factor/nitric oxide (NO), effects on vascular smooth muscle to maintain vasodilator tone, and vasoconstriction through release of endothelin and conversion of angiotensin-I to angiotensin-II. Thus, it has been shown that rhEPO, administered as epoetin alfa, administered to both patients with anemia of CKD as well as participants with normal renal function, impaired methacholine-stimulated increases in forearm blood flow, while no effect was seen for sodium nitroprusside-stimulated vasodilation. In addition, patients with renal failure who were dialysis-dependent and treated for their anemia with three-times weekly subcutaneous doses of rhEPO for 12 weeks, had an increase in forearm vascular resistance in response to norepinephrine as compared to pre-treatment values. These results suggest that epoetin alfa, and potentially other rhEPOs, can negatively affect endothelial function, an effect which may, at least in part, play a role in the increased CV risk seen with administration of these agents.

Daprodustat has demonstrated an ability to effectively raise Hgb concentrations with lower EPO levels than those observed after administration of rhEPOs. Therefore, daprodustat has the potential to treat anemia of CKD with a lower CV risk than is observed with the rhEPOs. While the effect of rhEPOs on endothelial function has been assessed, to date the effect of daprodustat or other prolyl hydroxylase inhibitor (PHI) compounds on endothelial function has not. Therefore, the purpose of this study is to compare the effect of daprodustat to darbepoetin alfa on endothelial function by assessing FBF in patients with anemia of CKD by using venous occlusion plethysmography as a means to estimate the potential for daprodustat to have a lower risk of CV events as

compared to rhEPO. Venous occlusion plethysmography is recognized as a robust methodology in the study of human vascular physiology when used in combination with intra-arterial administration of challenge agents to assess the effect of agents on FBF. In the present study, which involves two forearm blood flow procedures six weeks apart, it is planned to use acetylcholine (ACh) to assess the effect of daprodustat and darbepoetin alfa on endothelial-dependent vasodilation, sodium nitroprusside (SNP) for assessing endothelial-independent vasodilation, and L-N^G-monomethyl arginine acetate (L-NMMA), an inhibitor of NO synthase, as a measure of basal NO synthesis.

N.B. Throughout the document the use of these challenge agents is described on the assumption that they are available for both FBF visits for an individual participant. If either of the secondary endpoint challenge agents (SNP and L-NMMA) are unavailable, then that agent will be excluded from the FBF procedure for that individual participant.

Therefore, if L-NMMA is not available then the FBF procedure will be finished after the last SNP infusion. Similarly, if SNP is not available for any reason, then the L-NMMA (if available) will be given following the ACh challenge. If both challenge agents become unavailable for any reason, then only ACh will be given.

In the event that a challenge agent becomes available again while a patient is mid-study (i.e. between Day 1 and Day 42 FBF procedures), then the challenge agent will still not be used at Day 42 due to the lack of baseline comparator data.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
To compare the effect of daprodustat to darbepoetin alfa on endothelial function	• Change in FBF ratio from Day 1 to Day 42 in response to acetylcholine
Principle Secondary	
To further compare the effect of daprodustat to darbepoetin alfa on endothelial function	• Change in the absolute FBF from Day 1 to Day 42 in response to acetylcholine
Secondary	
To compare the effect of daprodustat to darbepoetin alfa on endothelium-independent vasodilation	• Change in FBF ratio from Day 1 to Day 42 in response to sodium nitroprusside • Change in the absolute FBF from Day 1 to Day 42 in response to sodium nitroprusside
To compare the effect of daprodustat to darbepoetin alfa on basal endothelial NO synthesis	• Change in FBF ratio from Day 1 to Day 42 in response to L-NMMA • Change in the absolute FBF on Day 1 to Day 42 in response to L-NMMA
To compare the effect of daprodustat on the FBF response to acetylcholine, sodium nitroprusside and L-NMMA between Day 42 and Day 1	• Change in FBF ratio in response to each individual challenge agent at Day 42 vs Day 1 in participants treated with daprodustat • Change in the absolute FBF in response to each individual challenge agent at Day 42 vs

Objectives	Endpoints
	Day 1 in participants treated with daprodustat
To compare the effect of darbepoetin alfa on the FBF response to acetylcholine, sodium nitroprusside and L-NMMA between Day 42 and Day 1	• Change in FBF ratio in response to each individual challenge agent at Day 42 vs Day 1 in participants treated with darbepoetin alfa • Change in the absolute FBF in response to each individual challenge agent at Day 42 vs Day 1 in participants treated with darbepoetin alfa
To compare the effect of daprodustat to darbepoetin alfa on vascular compliance	• Change in Augmentation Index (an indicator of arterial stiffness) as estimated by radial arterial pulse contours from Day 1 to Day 42 • Change in pulse wave velocity (PWV) from Day 1 to Day 42
Safety	
Assess safety and tolerability	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) including AEs of special interest • Reasons for discontinuation of randomized study treatment • Absolute values and changes from baseline in clinical laboratory parameters, electrocardiogram (ECG) parameters, blood pressure (BP) and heart rate (HR)
Exploratory	
To explore the relationship between diabetes status and endothelial function	• FBF response to each individual challenge agent in relation to diabetes status

Overall Design: This study will use a randomized, repeat dose, open label, parallel group design, in adult, male and female participants with anemia of CKD not on dialysis (ND) that are currently not treated with rhEPOs (i.e. no rhEPO use within the 12 weeks prior to the screening visit and through Day 1).

- The study will comprise three study periods: a screening period starting up to 30 days prior to Day 1, a 42 day (6 week) treatment period with 4 visits starting at Day 1, and a follow-up visit up to 14 days later.

- Participants will be randomized to either daprodustat or darbepoetin alfa. A central randomization approach will be used with stratification by center.

- All participants will be treated with the goal of achieving a Hgb level within the range of 10.0 to 12.0 g/dL, consistent with national guidelines.

- FBF and PWV will be conducted at the beginning (Day 1) and end (Day 42) of the treatment period.

- This study will include an optional, Non-Contact, Optical Forearm Plethysmography (NC-OFP) Sub-Study.

Number of Participants: A sufficient number of participants will be enrolled such that at least 50 participants comprise the Pharmacodynamic Per-Protocol (PDPP) Population (i.e. all randomized participants who provide pharmacodynamic (PD) data at Day 1 and Day 42 and complete the protocol without important deviations).

Treatment Groups and Duration: Eligible participants will be randomized to receive either daprodustat (Day 1 through Day 41) or darbepoetin alfa (administered Day 1, 14 & 28).

2. SCHEDULE OF ACTIVITIES (SOA)

Procedure	Screening[1]	Treatment Period[2]						Follow-up[3]
	Up to 30 Days	Day 1	Repeat Day 1[17]	Day 14	Day 28	Day 42	Repeat Day 42[17]	
Informed consent	X							
Eligibility Criteria	X	X[4]						
Physical examinations, Medical History, Demographics	X	X[5]						
HemoCue Hgb	X	X[6]	X	X	X	X	X	
eGFR	X							
Vital signs	X	X[6]	X	X	X	X	X	X
Clinical chemistry[7]	X					X		X
Hematology[7]	X	X		X	X	X		X
Serum pregnancy test (FRP only)	X							
Urine pregnancy test (FRP only)[8]		X	X	X	X	X	X	X
Folate & Vitamin B_{12}	X							
12 Lead ECG	X	X[6]				X		X
Ferritin & TSAT	X							
hs-CRP	X	X				X		
Females only: FSH[9]	X							
Brachial artery palpation	X							
Randomization		X						
Daprodustat administration[10]		<==========						
Darbepoetin alfa administration[10]		X[10]		X	X	See footnote 18		
Study treatment adjustment[11]					X			
Liver chemistry monitoring				X	X			
International Normalized Ratio (INR)[12]	X	X	X			X	X	

Procedure	Screening[1]	Treatment Period[2]						Follow-up[3]
	Up to 30 Days	Day 1	Repeat Day 1[17]	Day 14	Day 28	Day 42	Repeat Day 42[17]	
Hepatitis B and C screening[13]	X							
HbA1c, lipids[7]		X						
Forearm Blood Flow (FBF)[14]		X	X			X	X	
Pulse Wave Velocity (PWV)[14]		X	X			X	X	
AE and SAE Assessment	X[15]	X	X	X	X	X	X	X
Blood draw for storage biomarkers[16]		X				X		
Review concomitant medications	X	X	X	X	X	X	X	X

[1] All participants will undergo screening assessments within 30 days of enrolment.

[2] Allowable time window ± 2 days except for the forearm blood flow (FBF) & pulse wave velocity (PWV) as noted.

[3] Allowable time window ± 3 days. Follow up will occur 7-14 days ± 3 following completion of the last FBF procedure. See Section 8.2 for guidance regarding EW participants.

[4] Eligibility criteria to be assessed prior to performance of the FBF procedure include HemoCue hemoglobin (Hgb), vital signs & 12-lead electrocardiogram (ECG).

[5] Only a brief physical exam on Day 1.

[6] As detailed in Exclusion Criteria (Section 6.2).

[7] Clinical chemistry, hematology and other laboratory tests as listed in Appendix 2.

[8] Local urine pregnancy testing will be standard for protocol unless serum testing is required by local regulations or Institutional Review Board (IRB)/ Independent Ethics Committee (IEC).

[9] As detailed in Inclusion Criteria (Section 6.1).

[10] The initial dose of study treatment will be given **after** the completed Day 1/Repeat Day 1 FBF & PWV procedures. Participants will receive **EITHER** daprodustat **OR** darbepoetin alfa. The final dose of daprodustat will be Day 41 (i.e., the day prior to the FBF & PWV procedures), while the final dose of darbepoetin alfa will be Day 28. If repeat procedures are needed the dosing will be extended (See Section 9.6.1.4).

[11] See Section 7.2 for details on study treatment adjustment

[12] Applies only to participants on anti-coagulant therapy; further details concerning measurement of the international normalised ratio (INR) and subsequent actions can be found in the study reference manual (SRM).

[13] If test otherwise performed within 3 months prior to first dose of study treatment, testing at screening is not required

[14] The FBF & PWV assessments are to be performed following confirmation of INR < 3.0 for participants on anticoagulant therapy. The Day 42 FBF & PWV assessments can be delayed up to 1 week (i.e., Day 49)., while randomization (Day 1) should be delayed until the participant's INR < 3.0 (Section 9.6.1.1).

[15] Only serious adverse events (SAEs) assessed as related to study participation are collected at this visit. See Appendix 4 for additional details.

[16] As described in Section 9.8

[17] Indicates the procedures to be performed on the repeat visits only if the visit is deemed necessary. See Section 9.6.1.4

[18] Darbepoetin is only to be given on Day 42 if a Repeat Day 42 is needed.

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

3. INTRODUCTION

Daprodustat (GSK1278863) is a small molecule, oral inhibiter of the hypoxia-inducible factor (HIF) prolyl-4-hydroxylase (PHD) enzymes. This novel molecule may present several important advantages over recombinant, human erythropoietins (rhEPOs) and analogs for the treatment of anemia of chronic kidney disease (CKD). It is an oral medication and does not require cold-chain storage as do some rhEPOs, thus increasing ease of use for patients. Moreover, data indicate that - similar to other HIF-PHD inhibitors - daprodustat can effectively raise hemoglobin (Hgb) concentrations in CKD patients that are either hemodialysis-dependent (HD) or not on dialysis (ND) with lower erythropoietin (EPO) levels than those observed after administration of rhEPOs (Besarab, 2015; Holdstock, 2016). Because of the increased cardiovascular (CV) risk associated with raising Hgb concentrations through large increases in EPO levels (Pfeffer, 2009), daprodustat has the potential to treat patients with less CV risk than rhEPOs (*vida infra*). Additional potential benefits include improving iron availability for erythropoiesis and successfully treating rhEPO hypo-responders (Holdstock, 2016).

3.1. Study Rationale

Several recent studies have demonstrated that the treatment of patients with anemia of CKD that returns them to normal - as compared to lower - Hgb levels by using rhEPOs is associated with a higher mortality rate (Besarab, 1998), an increased risk for a composite endpoint of death, myocardial infarction, hospitalization for congestive heart failure and stroke (Singh, 2006), and an increased risk of death and major cardiovascular events (i.e. myocardial infarction, stroke, heart failure, myocardial ischemia; Pfeffer, 2009). The reason for the increased risk for mortality and CV events is not known, but has been hypothesized to be due to the dose of epoetin alfa administered rather than the level of Hgb achieved (Szczech, 2008), with higher doses associated with increased risk.

It is known that endothelial dysfunction may be predictive of CV events (Lerman, 2005; Ras, 2013). In this regard, it has been shown that endothelial dysfunction, as assessed by flow-mediated dilation of the brachial artery, was an independent predictor of major cardiovascular events in peritoneal dialysis patients; however, in this study it was not clear whether these patients received a rhEPO (Lee, 2014). It has increasingly become apparent that the vascular endothelium plays a critical role in normal vascular homeostasis through several mechanisms, in particular release of endothelium-derived relaxing factor/nitric oxide (NO), effects on vascular smooth muscle to maintain vasodilator tone, and vasoconstriction through release of endothelin and conversion of angiotensin-I to angiotensin-II (Deanfield, 2007). Thus, it has been shown that EPO, administered as epoetin alfa, administered to both patients with anemia of CKD as well as participants with normal renal function, impaired methacholine-stimulated increases in forearm blood flow, while no effect was seen for sodium nitroprusside-stimulated vasodilation (Annuk, 2006). In addition, patients with renal failure who were dialysis-dependent and treated for their anemia with three-times weekly subcutaneous doses of rhEPO for 12 weeks, had an increase in forearm vascular resistance in response to norepinephrine as compared to pre-treatment values (Hand, 1995). These results suggest that epoetin alfa, and potentially other rhEPOs, can negatively affect endothelial function,

an effect which may, at least in part, play a role in the increased CV risk seen with administration of these agents.

Daprodustat has demonstrated an ability to effectively raise Hgb concentrations with lower EPO levels than those observed after administration of rhEPOs (*vida supra*). Therefore, daprodustat has the potential to treat anemia of CKD with a lower CV risk than is observed with the rhEPOs. While the effect of rhEPOs on endothelial function has been assessed, to date the effect of daprodustat or other PHI compounds on endothelial function has not. Therefore, the purpose of this study is to compare the effect of daprodustat to darbepoetin alfa on endothelial function by assessing forearm blood flow (FBF) in patients with anemia of CKD by using venous occlusion plethysmography as a means to estimate the potential for daprodustat to have a lower risk of CV events as compared to rhEPO. Venous occlusion plethysmography is recognized as a robust methodology in the study of human vascular physiology when used in combination with intra-arterial administration of challenge agents to assess the effect of agents on FBF (Wilkinson, 2001). In the present study, it is planned to use acetylcholine (ACh) to assess the effect of daprodustat and darbepoetin alfa on endothelial-dependent vasodilation, sodium nitroprusside (SNP) (if available) for assessing endothelial-independent vasodilation, and L-N^G-monomethyl arginine acetate (L-NMMA) (if available), an inhibitor of NO synthase as a measure of basal NO synthesis (Wilkinson, 2001).

In addition to the assessment of endothelial function as a biomarker of potential rhEPO-induced cardiovascular risk, carotid to femoral pulse wave velocity (PWV) will be measured as an assessment of arterial stiffness. PWV is considered the 'gold standard' methodology for assessing arterial stiffness, with a growing body of evidence showing the predictive value of PWV and CV disease and risk (Pereira, 2015). In this regard, both normal values for PWV, from a population with no apparent risk factors, and values from a population with various CV risk factors have been proposed (Boutouyrie, 2010). Therefore, PWV will also be measured as an additional biomarker of potential rhEPO-induced cardiovascular risk.

3.2. Background

PHIs are an emerging class of agents under investigation for the treatment of anemia of CKD. These molecules stimulate erythropoiesis through inhibition of HIF-PHD enzymes (PHD1, PHD2, PHD3). This activity results in the accumulation of HIFα transcription factors which leads to increased transcription of HIF-responsive genes, stimulating components of the natural response to hypoxia. During hypoxia, PHD enzymes are inhibited, resulting in the accumulation of unhydroxylated HIFα subunits, which dimerize with HIFβ subunits to affect the transcription of HIF-responsive genes, including EPO and others involved in increasing oxygen availability and utilization. Other functions regulated by HIFs include iron metabolism and utilization, angiogenesis, extracellular matrix metabolism, apoptosis, energy and glucose metabolism, vascular tone, cell adhesion, and motility (Haase, 2013).

3.3. Benefit: Risk Assessment

Summaries of findings from both clinical and non-clinical studies conducted with daprodustat can be found in the daprodustat Investigator's Brochure (IB) and IB supplement(s), if applicable.

3.3.1. Risk Assessment

The potential risks of clinical significance, including adverse events of special interest (See Section 9.2.5 for details), and the mitigation strategies for this protocol taking into account the results of completed clinical and nonclinical studies with daprodustat are outlined in Appendix 7.

3.3.2. Benefit Assessment

In clinical trials of up to 24 weeks in duration, in participants with anemia of CKD, daprodustat has been shown to treat Hgb to target range. Data from prior studies with daprodustat suggest that the increases in Hgb are achieved with EPO exposure lower than those observed with rhEPO (Holdstock, 2016).

3.3.3. Overall Benefit: Risk Conclusion

Daprodustat demonstrates a positive benefit vs. risk based on the evidence as follows. In clinical trials up to 24 weeks in duration, daprodustat maintained Hgb to target range in participants with anemia of CKD (both ND and HD) with a safety profile consistent with the patient population.

This protocol employs precautions to mitigate known and potential risks to enrolled participants (see Appendix 7 for details). Given these precautions, as well as the potential benefit that daprodustat holds for the treatment of anemia of CKD compared to the current standard, the overall benefit risk balance is considered to be positive.

4. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To compare the effect of daprodustat to darbepoetin alfa on endothelial function	• Change in FBF ratio from Day 1 to Day 42 in response to acetylcholine
Principle Secondary	
To further compare the effect of daprodustat to darbepoetin alfa on endothelial function	• Change in the absolute FBF from Day 1 to Day 42 in response to acetylcholine
Secondary	
To compare the effect of daprodustat to darbepoetin alfa on endothelium-independent vasodilation	• Change in FBF ratio from Day 1 to 42 in response to sodium nitroprusside • Change in the absolute FBF from Day 1 to Day 42 in response to sodium nitroprusside
To compare the effect of daprodustat to darbepoetin alfa on basal endothelial NO synthesis	• Change in FBF ratio from Day 1 to Day 42 in response to L-NMMA • Change in the absolute FBF from Day 1 to Day 42 in response to L-NMMA
To compare the effect of daprodustat on the FBF response to acetylcholine, sodium nitroprusside and L-NMMA between Day 42 and Day 1	• Change in FBF ratio in response to each individual challenge agent at Day 42 vs Day 1 in participants treated with daprodustat • Change in the absolute FBF in response to each individual challenge agent at Day 42 vs Day 1 in participants treated with daprodustat
To compare the effect of darbepoetin alfa on the FBF response to acetylcholine, sodium nitroprusside and L-NMMA between Day 42 and Day 1	• Change in FBF ratio in response to each individual challenge agent at Day 42 vs Day 1 in participants treated with darbepoetin alfa • Change in the absolute FBF in response to each individual challenge agent at Day 42 vs Day 1 in participants treated with darbepoetin alfa
To compare the effect of daprodustat to darbepoetin alfa on vascular compliance	• Change in Augmentation Index (an indicator of arterial stiffness) as estimated by radial arterial pulse contours from Day 1 to 42 • Change in pulse wave velocity (PWV) from Day 1 to Day 42
Safety	
Assess safety and tolerability	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) including AEs of special interest • Reasons for discontinuation of randomized study treatment • Absolute values and changes from baseline in clinical laboratory parameters, ECG parameters, blood pressure (BP) and heart rate (HR)

Objectives	Endpoints
Exploratory	
To explore the relationship between diabetes status and endothelial function	• FBF response to each individual challenge agent in relation to diabetes status

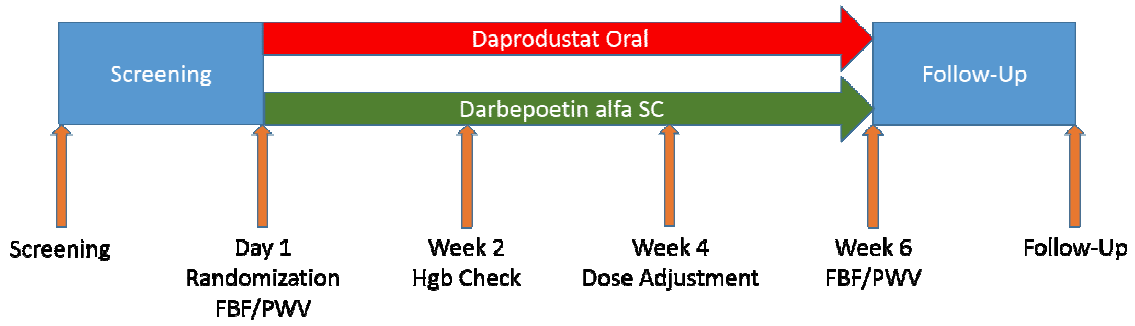
5. STUDY DESIGN

5.1. Overall Design

This study will use a randomized, repeat dose, open label, parallel group design, in adult, ND, male and female participants with anemia of CKD that are currently not treated with rhEPOs (i.e. no rhEPO use within the 12 weeks prior to the screening visit and through Day 1). The study design is outlined in Figure 1.

- The study will comprise three study periods: a screening period starting up to 30 days prior to Day 1, a 42 day (6 week) treatment period with 4 visits starting at Day 1, and a follow-up visit up to 14 days later (Figure 1). The total duration of participant involvement is up to 14 weeks.
- Participants will be randomized to either daprodustat or darbepoetin alfa. A central randomization approach will be used with stratification by center.
- All participants will be treated with the goal of achieving a Hgb level within the range of 10.0 to 12.0 g/dL, consistent with national guidelines (Medicines and Healthcare Products Regulatory Agency, 2007).
- FBF and PWV will be conducted at the beginning (Day 1) and end (Day 42) of the treatment period as outlined in the SoA (See Section 2) and described in Section 9.6.
- This study will include an optional, Non-Contact, Optical Forearm Plethysmography (NC-OFP) Sub-Study (See Appendix 8).

Figure 1 Study Outline



5.2. Number of Participants

A sufficient number of participants will be enrolled such that at least 50 evaluable participants comprise the Pharmacodynamic Per-Protocol (PDPP) Population (See Section 10.3.1).

5.3. Participant and Study Completion

A participant is considered to have completed the study if he/she has completed all phases of the study including the last visit or the last scheduled procedure shown in the SoA (See Section 2).

The end of the study is defined as the date of the last visit of the last participant in the study or last scheduled procedure shown in the SoA (See Section 2) for the last participant in the trial.

5.4. Scientific Rationale for Study Design

This study will use an open label, randomized, repeat dose, parallel group design, in adult, ND, male and female participants with anemia of CKD that are currently not treated with rhEPOs (i.e., no rhEPO use within the 12 weeks prior to the screening visit and through randomization (Day 1)).

- This will be an open label study as FBF and PWV are both objective measures and not likely to be biased by the knowledge of the participant's treatment. In addition, as daprodustat is administered as an oral tablet, and darbepoetin alfa is administered subcutaneously, it would be logistically challenging to blind treatments.
- Participants will be treated for 42 days (6 weeks) with study treatment. The treatment duration was chosen to coincide with the duration of treatment where previously increases in blood pressure were observed with administration of rhEPO (Santhanam, 2010).
- The planned patient population is participants with anemia of CKD that are not on dialysis. This population was chosen as it represents a relevant population for assessing endothelial functioning as a biomarker for the potential of CV events. In addition, this population is more likely to be untreated with rhEPO, as compared to the HD population. Finally, it would not be feasible to administer therapeutic doses of daprodustat or darbepoetin alfa to participants that are not anemic due to the resulting increases in Hgb.
- The challenge agents planned to be used in this study (ACh, SNP & L-NMMA) are well-established for assessment of endothelial function by venous occlusion plethysmography (Wilkinson, 2001).

5.5. Dose Justification

The daprodustat starting dose and dose adjustment algorithm are based on dose-response longitudinal modeling of Hgb data collected across the daprodustat Phase 2 program

(GlaxoSmithKline Document Number 2015N248947_00, 2016; Holdstock, 2016). In addition, the starting (2 mg) and maintenance (1, 2 & 4 mg) doses for all participants randomized to daprodustat have been studied previously in this population and have been shown to be effective, and generally well tolerated with an AE profile consistent with that of the patient population being studied (Holdstock, 2016). Starting doses, dose steps, and elements of the dose adjustment scheme for daprodustat are provided in Section 7.1.

The darbepoetin alfa starting dose and dose adjustments are to be based on the local prescribing information as well as clinical judgement.

Dosages of the challenge agents are based on a previous study (Cheriyan, 2011). An advantage of brachial artery infusion using venous occlusion plethysmography is that most of the administered challenge agents are metabolized prior to realizing substantial systemic exposures. This is certainly the case for ACh (used to induce NO endothelial production), which is often below a physiologically active concentration once it has traversed the entire arm length, L-NMMA (used to block NO synthesis) as well as sodium nitroprusside (infused to directly induce vasodilation at the level of the smooth muscle (i.e., non-endothelial based)).

6. STUDY POPULATION

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

6.1. Inclusion Criteria

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

Age

1. Participant must be at least 18 years of age inclusive, at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2. Participants who are Stage 3, 4 or 5 CKD defined by eGFR using the CKD Epidemiology Collaboration (CKD-EPI) formula (Levey, 2009).
3. Hgb as measured by HemoCue at screening visit and Day 1 is ≤ 11.0 g/dL (≤ 110 g/L).
4. Palpable brachial artery as assessed at screening.
5. Participants, if necessary, may be on stable maintenance oral iron supplementation (<50% change in overall dose and compliance of 80% of prescribed doses in the 4 weeks prior to and including the screening period). If participants have been on intravenous (IV) iron, then participants will not have received IV iron for 4 weeks prior to the Day 1 visit.

Sex

6. Male or female

   a. Female participants:

   A female participant is eligible to participate if she is not pregnant (see Appendix 5), not breastfeeding, and at least one of the following conditions applies:

   (i) Not a woman of childbearing potential (WOCBP) as defined in Appendix 5

   OR

   (ii) A WOCBP who has been on an approved form of contraceptive as defined in Appendix 5 for the 4 weeks prior to Day 1 and agrees to follow the contraceptive guidance in Appendix 5 until the follow-up visit.

Informed Consent

7. Capable of giving signed informed consent as described in Appendix 3 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

6.2. Exclusion Criteria

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

Medical Conditions

1. On dialysis or clinical evidence of impending need to initiate dialysis within 12 weeks of Day 1.
2. Planned kidney transplant within 12 weeks of Day 1.
3. Presence of an arteriovenous (AV) fistula

Prior/Concomitant Therapy

4. rhEPO use within the 12 weeks prior to the screening visit and through Day 1.
5. History of severe allergic or anaphylactic reactions or hypersensitivity to the study treatments or challenge agents, or excipients in the study treatments or challenge agents (see daprodustat IB for list of excipients, and the Study Reference Manual (SRM) for product sheets for darbepoetin alfa and the challenge agents).
6. Planned use of any prescription or non-prescription drugs or dietary supplements that are prohibited from screening until all assessments on Day 42 have been successfully completed (see Section 7.7.2).

Prior/Concurrent Clinical Study Experience

7. The participant has participated in a clinical trial and has received an experimental investigational product within the prior 30 days or within 5 half-lives of the investigational product (whichever is longer) prior to screening and through Day 1.

Diagnostic assessments

8. At or below the lower limit of the reference range at screening for Vitamin B_{12} (may rescreen in a minimum of 8 weeks).
9. Ferritin ≤ 50 ng/mL (≤ 50 µg/L) at screening.
10. Transferrin saturation (TSAT) ≤ 15% (0.15) at screening.
11. Folate < 2.0 ng/mL (4.5 nmol/L; may rescreen in a minimum of 8 weeks) at screening.
12. High sensitivity C-reactive protein (hs-CRP) ≥ 50 µg/mL (≥ 50 mg/L) at screening.

Other Exclusions

13. Myocardial infarction or acute coronary syndrome ≤ 12 weeks prior to screening and through Day 1.
14. Hospitalization for greater than 24 hours ≤ 12 weeks prior to screening and through Day 1.
15. Stroke or transient ischemic attack ≤ 12 weeks prior to screening and through Day 1.
16. Class IV heart failure, as defined by the New York Heart Association (NYHA) functional classification system.
17. Resting systolic blood pressure > 180 mmHg or diastolic blood pressure > 110 mmHg at screening visit or current uncontrolled hypertension as determined by the investigator.
18. QT interval corrected for heart rate using Bazett's formula (**QTcB)**: QTcB >500 msec, or QTcB >530 msec in participants with bundle branch block. There is no QTc exclusion for participants with a predominantly ventricular paced rhythm.
19. Active chronic inflammatory disease that could impact erythropoiesis. A partial list can be found in the Study Reference Manual (SRM).
20. History of bone marrow aplasia or pure red cell aplasia.
21. Conditions, other than anemia of CKD, which can affect erythropoiesis. A partial list can be found in the SRM.
22. Evidence of actively bleeding gastric, duodenal, or esophageal ulcer disease OR clinically significant GI bleeding from ≤ 8 weeks prior to screening and through Day 1.
23. Liver disease (any of the following):
    - Alanine transaminase (ALT) > 2x upper limit of normal (ULN; screening only)
    - Bilirubin > 1.5x ULN (screening only)
      NOTE: Isolated bilirubin > 1.5 x ULN is acceptable if bilirubin is fractionated and direct bilirubin < 35%
    - Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).

24. Major surgery within the 12 weeks prior to screening and through Day 1, or planned during the study.
25. Anticipated or planned vascular access surgery (i.e., AV fistula) within the 12 weeks prior to screening and through the Day 42 assessments.
26. Received a tissue heart valve replacement or repair within the 6 months prior to screening or has received a mechanical heart valve replacement.
27. Blood transfusion within 6 weeks prior to screening and through Day 1, or an anticipated need for blood transfusion during the study.
28. Clinical evidence of an acute infection, or history of infection requiring IV antibiotic therapy from 8 weeks prior to screening and through Day 1.
    NOTE: Prophylactic oral antibiotics are allowed.
29. History of malignancy within the two years prior to screening and through Day 1 or currently receiving treatment for cancer, with the exception of localized squamous cell or basal cell carcinoma of the skin definitively treated 12 weeks prior to Day 1.
30. Platelet count < 50,000/μL.(<50 GI/L)
31. History of a bleeding disorder (e.g., hemophilia).
32. Any other condition, clinical or laboratory abnormality, or examination finding that the investigator considers would put the participant at unacceptable risk, which may affect study compliance or prevent understanding of the aims or investigational procedures or possible consequences of the study.

6.3. Lifestyle Restrictions

6.3.1. Meals and Dietary Restrictions

Apart from a light breakfast (e.g., plain toast) and clear water which is allowed, participants are to refrain from any other food and drink for at least 8 h prior to the venous occlusion plethysmography performed on Days 1 and 42.

6.3.2. Caffeine, Alcohol and Tobacco

- Participants will be instructed to avoid caffeine/xanthine-containing products (i.e., coffee, tea, cola drinks, chocolate) for 6 hours prior to the venous occlusion plethysmography performed on Days 1 and 42.
- Participants will be instructed to avoid alcohol for 24 h prior to the venous occlusion plethysmography performed on Days 1 and 42.
- Participants will be instructed to avoid the use of nicotine and/or nicotine containing products for 24 h prior to the venous occlusion plethysmography performed on Days 1 and 42.

6.3.3. Activity

Participants will refrain from strenuous exercise for 24 h prior to the venous occlusion plethysmography performed on Days 1 and 42.

6.4. Screen Failures

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

Participants that fail screening are eligible to be rescreened up to an additional 2 times as soon as the investigator assesses (or 8 weeks later if low vitamin B_{12}) they may meet study entry criteria. If participants are rescreened, they must sign a new informed consent form.

7. TREATMENTS

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol. Eligible participants will be randomized to receive **either** daprodustat or darbepoetin alfa.

Participants randomized to daprodustat will receive this study treatment daily, starting Day 1 through Day 41 (i.e., day prior to FBF & PWV procedures). Participants randomized to darbepoetin alfa will receive this study treatment every 2 weeks, starting Day 1 through Day 28.

7.1. Treatments Administered

Study Treatment Name:	**Daprodustat**	**Darbepoetin alfa (Aranesp)**
Dosage formulation:	Tablet	Solution for injection
Unit dose strength(s)/ Dosage level(s):	1 mg, 2 mg, 4 mg tablet strengths/1 mg, 2 mg, 4 mg dosage levels	Darbepoetin alfa is commercially available in various single-dose vials and single-dose prefilled syringes
Route of Administration	Oral	Subcutaneous injection
Dosing instructions:	1 tablet to be taken daily without regard for food	Administered subcutaneously every 2 weeks as per product labelling
Packaging and Labeling	Study Treatment will be provided in a bottle. Each bottle will be labeled as per country requirement.	As per commercial packaging and product labelling
Manufacturer	GlaxoSmithKline (GSK)	Amgen

7.1.1. Study Treatments

7.1.1.1. Daprodustat Dosing Information

Daprodustat will be administered orally once daily starting **after** the completion of the FBF & PWV procedures on Day 1. The only exception to this is if a Repeat Day 1 is necessary (See Section 9.6.1.4).

Daprodustat Starting Dose

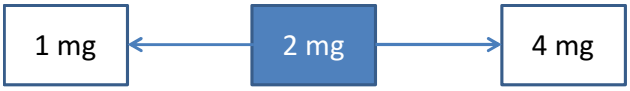
All participants randomized to the daprodustat group will be dosed with 2 mg orally once daily starting on Day 1 (See Section 5.5)

Daprodustat Dose Adjustment Algorithm

Participants randomized to daprodustat will have doses adjusted, as required, to target Hgb within the range of 10.0 to 12.0 g/dL. Dose adjustments will be assigned **automatically** via the IWRS (Interactive Web Response System) based on the participant's Hgb value via onsite HemoCue assessment according to the algorithm in Table 1 on Day 28 as noted in the SoA (See Section 2).

The available dose steps for daprodustat are outlined in Figure 2 (highlighted box indicates starting dose). Dose adjustments will result in the daprodustat dose being increased or decreased by **one dose step (up or down) at a time**.

Figure 2 Daprodustat Dose Steps



7.1.1.2. Darbepoetin Alfa Dosing Information

Darbepoetin alfa will be administered subcutaneously (SC) once every two weeks starting **after** the completion of the FBF & PWV procedures on Day 1.

Darbepoetin Alfa Starting Dose and Dose Adjustments

Participants randomized to darbepoetin alfa will receive study treatment according to local labelling, clinical practice guidelines, and clinical judgement to maintain Hgb in the target range (10.0-12.0 g/dL). For additional guidance on Hgb-based stopping criteria please refer to Section 8.1.2. Darbepoetin alfa is to be administered as a single subcutaneous injection once every two weeks as outlined in the SoA (See Section 2), although doses can be temporarily held or delayed if necessary to maintain patient safety (after consultation with the study medical monitor). Doses can only be modified on Day 28 as noted in the SoA (See Section 2).

7.1.2. Challenge Agents

The use of these challenge agents is described on the assumption they are available for both visits for an individual participant. If either of the secondary challenge agents (SNP and L-NMMA) are unavailable then that agent will be excluded from the procedure for that individual participant. If, for any reason, both SNP and L-NMMA become unavailable, the participant will receive ACh only.

All of the challenge agents will be infused in a fixed order (See Section 9.6.1, Figure 4) into the brachial artery, preferably the brachial artery of the nondominant (test) arm via a 27-guage needle inserted under local anesthesia. All challenge agents will be prepared

aseptically and diluted in sterile saline (0.9%). All infusions will be performed at a rate of 1 mL/min.

7.1.2.1. Acetylcholine Dosing Information

Endothelial function will be assessed by determining the vasodilator response to acetylcholine. Acetylcholine will be infused at 7.5, 15 & 30 µg/min each for 6 min per infusion into the brachial artery of the test arm.

7.1.2.2. Sodium Nitroprusside Dosing Information

Measurement of endothelium-independent vasodilation will be with sodium nitroprusside. Sodium nitroprusside will be infused at 3 and 10 µg/min each for 6 min per infusion into the brachial artery of the test arm.

7.1.2.3. L-N^G-monomethyl Arginine Acetate (L-NMMA) Dosing Information

The effects of daprodustat or darbepoetin alfa on basal NO synthesis will be assessed using L-NMMA at a doses of 2 and 8 µmol/min for 6 min each per infusion into the brachial artery of the test arm.

7.2. Dose Modification

Doses of daprodustat can be modified on Day 28 based on the participant's Hgb level as measured using the HemoCue device. The dose modifications, based on the Hgb value and change from previous measurement, are found in Table 1.

Doses of darbepoetin alfa can be modified on Day 28 based on the participant's Hgb level as measured using the HemoCue device based on local product labelling, clinical practice guidelines, and clinical judgement.

Table 1 Daprodustat Dose Modification Algorithm

Hgb (g/dL)	Hgb change since Day 1 visit	Dose Adjustment
< 7.5	Any change	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently discontinue study treatment and withdraw subject from the study (See Section 8.1.2).
≥7.5 to < 10.0	Decreasing or No change (i.e., < 0.5 g/dL increase)	Increase to the next higher dose step
≥7.5 to < 10.0	Increasing by at least 0.5 g/dL	Maintain dose
≥10.0 to ≤ 12.5	Any change	Maintain dose
> 12.5 to < 13.0	Increasing or No change	Decrease to the next lower dose step
	Decreasing	Maintain dose

Hgb (g/dL)	Hgb change since Day 1 visit	Dose Adjustment
≥13.0	Any change	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently discontinue study treatment and withdraw subject from the study (See Section 8.1.2).
≥7.5 - <13.0	≥1.3 g/dL increase in Hgb over the previous 2 weeks (as assessed on Day 28 only)[1]	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, decrease to next lower dose step (see Section 7.1.1)
≥7.5 - <13.0	>2 g/dL increase in Hgb over the previous 4 weeks.	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, decrease to next lower dose step (see Section 7.1.1)

[1] If increase in Hgb >1.3 g/dL is noted on Day 15, please refer to Hgb Stopping Criteria Table below (Section 8.1.2).

7.3. Method of Study Treatment Assignment

Participants will be stratified as outlined in Section 5.1 and randomized 1:1 to receive daprodustat or darbepoetin alfa.

A randomization number will be assigned in accordance with the randomization schedule prepared in advance of the study by Clinical Statistics, GlaxoSmithKline, using validated internal software (Registration and Medication Ordering System Next Generation (RAMOS-NG)).

7.4. Blinding

This is an open-label study. However, a central FBF reader, who will read and evaluate the FBF data, will be blinded to the treatment assignment. Details of the blinding procedures can be found in the SRM.

7.5. Preparation/Handling/Storage/Accountability

- Daprodustat is to be stored at up to 30°C (86°F). Maintenance of a temperature log (manual or automated) is required.
- Darbepoetin alfa, acetylcholine, sodium nitroprusside and L-NMMA are to be handled and stored as per package labeling.
- 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 the final disposition of unused study treatment are provided in the SRM.
    - Only participants enrolled in the study are to be administered any study treatments.

- Under normal conditions of handling and administration, study treatment is not expected to pose significant safety risks to site staff.
- 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.

- The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are to be reported and resolved before use of the study treatment.

7.6. Treatment Compliance

Participants that are randomized to daprodustat will be instructed to return all unused study treatment at each clinic visit. A record of the number of daprodustat tablets dispensed to and returned by each participant will be maintained and reconciled with study treatment and compliance records.

Participants that are randomized to darbepoetin alfa will have supervised administration of study treatment in the clinic, with the details of each administered darbepoetin alfa dose maintained and reconciled with study treatment and compliance records.

Study treatment start and stop dates and dosing details, including dates for study treatment dose increases or reductions, will be recorded in the electronic Case Report Form (eCRF).

7.7. Concomitant Therapy

Concomitant medications, including over-the-counter medications and supplements, taken during the study will be recorded in the eCRF. Start/stop dates and route of administration will be recorded for general concomitant medications. Additional details (e.g., changes in dose, reason for change, reason for addition or termination) will be recorded for certain medications at each visit (e.g., iron and anti-hypertensive medications).

NOTE: Participants should be maintained on the current dose and frequency of anti-hypertensive therapy, whenever possible.

7.7.1. Permitted Medications and Non-Drug Therapies

Unless specified as a prohibited medication in Section 7.7.2, all concomitant medications should be considered permitted provided they are not contraindicated for the individual participant concerned.

Participants are to be advised to maintain any anti-hypertensive medications at a consistent dosage and frequency; however, participants **are to delay administration of any anti-hypertensive medications until after all study-related procedures are completed on Days 1 & 42.**

Participants may take non-steroid anti-inflammatory drugs (NSAIDs) and aspirin during the treatment period; however, participants **are to avoid NSAIDs and aspirin for 24 hours prior to the FBF & PWV procedures until after all study-related procedures are completed on Days 1 & 42.**

7.7.2. Prohibited Medications and Non-Drug Therapies

No rhEPO use is permitted within the 12 weeks prior to the screening visit through Day 1.

Use of any of the following prohibited drugs from 1 week prior to screening until all assessments on Day 42 have been successfully completed is prohibited and will constitute a protocol violation, with the exception of strong cytochrome P_{450} 2C8 (CYP2C8) inhibitors or inducers, which can be used for up to 14 days.

- Strong inhibitors of CYP2C8 (e.g., gemfibrozil, high dose clopidogrel [300 mg])
- Strong inducers of CYP2C8 (e.g., rifampin/rifampicin)
- Intravenous administration of iron
- Any rhEPO other than darbepoetin alfa administered as study treatment as per protocol

7.7.3. Standard of Care

During the study, investigators are expected to monitor the participant's overall clinical status to ensure standards of care are met to enable consistency of practice with Kidney Disease: Improving Global Outcomes (KDIGO) guidelines or local equivalent.

7.7.4. Iron Management Criteria

Participants must be iron replete, as per local/regional guidelines prior to randomization and must remain iron replete throughout the study. Oral iron is allowed during the course of the study.

Participants that receive IV iron during the treatment period (i.e., from Day 1 through Day 42) are to discontinue study treatment (See Section 8.1) and be withdrawn (See Section 8.2).

7.8. Treatment after the End of the Study

Participants will not receive any additional treatment from GSK after completion of the study. The investigator is responsible for ensuring that consideration has been given to post-study care of the participant's medical condition.

8. DISCONTINUATION CRITERIA

8.1. Discontinuation of Study Treatment

Participants that discontinue study treatment will be withdrawn from the study. See Section 8.2 for a description of withdrawal procedures.

A participant must discontinue study treatment for the pre-specified reasons below.

- Meets Hgb stopping criteria (See Section 8.1.2)
- Receives a blood transfusion
- Receives an AV fistula or graft
- Receives a kidney transplant
- Participant initiating dialysis
- Receives IV iron
- Becomes pregnant or intends to become pregnant during the study
- Diagnosis of cancer (new or recurrent), with the exception of localized squamous cell or basal cell carcinoma of the skin
- Need for more than 14 days use of a strong CYP2C8 inhibitor or inducer (See Section 7.7.2)
- Any other condition which in the opinion of the PI makes the participant unsuitable to continue in the study.

8.1.1. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure participant safety and evaluate liver event etiology.

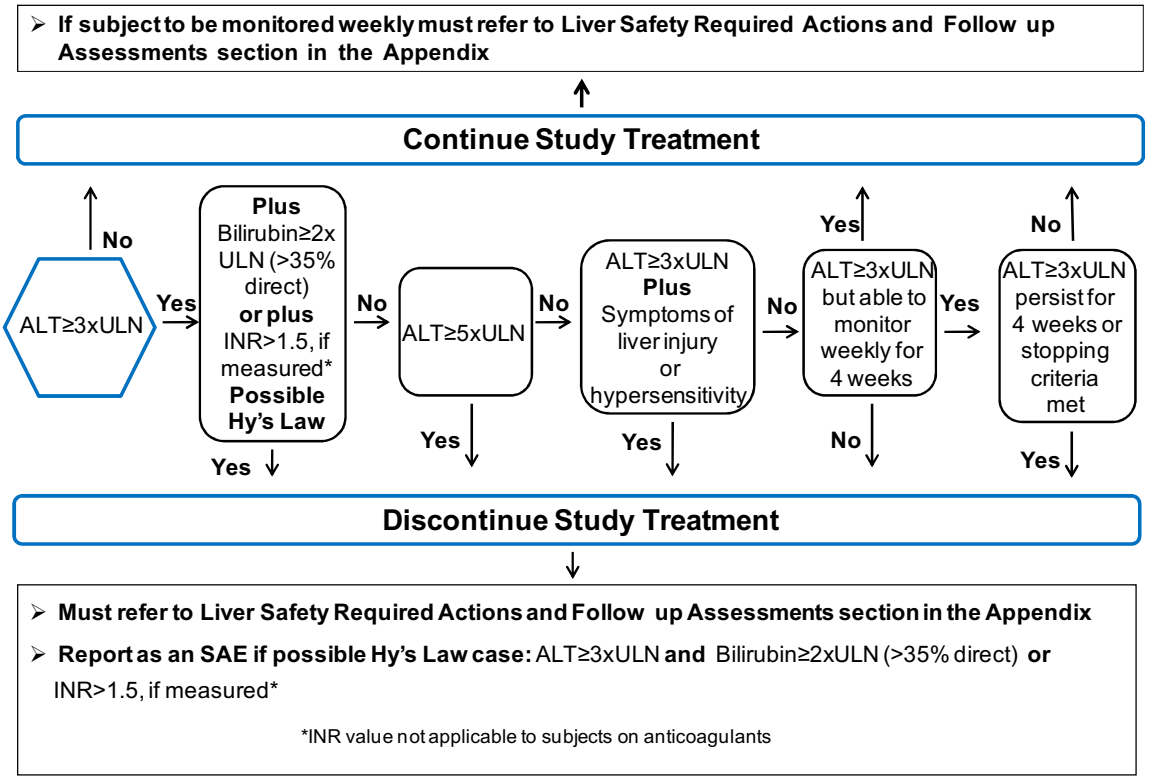
Discontinuation of study treatment for abnormal liver tests is required when:

- a participant meets one of the conditions outlined in Figure 3

  OR

- when in the presence of abnormal liver chemistries not meeting protocol-specified stopping rules, the investigator believes study treatment discontinuation is in the best interest of the participant.

Figure 3 Phase II Liver Chemistry Stopping and Increased Monitoring Algorithm



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

8.1.2. Hgb Stopping Criteria

GSK will supply a point-of-care Hgb analyzer (HemoCue) to each site for rapid and convenient monitoring of Hgb levels and to ensure consistency of Hgb measurements across sites participating in the study.

Blood samples for measurement of Hgb concentrations via HemoCue will be collected and recorded in the eCRF. Table 2 summarizes the Hgb values and corresponding action to be taken at each visit.

Table 2 Hgb Stopping Criteria

Hgb (g/dL) at visit		Action
<7.5		Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently discontinue study treatment and withdraw participant from the study.
≥7.5 - <13.0 with:	≥2.0 g/dL **decrease** in Hgb over the previous 2 weeks	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently discontinue study treatment and withdraw participant from the study.
≥7.5 - <13.0 with:	≥1.3 g/dL **increase** in Hgb over the previous 2 weeks (at Day 15 only)[1]	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently discontinue study treatment and withdraw participant from the study.
≥13.0		Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently discontinue study treatment and withdraw participant from the study.

[1] If increase in Hgb >1.3 g/dL is noted on Day 28, refer to Dose Modification (Section 7.2).

8.2. Withdrawal from the Study

Participants that are randomized but not dosed with study treatment will be considered for study withdrawal if Day 1 FBF measurement with ACh does not meet quality control criteria or they are unable to complete the initial FBF assessment, including a Day 1 FBF procedure where necessary (See Section 9.6.1.2). These participants will not be considered in the safety analysis as they have not received study treatment. Quality control procedures and criteria are described in the SRM. In all cases, the reason for withdrawal and the date of the last dose will be recorded in the participant's eCRF.

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance or administrative reasons.
- If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.
- The participant will be asked to attend the follow-up visit 7 to 14 days following the last dose of study treatment. Refer to the SoA (See Section 2) for data to be collected at the time of follow-up and for any further evaluations that need to be completed.

8.3. Lost to Follow Up

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

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

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

9. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA (See Section 2).
- Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment and be withdrawn.
- Adherence to the study design requirements, including those specified in the SoA (See Section 2), is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (e.g., Hgb levels) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA (See Section 2).
- The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 500 mL.
- Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

9.1. Efficacy Assessments

Efficacy is not evaluated in this study.

9.2. Adverse Events

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

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the participant to discontinue the study treatment (See Section 8.1).

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

- Any SAEs assessed as related to study procedure (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product will be recorded in the eCRF from the time a participant consents to participate in the study up to and including any follow-up contact.
- AEs and SAEs will be collected from randomization (Day 1) until the follow-up visit at the time points specified in the SoA (See Section 2).
- Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the eCRF and not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 h, as indicated in Appendix 4. The investigator will submit any updated SAE data to the sponsor within 24 h of it being available.
- Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the sponsor.
- The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

9.2.2. 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 participant is the preferred method to inquire about AE occurrence.

9.2.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs, and non-serious AEs of Special Interest (AESI; as defined in Section 9.2.5), will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 8.3). Further information on follow-up procedures is given in Appendix 4.

9.2.4. Regulatory Reporting Requirements for SAEs

- Prompt (i.e., within 24 h) notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

9.2.5. Adverse Events of Special Interest

AESIs have been identified based on non-clinical studies with daprodustat, clinical experience with rhEPOs, and current information regarding HIF-regulated pathways in mediating hypoxia-associated pathophysiology. The currently identified AESIs for daprodustat are as follows:

- Thrombosis and/or tissue ischemia secondary to excessive erythropoiesis
- Death, MI, stroke, heart failure, thromboembolic events, thrombosis of vascular access
- Cardiomyopathy
- Pulmonary artery hypertension
- Cancer-related mortality and tumor progression and recurrence
- Esophageal and gastric erosions
- Proliferative retinopathy, macular edema, choroidal neovascularization
- Exacerbation of rheumatoid arthritis
- Worsening of hypertension

9.2.6. Cardiovascular and Death Events

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

- 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

The CV eCRFs are presented as queries in response to reporting of certain CV Medical Dictionary for Regulatory Activities (MedDRA) terms. The CV information should be recorded in the specific cardiovascular section of the eCRF within one week of receipt of a CV Event data query prompting its completion.

The Death eCRF 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.

9.2.7. Possible Suicidality Related Adverse Events

If during the study there is an occurrence of an AE or SAE which in the investigator's opinion is possibly related to suicidality, the Possible Suicidality Related Adverse Events (PSRAE) eCRF form should be completed (in addition to the AE and SAE pages, as appropriate).

This event may include, but is not limited to, one that involves suicidal ideation, a preparatory act toward imminent suicidal behavior, a suicide attempt, or a completed suicide. The investigator will exercise his or her medical and scientific judgment in deciding whether an event is possibly related to suicidality.

9.2.8. Pregnancy

Details of all pregnancies in female participants and the outcome for the neonate, if applicable, will be collected from the start of study treatment.

If a pregnancy is reported then the investigator should inform the Sponsor within 2 weeks of learning of the pregnancy and should follow the procedures outlined in Appendix 5.

Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

9.3. Treatment of Overdose

There is no specific antidote for overdose with daprodustat. The expected manifestations of daprodustat overdosage include signs and symptoms associated with an excessive and/or rapid increase in Hgb concentration. Daprodustat is highly protein bound; thus, clearance of daprodustat by hemodialysis (HD) or peritoneal dialysis (PD) is very low and these are not effective methods to enhance the elimination of daprodustat. Daprodustat metabolites are, in part, cleared via hemodialysis. In the event of a suspected overdose, it is recommended that the appropriate supportive clinical care be instituted, as dictated by the participant's clinical status. Additionally, participants should be monitored closely for CV events, increased heart rate and hematologic abnormalities.

Consult the approved product label for information on overdose for darbepoetin alfa.

9.4. Safety Assessments

Planned time points for all safety assessments are provided in the SoA (See Section 2).

9.4.1. Physical Examinations

- A complete physical examination will include, at a minimum, assessments of the skin, cardiovascular, respiratory, gastrointestinal and neurological systems. Height and weight will also be measured and recorded at screening.
- Investigators should pay special attention to clinical signs related to previous serious illnesses, e.g., history of anemia or bleeding.

9.4.2. Vital Signs

- Temperature, pulse, respiratory rate, blood pressure, and weight will be assessed.
- Blood pressure and pulse measurements will be assessed in a semi-supine position with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones).
- Vital signs (to be taken before blood collection for laboratory tests) will consist of 1 pulse and 3 blood pressure measurements (3 consecutive blood pressure readings will be recorded at intervals of at least 1 minute). The average of the 3 blood pressure readings will be recorded on the eCRF.
- Participants with resting systolic blood pressure > 170 mmHg or diastolic blood pressure > 100 mmHg can delay the Day 42 assessment up to 1 week (i.e., Day

49) in order to allow the blood pressure to decrease. For participants that will have delayed FBF procedures performed, details can be found in Section 9.6.1.4.

9.4.3. Electrocardiograms

- ECG measurements will be obtained as outlined in the SoA (See Section 2). Full 12-lead ECGs will be recorded with the participant in a semi-supine position. Heart rate, PR interval, QRS duration, and QT (uncorrected) interval will be measured. QTcB will be calculated (machine read or manually).
- At each time point at which ECGs are required, two additional ECGs are required if the initial ECG measurement indicates prolonged QTc (See Section 6.2) using the automated or manually calculated QTcB value. The average QTcB value of all three ECGs will be used to determine eligibility.
- Participants with an abnormal ECG can delay the Day 42 assessment up to 1 week (i.e., Day 49) at the Principal Investigator's discretion. For participants that will have delayed FBF procedures performed, details can be found in Section 9.6.1.4.

9.4.4. Clinical Safety Laboratory Assessments

- Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA (See Section 2) for the timing and frequency.
- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- All laboratory tests with values 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 or are no longer considered significantly abnormal by the investigator or medical monitor.
- If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified.
- All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the laboratory manual and the SoA (See Section 2).

9.5. Pharmacokinetics

Pharmacokinetic (PK) parameters will not be evaluated in this study.

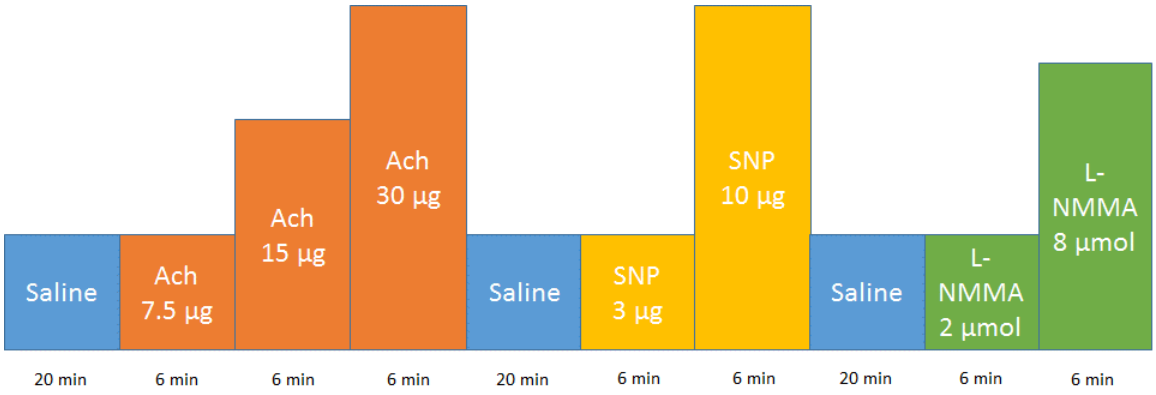
9.6. Pharmacodynamics

Detailed guidelines for the FBF and PWV procedures are provided in the SRM to ensure that the same procedure is followed at every site to avoid any inconsistencies. Brief descriptions of the procedures can be found in Section 9.6.1 and Section 9.6.2.

9.6.1. Venous Occlusion Plethysmography to Measure Forearm Blood Flow (FBF)

Venous occlusion plethysmography has been used extensively to study human vascular physiology *in vivo*, and is at its most powerful when combined with intra-arterial drug administration, usually into the forearm vascular bed. The procedure for conducting the FBF measurements has been previously described (Cheriyan, 2011). Briefly, in the morning, after a light breakfast and following an initial 30 minute rest period, participants will be placed in a supine position and a 27-gauge needle inserted into the brachial artery of the non-dominant arm. Mercury filled silastic strain gauges are attached to both arms, along with cuffs at the wrists and above the antecubital fossa (congesting cuff). A rapid inflation of the congesting cuff to 40 mmHg, (thereby blocking the venous outflow of blood) along with the 200 mmHg wrist cuffs to isolate the forearm from the complicated hemodynamic features of the hand, produces a rapid increase in forearm volume while the challenge agents are infused (Figure 4). This change is recorded via the strain gauges connected to the plethysmograph recorder. Congesting cuff inflation is maintained for 10-13 seconds, establishing a maximal volume, and repeated at approximately 15-20 second intervals for at least 4-5 time sequences. The maximal volume and the rate to reach plateau, is averaged and thereby recorded in both arms.

Figure 4 FBF Infusion Schedule



Saline will be infused prior to initating the challenge agent dosing and between the challenge agents for at least 20 minutes.

Endothelium-dependent function will be assessed by determining the vasodilator response to acetylcholine. Acetylcholine will be infused intra-arterially at 7.5, 15 and 30 µg/min each for 6 minutes per infusion; measurement of endothelium-independent vasodilatation will be with sodium nitroprusside. Sodium nitroprusside will be infused at 3 and 10 µg/min each for 6 minutes. Effects on basal NO synthesis will be assessed using L-NMMA at a doses of 2 and 8 µmol/min each for 6 minutes. If L-NMMA is not

available then the FBF procedure will be finished after the last SNP infusion. Similarly, if the SNP is not available for any reason, then the L-NMMA (if available) will be given following the ACh challenge.

Measures as noted above are made in both arms concurrently, and thus the maximal strain gauge measure from the infused arm is divided by the same from the contralateral (non-infused) arm, producing a ratio.

9.6.1.1. Participants on Anti-coagulant Therapy

In order to minimize blood loss from the FBF procedure, participants on anti-coagulant therapy are to have INR assessed prior to initiation of the procedure. Only participants with INR < 3.0 are to undergo the FBF & PWV assessments. Participants with INR ≥ 3.0 can delay the Day 42 assessment up to 1 week (i.e., Day 49) in order to allow INR to decrease below 3.0 (See SRM for further details). For participants that will have delayed FBF procedures performed, details can be found in Section 9.6.1.4.

9.6.1.2. Participants Unable to Complete FBF Procedure

If a participant is unable to complete the Day 1 FBF procedure with all three doses of the ACh infusion and there is reasonable expectation a second attempt will be successful, one additional attempt may be made in a subsequent visit up to 1 week later, if the participant agrees. In this case, the participant should not be dosed with study drug until after the FBF procedures are completed at the repeat visit.

If participants are unable to complete the Day 42 FBF procedure with all three doses of the ACh infusion, one additional attempt may be made in a subsequent visit up to 1 week later, if the participant agrees. For participants that will have repeat FBF procedures performed, details can be found in Section 9.6.1.4.

9.6.1.3. FBF Quality Control Criteria

After both the Day 1 and Day 42 FBF procedures, the results will be checked for data quality as defined in the Study Reference Manual (SRM). If the FBF fails the quality control (QC) criteria following the Day 42 procedure, one additional attempt may be made in a subsequent visit up to 1 week later, if the participant agrees. For participants that will have repeat FBF procedures performed, details can be found in Section 9.6.1.4.

9.6.1.4. Delayed or Repeat FBF Procedures

Delayed FBF Procedures: In the instance that the FBF procedure needs to be delayed, participants should either continue receiving the daprodustat dispensed on Day 28, while the participants randomized to darbepoetin alfa should receive an appropriate dose on Day 42. The assessments that are scheduled to be conducted on Day 42 are to be followed (See Section 2).

Repeat FBF Procedures: If the FBF procedure is determined to be repeated for either Day 1 or Day 42, the assessments should be performed as per the SoA Table at the additional FBF visit. The PWV procedures should also be repeated.

In the instance that the Day 1 FBF procedure needs to be repeated, the participant should not be dosed with study drug until after the repeat/completed procedure. The timing of all subsequent visits will begin from that completed procedure day.

In the instance that the Day 42 FBF procedure needs to be repeated, participants should either continue receiving the daprodustat dispensed on Day 28, while the participants randomized to darbepoetin alfa should receive an appropriate dose on Day 42. The safety follow up visit will take place 7-14 days after the final procedure.

9.6.2. Pulse Wave Analysis and Pulse Wave Velocity to measure vascular compliance

Pulse wave analysis (PWA) is a reproducible, noninvasive method for assessing central blood pressure and augmentation index (AIx, a measure of the contribution that wave reflection makes to the arterial pressure waveform). The amplitude and timing of the reflected wave ultimately depends on the stiffness of the small (pre-resistance) vessels and large arteries, and thus, AIx provides a measure of systemic arterial stiffness (Wilkinson, 2002).

Pulse wave analysis will be used to determine systemic arterial stiffness (SphygmoCor). A high-fidelity micromanometer will be used to obtain accurate readings of the peripheral pressure waveforms by flattening, but not occluding, the radial artery of the dominant arm using gentle pressure. Data will be collected to produce a central pressure waveform, which has been correlated with the aortic waveform. Augmentation represents the difference between the second and first systolic peaks of the central pressure waveform. AIx is defined as the augmentation (difference between systolic peaks) expressed as a percentage of the overall pulse pressure.

Pulse wave velocity will be assessed with similar equipment as described above, using the carotid and femoral arteries as the two points of measure.

9.7. Genetics

Genetics will not be evaluated in this study.

9.8. Biomarkers

Biomarkers will not be evaluated in this study. However, blood (serum and plasma) samples may be collected by investigators as outlined in the SoA (See Section 2) for potential future analysis of biomarkers of cardiovascular physiology and vascular function, with the consent of the participant. These samples, if any, will be managed by the Investigator or designee, and are considered outside the scope of this protocol.

9.9. Health Economics OR Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters will not be evaluated in this study.

10. STATISTICAL CONSIDERATIONS

Statistical analyses will be performed by, or under the direct auspices of Clinical Statistics and Programming, GSK.

The null hypothesis is that there is no difference between daprodustat and darbepoetin alfa on the acetylcholine-induced change in FBF ratio at Day 42 vs the alternative hypothesis that there is a difference.

10.1. Sample Size Determination

A sufficient number of participants will be enrolled such that at least 50 participants comprise the Pharmacodynamic Per-Protocol (PDPP) Population (See Section 10.3.1). The sample size may be adjusted following the sample size re-estimation (See Section 10.1.2).

Based on a coefficient of variation of 0.277 of change from baseline FBF ratio, a sample size of 25 participants per treatment arm, provides 80% power to show a statistically significant difference between groups, assuming a true difference of at least 25% (Cheriyan, 2011*)* at the alpha = 5% level of significance.

10.1.1. Sample Size Sensitivity

A sensitivity analysis was performed to evaluate the impact of a range of sample sizes and estimates of variability on the power to detect differences in the primary endpoint.

Power of the Primary Analysis*			
CV	Total Evaluable Sample Size		
	40	46	50
0.249	80%	85%	88%
0.277	72%	77%	81%
0.305	64%	70%	74%

*Under an assumed 25% increase for daprodustat compared to darbepoetin (on normal scale), and a 5% alpha level

10.1.2. Sample Size Re-estimation

A blinded sample size re-estimation assessment may be conducted after approximately 20 subjects have completed the Day 42 FBF procedure in order to adjust the final sample size if the variability of the study differs significantly from the variability estimate used for the original sample size calculation. This blinded assessment will be performed before any unblinded interim analyses are conducted (see Section 10.3.4).

10.2. Populations for Analyses

The primary population of interest is the pharmacodynamic per-protocol (PDPP) population as defined in Table 3.

Table 3 Description of Analysis Populations

Population	Description
Screened Population	All participants who signed an ICF to participate in the clinical trial. This population will be used for summarizing screening failure rates and reasons for screening failure.
Safety Population	All randomized participants who receive at least one dose of study treatment will be included in the safety population. This population will be used in the evaluation of safety and tolerability.
Pharmacodynamic Per-Protocol (PDPP) Population	All randomized participants who provide pharmacodynamic (PD) data at Day 1 and Day 42 that meets the QC criteria (See Section 9.6.1.3) and completes the protocol without important deviations will be included in the PDPP population. This population will be used in the evaluation of all endpoints with the exception of safety endpoints.

Because the current study is designed to assess the effects of study treatment on pharmacodynamic parameters, an intent-to-treat population will not be defined.

10.2.1. Treatment Comparisons

10.2.1.1. Primary Comparisons

The primary analysis of interest is the comparison of acetylcholine-induced response between daprodustat and darbepoetin alfa based on the change in FBF ratio from Day 1 to Day 42.

The pharmacodynamic per-protocol population will be used for analyzing the primary comparison.

10.2.1.2. Secondary Comparisons

The primary comparison will be repeated for the two remaining challenge agents. If an insufficient number of subjects in the PDPP population have complete Day 1 and Day 42 data for either of the two remaining challenge agents, then that challenge agent will not be statistically analysed. Instead, a summary table and subject level listing will be generated. Details regarding the exclusion from analysis will be included in the RAP. (Section 4).

Comparisons within and between treatment for daprodustat and darbepoetin alfa will be made for other PD endpoints as well.

The pharmacodynamic per-protocol population will be used for analysing all secondary comparisons.

10.3. Statistical Analyses

This section provides an overview of the analysis of data. The Reporting and Analysis Plan (RAP) which will be finalised before the interim analyses described in Section 10.3.4 are performed, will contain the full details of the planned analysis.

10.3.1. Efficacy Analyses

Not applicable.

10.3.2. Safety Analyses

All safety data will be summarised using the Safety Population.

Safety endpoints will include all AEs, clinical laboratory test parameters, HemoCue Hgb measures, vital signs, ECG parameters and physical examination.

No formal statistical analyses will be performed for the safety endpoints. These data will be listed by treatment using suitable displays for each endpoint.

10.3.3. Other Analyses (Pharmacodynamic)

The primary analysis model will be based on the acetylcholine challenge agent data.

Following log-transformation the change in FBF ratio from Day 1 to Day 42 will be analyzed using analysis of covariance (ANCOVA) model including terms for the treatment group (daprodustat/darbepoetin alfa), challenge agent dose, treatment*challenge agent dose interaction term, FBF ratio on Day 1, gender, and site stratification variable. The primary comparison of interest is the difference between treatment groups in the primary endpoint associated with the acetylcholine challenge. The point estimate and corresponding 95% confidence interval will be derived, using the residual error from the model, for the comparison of the treatment groups. These estimates will be back-transformed to provide the point estimate and corresponding 95% confidence interval (CI) for the ratio of daprodustat/darbepoetin alfa for the primary analysis.

In addition, this model will provide the supporting analysis to the primary analysis deriving the estimates and 95% CIs for (1) Change in FBF ratio from Day 1 to Day 42 for each treatment group and (2) Difference in FBF ratio between the challenge agent and saline on both Day 1 and Day 42. These estimates will be back-transformed to provide the point estimates and corresponding 95% CIs for the ratios for “Day 42/Day 1” and “challenge agent/saline” (for each challenge agent dose level) where saline is considered as providing assay sensitivity for the challenge agent.

The first secondary endpoint will use the same model as the primary endpoint with absolute FBF instead of FBF ratio as the dependent variable. The other challenge agents, sodium nitroprusside and L-NMMA, will utilize a similar model. If an insufficient number of subjects in the PDPP population have complete Day 1 and Day 42 data for either of the two remaining challenge agents, then that particular challenge agent will not

be statistically analysed. Instead, a summary table and subject level listing will be generated.

The secondary endpoints evaluating the within treatment comparison of daprodustat and darbepoetin alfa will utilize an ANCOVA model including terms for challenge agent dose, FBF measurement on Day 1, gender, and site. This model will be run for both treatment groups for each of the three challenge agents and for FBF ratio and absolute FBF.

The other secondary endpoint evaluating vascular compliance will make use of two models. The first model for augmentation index will utilize an ANCOVA model with terms for treatment group (daprodustat/darbepoetin alfa), challenge agent dose, treatment*challenge agent dose interaction term, augmentation index on Day 1, gender, and site. The second model for pulse wave velocity will utilize an ANCOVA model with terms for the treatment group (daprodustat/darbepoetin alfa), challenge agent dose, treatment*challenge agent dose interaction term, PWV on Day 1, gender, and site.

The exploratory endpoint looking at the relationship between diabetes status and endothelial function will use a similar ANCOVA model as our primary endpoint, adding in the indicator variable diabetes. This will be explored for all three challenge agents and modeled for FBF ratio and absolute FBF.

The last exploratory endpoint will analyze the FBF dose response relationships for each of the three challenge agents.

Sensitivity analyses will be performed for all endpoints analysing FBF ratio. Instead of FBF ratio, percent change from saline infusion and absolute change from saline infusion will be modelled.

Distributional assumptions underlying the statistical analyses will be assessed by visual inspection of the residual plots. Normality will be examined by normal probability plots, while homogeneity of variance will be assessed by plotting the residuals against the predicted values for the model.

Descriptive statistics (n, arithmetic mean and corresponding 95% confidence interval, standard deviation (SD), minimum, median, maximum) will be calculated by treatment group for all PD endpoints. In addition, summary of data for all FBF ratio endpoints will be provided including geometric means (and corresponding 95% confidence interval) and between-subject coefficients of variation (CVb) will be calculated by treatment group, where:

$CVb(\%) = \text{sqrt}[\exp(SD^2)-1] \text{ X } 100$ where SD is the standard deviation of the $\log_e$-transformed data.

Mean (standard error [SE]) plots of the change from baseline FBF ratio data will be produced by treatment group. Separate plots will be produced for the FBF ratio for each challenge agent.

Further details will be included in the RAP.

10.3.4. Interim Analyses

An interim analysis may be conducted after approximately 24 subjects have completed the Day 42 FBF procedure, unless the blinded sample size re-estimation suggests a different sample size. If the interim analysis for this study is expected to occur after the interim analysis of the larger Phase III ASCEND program, then this interim analysis may occur sooner.

If the observed percent change between daprodustat and darbepoetin alfa for FBF ratio from Day 1 to Day 42 in response to acetylcholine is less than 8%, then the study will be stopped. Assuming a true percent change of 25% and a standard deviation of 0.272, the decision guideline was determined to have favorable operating characteristics, such as an unconditional power of <30%, and an acceptable low probability of false stop (<10%). Since the study will not be stopped for positive results, type I error will not be inflated. If the sample size changes based on the blinded sample size re-estimation, then the operating characteristics may be revised to reflect a different standard deviation. Any such changes will be documented in the RAP.

While the above is a guideline for stopping the trial due to futility, the totality of the data will be considered when making the decision at the time of the interim analysis.

Further details of the interim analysis will be included in the RAP.

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

ACh	Acetylcholine
AE	Adverse event
AESI	Adverse event of special interest
AI	Augmentation Index
ALT	Alanine transaminase
ANCOVA	Analysis of Covariance
AST	Aspartate transaminase
AUC	Area under curve
AV	Arteriovenous
BCRP	Breast cancer resistance protein
BP	Blood pressure
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CKD	Chronic kidney disease
CFR	Code of Federal Regulations
CL/F	Total body clearance
Cmax	Maximum plasma concentration
CONSORT	Consolidated Standards of Reporting Trials
CPK	Creatine phosphokinase
CSR	Clinical Study Report
CV	Cardiovascular
CVb	Between-subject coefficients of variation
CYP	Cytochrome P_{450}
dL	Decilitre
ECG	Electrocardiogram
ECHO	Echocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
EPI	Epidemiology
EPO	Erythropoietin
FBF	Forearm Blood Flow
FRP	Females of reproductive potential
FSH	Follicle stimulating hormone
g	Gram
GCP	Good Clinical Practice
GI	Gastrointestinal
GSK	GlaxoSmithKline
HbA1c	Glycated hemoglobin
hCG	Human chorionic gonadotrophin
HD	Hemodialysis dependent
HDL-c	High density lipoprotein-C
Hgb	Hemoglobin

HIF	Hypoxia-inducible factor
HIPAA	Health Insurance Portability and Accountability Act
HPLC	High-performance liquid chromatography
HR	Heart rate
HRT	Hormone replacement therapy
hs-CRP	High sensitivity C-reactive protein
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IgM	Immunoglobulin M
INR	International Normalized Ratio
IRB	Institutional Review Board
IP	Investigational Product
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
IV	Intravenous
IWRS	Interactive Web Response System
KDIGO	Kidney Disease: Improving Global Outcomes
Kg	Kilogram
L	Liter
LDH	Lactate dehydrogenase
LDL-C	Low density lipoprotein-C
L-NMMA	NG-monomethyl arginine acetate
LVEF	Left Ventricular Ejection Fraction
µg	Microgram
µmol	Micromole
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial infarction
Mg	Milligram
mmHg	Millimeters of mercury
mL	Milliliter
MSDS	Material Safety Data Sheet
Msec	Millisecond
NC-OFP	Non-Contact, Optical Forearm Plethysmography
ND	Not ondialysis
ng	Nanogram
nmol	Nanomole
NO	Nitric oxide
NSAID	Non Steroidal Anti Inflammatory Drug
NYHA	New York Heart Association
PAH	Pulmonary artery hypertension

PASP	Pulmonary artery systolic pressure
PCI	Percutaneous Coronary Intervention
PCM	Progressive cardiomyopathy
PD-PP	Pharmacodynamic Per-Protocol Population
PED	Peritoneal dialysis
PHD	Prolyl hydroxylase
PHI	Prolyl hydroxylase inhibitor
PK	Pharmacokinetic
PRVP	Peak Right Ventricular Pressure
PSRAE	Possible suicidality related adverse events
PWV	Pulse Wave Velocity
QC	Quality Control
QTcB	QT duration corrected for heart rate by Bazett's formula
RAP	Reporting and Analysis Plan
RBC	Red blood cell
RDW	Red blood cell distribution width
RNA	Ribonucleic Acid
rhEPO	Recombinant human erythropoietin
SAE	Serious adverse event
SC	Subcutaneous
SD	Standard deviation
SNP	Sodium nitroprusside
SoA	Schedule of Events
SRM	Study Reference Manual
SUSAR	Suspected Unexpected Serious Adverse Reactions
TSAT	Transferrin saturation
U	Units
ULN	Upper limit of normal
VEGF	Vascular Endothelial Growth Factor
WBC	White blood cell
WOCBP	Woman of Child Bearing Potential
UK	United Kingdom

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
Aranesp
HemoCue
MedDRA
RAMOS-NG
SphygmoCor

12.2. Appendix 2: Clinical Laboratory Tests

All study-required laboratory assessments will be performed by a central laboratory with the exception of HemoCue Hgb, INR measurements and urine pregnancy tests which will be performed at the clinical site. The results of each HemoCue Hgb must be entered into the participant's eCRF.

A local Hematology lab will be allowed in the event that central labs are unavailable.

Tests included in Table 4 must be conducted in accordance with the Laboratory Manual and Study Protocol SoA (See Section 2). Laboratory requisition forms must be completed and samples must be clearly labeled with the participant 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.

Refer to the SRM for appropriate processing and handling of samples.

Table 4 Protocol Required Laboratory Assessments

Laboratory Assessments	Parameters		
Hematology	Platelet count	*RBC indices:*	*WBC count with Differential*
	RBC count	MCV	Neutrophils
	Reticulocyte count	MCH	Lymphocytes
	Hgb	MCHC	Monocytes
	Hematocrit	RDW	Eosinophils
	WBC count (Absolute)		Basophils
Clinical Chemistry[1]	Sodium (serum)	AST[1]	Carbon Dioxide (total)
	Potassium (serum)	ALT[1]	Albumin
	Creatinine (serum)	Chloride (serum)	Urea (serum)
	Glucose	Bilirubin (total and direct/indirect)	Alkaline Phosphatase
Other laboratory tests	Serum ferritin	TSAT	hs-CRP
	Total cholesterol	LDL-C (direct)	HDL-C
	Triglycerides	Serum/Urine hCG pregnancy test[2]	FSH[3]
	HbA1c	Folate	Vitamin B_{12}
	Serology (hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody)		

Abbreviations: RBC: red blood cells, WBC, white blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width, AST, aspartate transaminase; LDL-C, low density lipoprotein-C; HDL-C, high density lipoprotein-C, hCG= human chorionic gonadotropin.

[1]Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 8.1.1 and Section 12.6.

[2]For females of reproductive potential only.

[3]Screening only. As needed in postmenopausal women were their menopausal status is in doubt (see Inclusion Criteria Section 6.1).

All laboratory tests with values that are considered clinically-significantly abnormal during participation in the study or within 14 (±3) 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 sponsor should be notified.

12.3. Appendix 3: Study Governance Considerations

Regulatory and Ethical Considerations

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

Financial Disclosure

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

Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of

informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.

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

The ICF may contain a separate section that addresses the use of remaining mandatory samples for optional exploratory research in accordance with SOP-GSKF-410. The investigator or authorized designee will explain to each participant the objectives of the exploratory research. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a participant's agreement to allow any remaining specimens to be used for exploratory research. Participants who decline to participate will not provide this separate signature.

Data Protection

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

Publication Policy

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

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

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

Dissemination of Clinical Study Data

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

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

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

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

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

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

Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the

currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final Clinical Study Report(CSR) or equivalent summary unless local unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Source Documents

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

Study and Site Closure

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

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

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

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study treatment development

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

Definition of AE

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

Events <u>Meeting</u> the AE Definition
• Any abnormal laboratory test results (hematology or clinical chemistry) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE. • The signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" constitutes an AE or SAE.

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

Definition of SAE

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

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d. Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct

normal life functions.

- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

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 or any event that may potentially be one of these categories:

- 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

Recording AE and SAE

AE 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)

related to the event.

- The investigator will then record all relevant AE/SAE information in the eCRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

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

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

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

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to** GSK**.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

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

Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool

- The primary mechanism for reporting SAE to GSK will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- The investigator or medically-qualified sub-investigator must show evidence within the eCRF (e.g., check review box, signature, etc.) of review and verification of the relationship of each SAE to IP/study participation (causality) within 72 hours of SAE entry into the eCRF
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives

updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the [medical monitor/SAE coordinator by telephone.

- Contacts for SAE reporting can be found in the SRM.

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

Definitions

Woman of Childbearing Potential (WOCBP)

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

Women in the following categories are not considered WOCBP

- Premenarchal
- Premenopausal female with ONE of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy

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

Contraception Guidance

Female participants

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in Table 5.

Table 5 Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent[1] *Failure rate of <1% per year when used consistently and correctly.*
Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation[2] • oral • intravaginal • transdermal
Progestogen-only hormonal contraception associated with inhibition of ovulation[2] • injectable
Highly Effective Methods That Are User Independent
• Implantable progestogen-only hormonal contraception associated with inhibition of ovulation[2] • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • bilateral tubal occlusion
Vasectomized partner *(A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.)*
Sexual abstinence (*Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)*

NOTES:
[1]Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants in clinical studies.
[2]Hormonal contraception may be susceptible to interaction with the study drug, which may reduce the efficacy of the contraceptive method. In this case two highly effective methods of contraception should be utilized during the treatment period and for at least 1 day, corresponding to time needed to eliminate study treatment plus 30 days for study treatments with genotoxic potential after the last dose of study treatment.

Pregnancy Testing

WOCBP should only be included after a confirmed menstrual period and a negative urine pregnancy test.

Additional pregnancy testing should be performed at monthly intervals during the treatment period and seven days after the last dose of study treatment and as required locally.

Urine pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected.

Pregnancy testing will be performed using the test kit provided by the central laboratory and in accordance with instructions provided in its package insert.

Collection of Pregnancy Information

Female Participants who become pregnant

- Investigator will collect pregnancy information on any female participant, who becomes pregnant while participating in this study.
- Information will be recorded on the appropriate form and submitted to GSK within 24 hours of learning of a participant's pregnancy.
- Participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow up information on participant and neonate, which will be forwarded to GSK. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date.
- Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE.
- A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any SAE occurring as a result of a post-study pregnancy which is considered reasonably related to the study treatment by the investigator, will be reported to GSK as described in Appendix 4. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female participant who becomes pregnant while participating must permanently discontinue study treatment. Participants will be expected to attend study visits through the Follow-up visit, according to the study visit schedule, unless consent is actively withdrawn.

12.6. Appendix 6: 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)
INR[2]	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) • If restart/rechallenge **not allowed per protocol or not granted**, permanently discontinue study treatment and may continue subject in the study for any protocol specified follow up assessments	• Viral hepatitis serology[4] • Blood sample for pharmacokinetic (PK) analysis, obtained within 24 hrs 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

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]). • 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.

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12.7. Appendix 7: Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Daprodustat		
Excessive erythropoiesis leading to thrombosis and/or tissue ischemia	In animal studies, excessive erythropoiesis (Hgb/Hct > upper limit normal) attributed to daprodustat was associated with vascular congestion/inflammation, microthrombi, and tissue ischemia in a number of organs. In the phase 2 proof of concept study, a high incidence of discontinuation due to hemoglobin stopping criteria (Hgb > 13.5 g/dL or Hgb increased > 1 g/dL over any 2-week period) was observed. In non-dialysis subjects administered 10 mg, 25 mg, 50 mg or 100 mg of daprodustat daily, a total of 21 of 61 subjects (34%) met these criteria. In hemodialysis-dependent subjects administered either 10 mg or 25 mg of daprodustat daily, a total of 8 of 31 subjects (26%) met these criteria. Phase 2 dose-ranging studies, and associated statistical and exposure response modelling has informed Phase 3 dose rationale, starting doses, dose levels, and dose adjustment scheme to optimize Hgb management. Integrated AE data [including 2 global phase 2b studies (24-week treatment duration) and 2 Japanese phase 3 studies (52-week treatment duration)]: Few subjects experienced a possible thrombosis related adverse event in the setting of excessive erythropoiesis [3/688 (0.5%) subjects on daprodustat vs. 0/404 on rhEPO]. Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat when dose is managed appropriately according to target Hgb. However, experience with daprodustat is currently insufficient to fully characterize this risk.	• Specific eligibility criteria related to requirements for entry Hgb are detailed in Section 6.1 • Hgb will be closely monitored throughout the dosing period as outlined in the SoA (See Section 2) • Specific guidance for dose adjustment, dose interruption, or discontinuation of daprodustat based on achieved Hgb (including rate of change) is provided in Section 7.2 and Section 8.1 • Instream monitoring of safety data by internal safety review team
Worsening hypertension	In a dog cardiovascular study, single oral doses of daprodustat (up to 90 mg/kg) did not produce effects on blood pressure.	• Specific eligibility criteria related to blood pressure, including exclusion of subjects with uncontrolled hypertension, are detailed in

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	Marketed rhEPO and its analogues have been associated with risks related to uncontrolled hypertension, including the need for initiation of or increases in antihypertensive therapy when used in patients with anemia of CKD (i.e. 25% Epogen, 27% Mircera, and 40% Aranesp treated patients with renal anemia required initiation or increase in their anti-hypertensive medications; hypertensive encephalopathy and seizures have been reported. The contribution of rhEPO-associated hypertension to the unfavourable effects on cardiovascular outcomes remains uncertain). Integrated AE data from clinical trials with daprodustat [including 2 global phase 2b studies (24-week treatment duration) and 2 Japanese phase 3 studies (52-week treatment duration)]: o The majority (>90%) of subjects had baseline history of hypertension. o No meaningful difference was seen between treatment groups in AEs (preferred term) of "hypertension" [29/688 (4%) daprodustat vs. 19/404 (4%) rhEPO; 0.91 relative risk (RR) (95% confidence interval: 0.5, 1.67)] or "blood pressure increased" [16 (2%) daprodustat vs. 7 (2%) rhEPO; RR 1.22 (0.48,3.11)]. Results were not substantively different between non-dialysis and haemodialysis subjects. o Although no clinically meaningful changes in blood pressure were observed, subjects in both treatment groups required increases in anti-HTN medications: o In the 24-week global phase 2b studies, 25/170 (15%) of ND subjects receiving daprodustat vs. 18/80 (14%) control and 22/177 (12%) of HD subjects receiving daprodustat vs. 2/39 (5%) control. o In the 52-week Japan phase 3 studies, 57/149 (38%) of ND subjects receiving daprodustat vs. 68/150 (45%) rhEPO and 51/136 (38%) of HD subjects receiving daprodustat vs. 66/135	Section 6.2 • Blood pressure will be closely monitored throughout the dosing period as outlined in the SoA Table Section 2. • Monitoring of emerging safety data by an internal GSK Safety Review Team.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	(49%) for rhEPO. The data received to date from completed clinical trials with daprodustat are insufficient to refute this risk.	
Death, MI, stroke, heart failure, thromboembolic events, thrombosis of vascular access at Hgb levels which are within the normal range (i.e., not polycythemic conditions)	Marketed rhEPO and its analogs have been associated with an increased risk for death and serious cardiovascular events when used in patients with anemia of CKD. Clinical studies with marketed rhEPO/analogs have suggested "higher" target hemoglobin, rate of hemoglobin rise of greater than 1 g/dL in any 2-week period, and/or higher doses may contribute to these risks. Integrated AE data from clinical trials with daprodustat [including 2 global phase 2b studies (24-week treatment duration) and 2 Japanese phase 3 studies (52-week treatment duration)]: No meaningful difference was seen between treatment groups in the overall incidence of this AESI: [39/688 (5.5%) daprodustat vs. 25/404 (6%) rhEPO; 0.92 relative risk (95% confidence interval: 0.55, 1.53)]. Within this composite AESI, the most frequent event types were heart failure (at least 12 events daprodustat vs. at least 13 events rhEPO) and thrombosis (at least 14 events daprodustat vs. at least 8 event rhEPO); and a numerical imbalance was noted in events of myocardial ischemia (at least 7 events daprodustat vs. at least 1 event rhEPO). The small number of events makes it difficult to draw any firm conclusions. The clinical data received to date from completed clinical trials with daprodustat are insufficient to substantiate or refute this risk.	• Specific eligibility criteria related to CV risk are outlined in Section 6.2 • Hgb will be closely monitored throughout the dosing period as outlined in the SoA (See Section 2) • Instream monitoring of safety data by internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Esophageal and gastric erosions	In animal studies, undesirable GI effects including emesis, abnormal feces and/or decreased food consumption/body weight loss and stomach erosions/ulcers with hemorrhage were observed with daprodustat. In rodents, stomach erosions were observed with intravenous and oral administration of daprodustat. Gender-averaged systemic exposure (AUC) at the no observed adverse effect levels (NOAEL) are 3.3 -fold (monkeys) and 737 -fold (rats) above human exposure (25 mg daprodustat). In clinical trials to date with daprodustat, mild-moderate GI signs and symptoms represent the most frequently-reported adverse event, however causal association has not been established. Integrated AE data from clinical trials with daprodustat [including 2 global phase 2b studies (24-week treatment duration) and 2 Japanese phase 3 studies (52-week treatment duration)]: No meaningful difference was seen between treatment groups in reports of this AESI [17 (2.7%) daprodustat vs. 10 (2.3%) rhEPO; 1.16 relative risk (95% confidence interval: 0.52, 2.58)]. Following review of clinical data received to date, GI erosions have not been identified as a safety concern for daprodustat.	• Suspected GI bleeding or significant symptoms consistent with erosion or ulcers should be investigated diagnostically (i.e., endoscopic examination) as clinically warranted • Instream monitoring of safety data by internal safety review team
Cancer-related mortality and tumor progression and recurrence	Marketed rhEPO and its analogs have been associated with increased risk of cancer related morbidity and mortality when used in patients with cancer. Administration of 60mg/kg daprodustat to mice caused minimal increases in circulating vascular endothelial growth factor (VEGF) while significant EPO increases were observed. In clinical studies with daprodustat up to 4 weeks duration, a dose-ordered increase in VEGF plasma concentrations, an angiogenic factor that has been implicated in tumor growth, was observed at doses ranging from 10 to 150 mg. In clinical studies up to 24 weeks duration at doses up to 25 mg, changes in VEGF plasma concentrations were variable but similar relative to control. Integrated AE data [including 2 global phase 2b studies (24-week treatment duration) and 2 Japanese phase 3 studies (52-week treatment duration)]: No	• Specific eligibility criteria related to personal history of malignancy is outlined in Section 6.2 • Stopping criteria for participants with treatment emergent malignancy are outlined in Section 8.1 • Instream monitoring of safety data by internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	meaningful difference was seen between treatment groups in the occurrence of this AESI: [8/688 (1.1%) daprodustat vs. 4/404 (0.9%) rhEPO; 1.14 relative risk (95% confidence interval: 0.31, 4.28)]. Clinical experience to date is not yet sufficient to substantiate or refute this as a safety concern for daprodustat.	

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Pulmonary artery hypertension (PAH)	A role for HIF-regulated pathways in the pathophysiology of PAH has been suggested based on well established effects of acute and chronic hypoxia in man on the pulmonary vasculature (vasoconstriction), and by findings in patients with naturally occurring mutations that result in decreased HIF degradation [Smith, 2006; Formenti, 2011]. There have been no histopathologic findings suggestive of PAH in pre-clinical safety studies with daprodustat (up to 13-weeks duration in dogs, up to 2-years in rats and mice, and up to 39-weeks in monkeys. Acute hypoxic challenge (rats): Daprodustat produced increases in peak right ventricular pressure (PRVP) during acute hypoxia that were slightly higher than the vehicle control group. However, these hypoxia-induced PRVP changes were within the range of PRVP changes noted among untreated rats. • Results from a clinical study of acute hypoxic challenge in healthy volunteers demonstrated that short-term (5 days) therapy with daprodustat 5mg or 100mg had no clinically significant effect on transthoracic echocardiographically (ECHO) estimates of pulmonary artery systolic pressure (PASP) under either normoxic or hypoxic conditions. ECHO assessments performed in Phase 2b studies (24 weeks treatment duration) did not identify any clinically meaningful changes in PASP in participants not on dialysis for daprodustat. In hemodialysis participants, mean absolute change from baseline in PASP was similar for both treatment groups; however, there was a numeric imbalance (daprodustat Total: 8 [7%]; Control 0) in participants reaching the PASP PCI (>20 mmHg increase from baseline). Regarding this imbalance, there were a number of confounding factors in the study, most notably a 4.5:1 randomization scheme and inconsistency in timing of ECHOs relative to dialysis day. Additionally, 2 of 3 participants with resolution of PASP on safety follow-up ECHOs had confounding conditions that could contribute to resolution other than discontinuation of study drug; and there was no dose relationship for participants meeting the PASP percutaneous coronary intervention (PCI) criterion. Overall, there is insufficient evidence to conclude a relationship to treatment with daprodustat. A post-hoc analysis was performed using a definition of PAH cited in the literature [Navaneethan, 2016]. Subjects with	• Instream monitoring of safety data by internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	sPAP >35 mmHg and/or tricuspid regurgitation maximum jet velocity (TRV) >2.5 m/s were considered as having PAH. Regardless of baseline status of PAH, there was no clinically meaningful difference in the proportion of subjects with on-treatment PAH between the two treatment groups: ○ Subjects with PAH at baseline: 35/113 (31%) vs. 21/54 (39%) (ND) and 37/115 (32%) vs. 7/21 (33%) (HD), daprodustat vs. control, respectively. ○ Subjects without PAH at baseline: 25/113 (22%) vs. 12/54 (22%) (ND) and 22/115 (19%) vs. 6/21 (29%) (HD), daprodustat vs. control, respectively. Integrated AE data [including 2 global phase 2b studies (24-week treatment duration) and 2 Japanese phase 3 studies (52-week treatment duration)]: Four (0.5%) non-serious AEs in the daprodustat group vs 0 in rhEPO. • Review of subject level information did not suggest adverse treatment effect: 2 subjects from phase2b that met protocol specified stopping criteria on scheduled ECHO had non-serious AEs of 'pulmonary arterial pressure increased' and 2 subjects from Japan Phase 3 had non-serious AE 'pulmonary hypertension' in setting of concurrent serious AEs of acute pulmonary embolus and mitral regurgitation identified during hospitalization for coronary angiography Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat.	
Cardiomyopathy	Published data suggest that cardiac effects of HIF stabilization are likely a function of the mechanism, extent, and duration of the effects, and can range from protective to detrimental depending upon the specific model and experimental conditions utilized. Small increases in cardiac troponin in 6 month rat study with daprodustat were consistent with the background finding of spontaneous rodent cardiomyopathy. There were no elevations observed in cardiac troponin in 9 month monkey study with daprodustat.	• Instream monitoring of safety data by internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	Cardiomyopathy has not been associated with naturally occurring mutation in man which results in increased HIF stabilization. ECHO assessments performed in Phase 2b studies (24 weeks treatment duration) did not identify any clinically-meaningful changes in left ventricular ejection fraction (LVEF) with daprodustat. Integrated AE data from clinical trials with daprodustat [including 2 global phase 2b studies (24-week treatment duration) and 2 Japanese phase 3 studies (52-week treatment duration)]: No meaningful difference was seen between treatment groups in reports of this AESI [1 (0.1%) daprodustat vs. 1 (0.2%) rhEPO; 0.64 relative risk (95% confidence interval: 0.02, 18.07)]. Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat.	
Proliferative retinopathy, macular edema, choroidal neovascularization	Increases in local (ocular) VEGF production with retinal neovascularization and macular edema observed in diabetic retinopathy and to choroidal leakage, edema and neovascularization seen in age-related macular degeneration [Campochiaro, 2006]. Administration of 60mg/kg daprodustat to mice caused minimal increases in circulating VEGF while significant EPO increases were observed. No ocular abnormalities with daprodustat were seen in non-clinical studies of up to 13 weeks duration in mice and dogs, 26 weeks in rats, and 39 weeks in monkeys. In clinical studies with daprodustat up to 4 weeks duration, a dose-ordered increase in VEGF plasma concentrations was observed at doses ranging from 10mg to 150mg administered once daily and from 10mg to 30mg administered three times weekly. In studies up to 24 weeks duration at doses up to 25mg, changes in VEGF plasma concentrations were variable but similar relative to control. Ophthalmologic assessments performed in Phase 2b studies (24 weeks treatment duration) did not identify any clinically meaningful changes in proliferative retinopathy, macular edema, or choroidal neovascularization from	• Instream monitoring of safety data by internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	daprodustat. Integrated AE data [including 2 global phase 2b studies (24-week treatment duration) and 2 Japanese phase 3 studies (52-week treatment duration)]: No meaningful difference was seen between treatment groups in reports of this AESI [9 (2.9%) daprodustat vs. 6 (2.5%) rhEPO; 1.19 relative risk; (95% confidence interval: 0.42, 3.43)]. Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat.	
Exacerbation of rheumatoid arthritis	In inflamed rheumatic joints, activation of HIF-related genes secondary to decreased oxygen and pro-inflammatory cytokines has been postulated to contribute to the neo-angiogenesis, proliferation and infiltration of rheumatoid synovial fibroblasts [Westra, 2010; Muz, 2009]. No abnormalities were seen in non-clinical studies conducted to date for daprodustat. Integrated AE data [including 2 global phase 2b studies (24-week treatment duration) and 2 Japanese phase 3 studies (52-week treatment duration)]: No meaningful difference was seen between treatment groups in reports of this AESI [2 (0.3%) daprodustat vs. 1 (0.2%) rhEPO; 1.20 relative risk; (95% confidence interval: 0.07, 20.87) and the incidence of musculoskeletal AEs was generally lower in the daprodustat treatment group]. Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat.	• Instream monitoring of safety data by internal safety review team
Drug-drug interactions	Daprodustat is a substrate of CYP2C8: Co-administration of daprodustat with a strong CYP2C8 inhibitor (i.e., gemfibrozil) increased the maximum plasma concentration (Cmax) and area under curve (AUC) of daprodustat, 4- and 19-fold, respectively, while co-administration of a weak inhibitor (i.e., trimethoprim) increased the Cmax and AUC of daprodustat by 1.3- and 1.5-fold, respectively. Population PK analysis from completed Phase 2 studies suggests that co-administration of daprodustat with a moderate CYP2C8 inhibitor (i.e., clopidogrel) leads to a ~ 2-fold increase in AUC, with no clinically-significant	• Co-administration of daprodustat with strong CYP2C8 inhibitors (e.g., gemfibrozil) and inducers (e.g., rifampin/rifampicin) is not permitted as outlined in Section 7.7.2 • Co-administration of daprodustat with moderate CYP2C8 inhibitors (i.e., clopidogrel, teriflunomide, deferasirox) should be performed with caution. If one of these medications is started, stopped or the

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	increase in the measured Hb response. Although CYP2C8 induction studies were not performed, co-administration of daprodustat with an inducer of CYP2C8 (e.g., rifampin/rifampicin) may decrease the exposure of daprodustat. Even though co-administration of daprodustat with strong inhibitors and inducers of CYP2C8 is prohibited, inadvertent co-administration may occur. Due to the known time delay in enhancing erythropoiesis by daprodustat, co-administration with strong CYP2C8 inhibitors for up to 14 days is not anticipated to lead to immediate marked increases in hemoglobin levels. Therefore, there is adequate time to change to alternate therapy that does not inhibit CYP2C8. Additionally, as the time for maximum induction of CYP2C8 occurs after approximately 10-14 days of dosing with rifampin (Brodie, 2013; Ohnhaus, 1989), daprodustat systemic exposure will decrease over time which will result in a lag period before an effect on Hgb is recognized and is of clinical concern. Daprodustat is an inhibitor of CYP2C8: A clinical drug interaction study between 25mg and 100mg daprodustat with a CYP2C8 substrate (i.e., pioglitazone) showed that there is no PK interaction at these doses of daprodustat. Daprodustat is a substrate of Breast cancer resistance protein (BCRP): Population PK analysis from Phase 2 studies suggested that while BCRP inhibitors were a covariate for daprodustat apparent total body clearance (CL/F) (8.6% lower clearance) the predicted change in exposure was not considered to be of clinical relevance. Daprodustat is an inhibitor of organic anion transporter polypedtides (OATP)1B1/1B3: A clinical drug interaction study between 25mg and 100mg daprodustat with an OATP1B1/1B3 substrate (i.e., rosuvastatin) showed no PK interaction at these doses of daprodustat.	dose is changed, Hgb should be monitored every 4 weeks Specific guidance on the management of potential drug-drug interactions and concomitant medications is provided in Section 7.2 • Hgb will be closely monitored throughout the dosing period as outlined in the SoA (See Section 2) • Specific guidance for dose adjustment, dose interruption, or discontinuation of daprodustat based on achieved Hgb is provided in Section 7.2. • Instream monitoring of safety data by internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Cyst progression in patients with autosomal dominant polycystic kidney disease (ADPKD)	Published data provide in vivo evidence for a potential role of HIF-1a in the growth of polycystic kidneys; Hif-1a deletion was sufficient to significantly mitigate a progressive polycystic phenotype in an ADPKD mouse model, while conversely pharmacologic HIF-1a stabilization was sufficient to convert a mild polycystic disease into a severely aggravated phenotype with marked loss of renal function (Kraus, 2018; Hofherr, 2018). A review of the non-clinical data from toxicity studies conducted with daprodustat does not indicate an exacerbation in incidence or severity of kidney cysts in daprodustat-treated animals in comparison to controls. However, the wild type animals used in these toxicity studies have a very low background incidence of renal cysts and are not comparable to the mice used in the Kraus article (Kraus, 2018) which are an inducible kidney epithelium-specific Pkd1-deletion model. There is limited experience with daprodustat in subjects with ADPKD in completed clinical trials. In the Japan phase 3 study in non-dialysis subjects, there were 5 subjects with ADPKD (all CKD stage 5) in each treatment group. Mean baseline eGFR was 10 mL/min/1.73m2 in the daprodustat subjects vs. 16 mL/min/1.73m2 in the rhEPO subjects. The mean (SD) percent change from baseline at Week 52 in eGFR was: -18% (8) vs. -21% (14) in daprodustat vs. rhEPO, respectively. Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat.	• Kidney function will be monitored throughout the dosing period as outlined in the SoA (Section 2). • Monitoring of emerging safety data by an internal GSK Safety Review Team.
Other		
Darbepoetin alfa (Control)	See risks outlined in table for daprodustat for excessive erythropoiesis (polycythemia) leading to thrombosis and/or tissue ischemia; Death, MI, stroke, heart failure, thromboembolic events, thrombosis of vascular access; and for increased cancer-related mortality and tumor progression. Uncontrolled hypertension. Pure red cell aplasia.	• See mitigation strategies outlined in table for daprodustat for Excessive erythropoiesis (polycythemia) leading to thrombosis and/or tissue ischemia; Risk of death, MI, stroke, heart failure, throboembolic events, thrombosis of vascular access; and for Increased cancer-related mortality and tumor progression. • Specific eligibility criteria related to current uncontrolled hypertension are outlined in Section

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		6.2 • Specific eligibility criteria related to personal history of pure red cell aplasia are outlined in Section 6.2
Challenge Agents		
Acetylcholine	Acetylcholine stimulates the production by the vascular endothelium of nitric oxide, a potent vasodilatory agent. Distribution of acetylcholine to the peripheral vasculature has the potential to lead to a decrease in blood pressure; however acetylcholine is rapidly metabolized by acetylcholinesterase, minimizing the potential for pharmacological concentrations distant from the infusion site.	• Study undertaken only at specialist centers by experienced study personell trained to conduct forearm blood flow assessment • Patients monitored continously throughout technique • Minimum dose given for required physiological response • Patients with recent cardiovascular events excluded from the study
N-monomethyl-L-arginine (NMMA)	L-NMMA inhibits nitric oxide synthase, leading to decreased production of nitric oxide, which can lead to increases in blood pressure.	• Study undertaken only at specialist centers by experienced study personnel trained to conduct forearm blood flow assessment • Patients monitored continously throughout technique • Minimum dose given for required physiological response • Patients with recent cardiovascular events excluded from the study
Sodium nitroprusside	Sodium nitroprusside directly causes vasodilation leading to decreases in blood pressure. Reported side effects of sodium nitroprusside administration are excessive hypotension and excessive accumulation of cyanide at high rates of infusion.	• Study undertaken only at specialist centers by experienced study personnel trained to conduct forearm blood flow assessment • Patients monitored continously throughout technique • Minimum dose given for required physiological

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		response • Patients with recent cardiovascular events excluded from the study
Study Procedures		
Venous occlusion plethysmography	Primary risk associated with venous venous occlusion plethysmography is related to the cannulation of the brachial artery. This has the potential for causing damage to the brachial artery, thrombosis, digital ischemia, and hemorrhage, as well as discomfort at the infusion site.	• Study undertaken only at specialist centers by experienced study personnel trained to conduct forearm blood flow assessment • Cannulation of the brachial artery for infusion of the challenge agents will be done with a fine (27 guage) needle to minimize any injury to the artery • Cannulation will be done under a local anesthetic to minimize participant discomfort

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12.8. Appendix 8: Non-Contact, Optical Forearm Plethysmography (NC-OFP) Sub-Study

12.8.1. Introduction and Rationale

The purpose of this pilot sub-study is to evaluate and compare the effectiveness of measuring FBF via a non-contact, alternative method in participants as compared to strain-gauge forearm plethysmography assessment. The proposed method could be a step in identifying alternate strategies to safely and effectively evaluate FBF in participants undergoing cardiovascular drug studies (Wilkinson, 2001).

12.8.2. Objectives and Endpoints

Objective	Endpoints
Primary	
To compare FBF measurements between non-contact plethysmography and strain-guage plethysmography	• Compare the FBF ratio and absolute FBF, as measured by non-contact plethymography, to FBF ratio and absolute FBF, as measured by strain-gauge plethysmography technique

12.8.3. Study Design

This is a sub-study of the 205767 study in order to compare measurement of forearm blood flow by a non-contact optical method to assessment by use of a strain gauge. Approximately 6 participants from one to two sites participating in the main study will be invited to be part of this sub-study. Every attempt will be made to have equal numbers of participants from each study treatment group.

12.8.4. Additional Inclusion Criteria

Signed written informed consent prior to beginning sub-study-related procedures (subject must understand the aims, procedures, and possible consequences of the sub-study).

Note: Consent to participate in the sub-study is separate from consent to participate in the main study.

12.8.5. Study Assessments

Non-contact plethysmography will be conducted in parallel with the main study FBF assessment, using a combination of an infrared camera and Raspberry Pi portable computer which will capture high-resolution, subdermal images of the forearm. The device will be placed at approximately 30 cm from participants' forearm while they are also undergoing the venous occlusion strain-gauge plethysmography assessment. The device will capture and store visual information and utilizing proprietary software, extract plethysmographic data.

The device will utilize the time of flight data gathered from the infrared camera to obtain three dimensional data of the forearm. The high resolution and high frequency images

will undergo image processing to allow for the extraction of the vasculature data separately from the acquisition (data will be stored and sent to the study team for analysis off-site). The data will be analysed in software to estimate the volume change of the vessels and therefore the forearm. A single-point time of flight sensor placed intermittently within the enclosure will measure how long it takes for light to return to the sensors, effectively measuring distance. The sensor will ensure correct placement of the system and allow for any variance in distance to be accounted for when making calculations. The combination of the sensors and cameras in the enclosure will allow for plethysmographic data to be extracted along with vasculature change data.

12.8.6. Withdrawal from Non-Contact, Optical Plethysmography Sub-Study

If a participant in this sub-study withdraws from the sub-study, the reason for the withdrawal must be recorded in the eCRF. The participant will remain in the main study unless the subject withdraws consent from the main study.

12.8.7. Sample Size

The target evaluable sample size is 6. The sample size is based on feasibility.

12.8.8. Analyses Plan

All participants with a minimum baseline FBF assessed via non-contact and strain-gauge method will be evaluated in the sub-study. Additional details regarding the analysis plan will be provided in the RAP of the main study.

12.9. Appendix 9: Protocol Amendment History

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

Previous Amendment:

Amendment 1 29-JAN-2018

Overall Rationale for the Amendment: The study team was required per regulatory agencies to change the protocol to include a serum pregnancy testing at screening. During the amendment process, minor updates were made for study optimization.

Section # and Name	Description of Change	Brief Rationale
1 and 4 Objectives and Endpoints	The first secondary endpoint was designated as the Principle Secondary endpoint.	For emphasis of the aim of the study.
2 Schedule of Activities	The word “examinations” was added to the Physical.	To be more consistent with wording later in the protocol.
2 Schedule of Activities	Serum pregnancy test [FRP only] was added to the table and indicated to be done at screening by an X in that column. The urine pregnancy test for that time point was deleted.	Required to amend this by regulatory agency.
6.3.2 Caffeine, Alcohol and Tobacco	Added in text regarding participant instruction on caffeine avoidance prior to certain study visits.	To have more consistency across sites on the participant preparation prior to procedures.
9.4.1 Physical Examinations	Height and weight was added to the the physical exam at screening.	Added for more complete assessment of the participant.
9.4.2 Vital Signs	Weight was added as a parameter to the vital signs.	Added for more complete assessment of the participant.
9.6.1 Venous Occlusion Plethysmography to Measure Forearm Blood Flow (FBF)	Minor wording changes to indicate the subject is not fasting during the procedures, but can have a light breakfast.	Added for clarity.
12.2 Appendix 2	Serum pregnancy test was added to Table 4: Protocol Required Laboratory Assessments	For consistency with the Schedule of Activities table.
Throughout	Minor editorial and grammatical revisions	Minor, therefore have not been summarized.