TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	A randomized, open label study to evaluate the effect of daprodustat on blood pressure in subjects with anemia associated with chronic kidney disease on hemodialysis switched from a stable dose of an erythropoiesis-stimulating agent

Short Title: **A**nemia **S**tudies in **C**KD: **E**rythropoiesis via a **N**ovel PHI **D**aprodustat – **B**lood **P**ressure (**ASCEND-BP**)

Compound Number: GSK1278863

Development Phase: IIB

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Protocol Amendment Number: 04

Sponsor Name and Legal Registered Address:

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Regulatory Agency Identifying Number(s): IND Number: 101,291

Revision Chronology

GlaxoSmithKline Document Number	**Date**	**Version**
2015N267693_00	2016-AUG-02	Original
2015N267693_01	2016-NOV-21	Amendment No. 01
This protocol amendment was written to clarify specific biomarkers being drawn, modify time points of biomarkers, clarify safety laboratory studies, and clarify day/time of assessments. An additional potential study visit is added.		
2015N267693_02	2017-JUL-31	Amendment No. 2
This protocol amendment was written to clarify the descriptive statistics and include them in the objectives and endpoints table(s) as well as remove one blood pressure measurement. Additional minor changes were made for clarity of study procedures.		
2015N267693_03	2018-MAY-21	Amendment No. 3
This protocol amendment was written to streamline recruitment of subjects into the study and maintain appropriate Hgb levels while in the study. This includes removing study visits, altering entry requirements as well as stopping criteria, changing dosing and dose adjustments, and restructuring Hgb parameters.		
2015N267693_04	2019-OCT-23	Amendment No. 4
This protocol amendment was written to remove the interim analysis from the statistical section due to more rapid recruitment than anticipated. During the update, the objectives and endpoints were streamlined, the stratification variable of previous ESA dose was added to the models as it was inadvertently left out, the daprodustat dosing algorithm was updated, and exploratory endpoints were added to further clarify results. During this time the safety language was updated as well as clarification of inclusion/exclusion criteria in response to an internal audit.		

SPONSOR SIGNATORY

PPD

10/23/2019

Alexander R. Cobitz, M.D., Ph.D.
Executive Director, Clinical Development
Metabolic Pathways and Cardiovascular Unit
GlaxoSmithKline

Date

PPD

PROTOCOL AGREEMENT PAGE

For protocol number 205665.

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

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

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

Investigator Name:	
Investigator Address:	
Investigator Phone Number:	

Investigator Signature	Date

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1. PROTOCOL SYNOPSIS FOR STUDY 205665

Rationale

The purpose of this study is to compare the effects on blood pressure of daprodustat to epoetin alfa in hemodialysis-dependent (HD) subjects with anemia associated with chronic kidney disease (CKD). The study will also assess various biomarkers associated with blood pressure physiology that may provide insight into the mechanism(s) of the hypertensive effects associated with erythropoiesis stimulating agent(s) (ESAs).

Objective(s)/Endpoint(s)

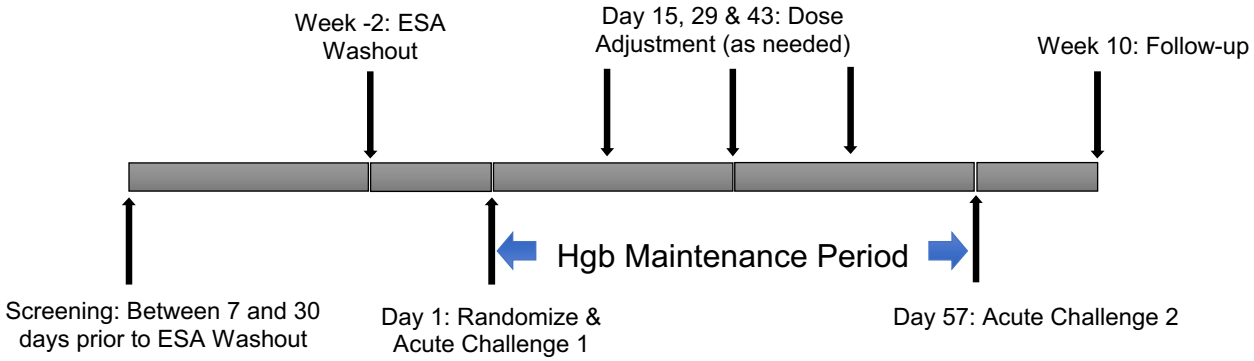
Objectives	Endpoints
Primary	
• To compare the effect of daprodustat to epoetin alfa on blood pressure (BP) after Acute Challenge 2 (8 weeks of hemoglobin [Hgb] maintenance therapy)	• Average of systolic blood pressure (SBP) as measured by ambulatory blood pressure monitoring (ABPM) over 6-hr post-dosing after 8 weeks of Hgb maintenance therapy
Secondary	
• To compare the initial effect of daprodustat to epoetin alfa on BP after Acute Challenge 1 (2 weeks of erythropoiesis-stimulating agent [ESA] washout)	• Average of SBP, diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR) as measured by ABPM over 6-hr post-dosing at Day 1 • Area under the effect curve (AUEC) of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 1
• To compare the effect of daprodustat to epoetin alfa on BP after Acute Challenge 2	• Average of DBP, MAP, and HR as measured by ABPM over 6 hr post-dosing at Day 57. • AUEC of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 57.
• To estimate the initial effect of daprodustat on SBP, DBP, HR and MAP after Acute Challenge 1	• Change from pre-dose in SBP, DBP, HR, and MAP at each timepoint at Day 1
• To characterize the pharmacokinetics of daprodustat	• Plasma concentrations of daprodustat and metabolites and derived pharmacokinetic parameters including maximum observed concentration (Cmax), time of occurrence of Cmax (tmax), terminal phase half-life (t½) and area under concentration-time curve from time zero to 24 hours (AUC[0-24]) as appropriate
Safety	
• To assess the safety and tolerability of daprodustat	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs)

Objectives	Endpoints
	• Reasons for discontinuation of study treatment • Absolute values and changes from baseline over time in laboratory parameters, electrocardiograms (ECGs) and vital signs
Exploratory	
• To investigate the effect of daprodustat and epoetin alfa on vasoactive mediators of blood pressure	• Plasma concentrations and derived parameters including Cmax, tmax, t½ and AUC(0-24) as appropriate (to include erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II and metabolites, and noradrenalin)
• To summarize the effect of daprodustat and epoetin alfa on SBP after Acute Challenge 1 and Acute Challenge 2	• AUEC of SBP as measured by ABPM over 24-hr post-dosing
• To compare the effect of daprodustat to epoetin alfa on SBP after 8 weeks of Hgb maintenance therapy	• Change from Day 1 pre-dose SBP to Day 57 pre-dose
• To compare the effect of daprodustat to epoetin alfa on SBP at Cmax of each study treatment, as measured by ABPM.	• Change in SBP from pre-dose on Day 1 to Cmax of the study treatment on Day 1 • Change in SBP from pre-dose on Day 1 to Cmax of the study treatment on Day 57 • Change in SBP from pre-dose on Day 57 to Cmax of the study treatment on Day 57
• To compare the effect of daprodustat to epoetin alfa on SBP at respective Cmax of the erythropoietin level, as measured by ABPM.	• Change in SBP from pre-dose on Day 1 to Cmax of erythropoietin on Day 1 • Change in SBP from pre-dose on Day 1 to Cmax of erythropoietin on Day 57 • Change in SBP from pre-dose on Day 57 to Cmax of erythropoietin on Day 57

Overall Design

This study will be an open-label, randomized, parallel-group design in HD subjects with anemia associated with CKD. There will be a screening period, a 2-week ESA washout period, an initial 24-hour acute challenge, an 8-week Hgb-maintenance period, a second 24-hr acute challenge and a follow-up visit 14±3 days after completing treatment. The study design is outlined below:

Study Design



Subjects will be screened for eligibility within 7 - 30 days prior to the start of the ESA washout period.

Subjects that qualify for enrolment will be washed out of their ESA for 2 weeks; the start of the ESA washout should be timed to coincide as closely as possible with their next planned ESA dose. Subjects are enrolled once they enter the ESA washout period.

Following the 2-week ESA washout, subjects will be randomized 1:1 and stratified by prior ESA dose, based on the average weekly (for epoetins or darbepoetin) or monthly (for methoxy polyethylene glycol (PEG)-epoetin beta) dosing during the 12 weeks prior to ESA washout as follows:

- Low ESA dose: <100 U/kg/week epoetin alfa OR <0.5 µg/kg/week darbepoetin OR <0.6 µg/kg/week methoxy PEG-epoetin beta
- High ESA dose: ≥100 U/kg/week epoetin alfa OR ≥0.5 µg/kg/week darbepoetin OR ≥0.6 µg/kg/week methoxy PEG-epoetin beta

On Day 1, subjects will be randomized and undergo Acute Challenge 1, a single dose challenge to compare the acute effects on BP of the highest planned once-daily maintenance dose of daprodustat (24 mg) to the highest starting dose of epoetin alfa (100 U/kg). Subjects will have BP monitored for 24 hr using ABPM, and will have serial blood sampling to assess the pharmacokinetics of daprodustat and the time-course of various biomarkers. Acute Challenge 1 will be started promptly following completion of a mid-week dialysis session.

After completing Acute Challenge 1, all subjects will enter the 8-week Hgb maintenance period where doses of either daprodustat or epoetin alfa will be administered and adjusted, as needed.

On Day 57, following the 8-week Hgb maintenance period, subjects will undergo Acute Challenge 2; this challenge will, similar to Acute Challenge 1, involve assessing the effect of daprodustat and epoetin alfa on BP as assessed by 24-hr ABPM measurement. The subjects will be administered the same dose of either daprodustat or epoetin alfa that was given in Acute Challenge 1, and serial blood sampling will be performed to assess

daprodustat pharmacokinetics and to characterize the time course of various biomarkers. Acute Challenge 2 will be started following completion of a mid-week dialysis session.

Subjects will attend a follow-up visit 2 weeks after completion of Acute Challenge 2. Subjects will be asked to not reinitiate anemia treatment until after the follow-up visit unless deemed medically necessary

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the QC criteria following Acute Challenge 2, one additional Acute Challenge may be made after a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed.

The Acute Challenge 2 may be delayed one week if the subject's Hgb ≥11.5.

Treatment Arms and Duration

The total maximum duration of subject involvement is up to 16 weeks (Screening to Follow-up) as described below:

- Screening: Screening will occur at least one week prior to but not more than 30 days prior to washout.

- ESA Washout: Subjects that meet eligibility criteria will begin the 2-week ESA washout.

    - This should be timed such that this day occurs on the date that would have been the next scheduled dose of ESA (or as close as practicable).

    - For subjects with a three-times weekly dialysis schedule, this day must not occur on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday).

    - For subjects with a four- to five-times weekly dialysis schedule, this day can be on any hemodialysis session of the week.

- Randomization & Acute Challenge 1 (Day 1): Subjects will be randomized 1:1 to two treatment arms and will begin a 24-hr Acute Challenge where the acute BP effects of daprodustat and epoetin alfa will be evaluated. This challenge will be initiated promptly following the subject's dialysis session.

- Hgb Maintenance Period: Subjects randomized to daprodustat will start an 8-week Hgb maintenance period based on a starting dose and dose adjustment algorithm. Subjects randomized to epoetin alfa will begin an 8-week Hgb maintenance period where Hgb will be managed in accordance with local labelling.

- Acute Challenge 2 (Day 57): At the end of the 8-week Hgb maintenance period subjects will repeat the procedures of Acute Challenge 1 utilizing the same treatment dose administered in Acute Challenge 1. This challenge will be initiated promptly following the subject's dialysis session.

- Follow-up: A Follow-up visit will be scheduled to occur 14 ± 3 days after completing treatment.

Treatment Arm Descriptions

Treatment Arm	Acute Challenge 1	Hgb Maintenance Phase	Acute Challenge 2
A	100 U/kg IV epoetin alfa	IV epoetin alfa according to label	100 U/kg IV epoetin alfa
B	24 mg daprodustat	QD daprodustat according to dose adjustment algorithm	24 mg daprodustat

Type and Number of Subjects

- The study will enroll hemodialysis-dependent subjects with anemia associated with CKD currently treated with an ESA. Subjects with Hgb values of 8.5 - 11.5 g/dL, inclusive at both Screening and Day 1, are eligible, and the subjects must currently be on an ESA product.
- Approximately 124 subjects will be screened to achieve approximately 62 randomized and 50 evaluable subjects for a total of 25 evaluable subjects per treatment group.
- If subjects prematurely discontinue the study, additional replacement subjects may be randomized and assigned to the same treatment arm at the discretion of the Sponsor in consultation with the investigator.

Analysis

- The primary endpoint for this study is average systolic blood pressure (SBP) over 6 hours of measurements taken at 15-minute intervals after the administration of study treatment for the Acute Challenge 2. The primary comparison of interest is daprodustat versus epoetin alfa for this challenge.
- The primary analysis of average SBP over 6 hr post-dose during Acute Challenge 2 will be an analysis of covariance (ANCOVA) with terms for treatment, prior ESA dose (low/high), post-HD/pre-Acute Challenge 1 SBP, difference between post-HD/pre-Acute Challenge 2 SBP and post-HD/pre-Acute Challenge 1 SBP, and treatment by (difference in post-HD SBP between Acute Challenge 1 and 2) interaction. Note that the pre-challenge SBP may change over time between Acute Challenge 1 and Acute Challenge 2 as a result of the maintenance therapy, and thus the pre-challenge SBP at Acute Challenge 2 is not a true pre-treatment covariate. As a result, the interpretation of the effect of the Acute Challenge 2 may be confounded by the impact of treatment phase on the covariate. Therefore, if the interaction term in this model is significant at the 0.10 level, then the primary model will be ANCOVA with terms for treatment, prior ESA dose (low/high), and post-HD/pre-Acute

Challenge 1 SBP. The primary model will provide a point estimate and two-sided 95% CI for the treatment effect and a p-value for the superiority assessment. Superiority will be established if the p-value is <0.05.

- The comparison of daprodustat versus epoetin alfa on 6 hr average SBP during Acute Challenge 1 is a secondary endpoint. Average SBP over 6 hr post-dose in Acute Challenge 1 will be analyzed using ANCOVA with terms for treatment, prior ESA dose (low/high), and post-HD/pre-Acute Challenge 1 SBP. Similar analyses will be performed for DBP, MAP, and HR.
- ANCOVA using the primary model will be used for the analysis of average DBP, MAP, and HR over 6 hr post-dose in Acute Challenge 2 replacing the baseline SBP term with the analogous baseline measurement for DBP, MAP, and HR, respectively.
- AUECs of SBP, DBP, MAP, and HR post Acute Challenge 2 and 1 will be analyzed using ANCOVA with terms for treatment and prior ESA dose (low/high) with 95% CIs and p-values provided for the treatment effect.
- Null Hypothesis: The difference between 24 mg daprodustat versus 100 IU/kg epoetin alfa on 6 hr average SBP under a background of treatment (i.e., during Acute Challenge 2) is zero.
- Alternative Hypothesis: The difference between 24 mg daprodustat versus 100 IU/kg epoetin alfa on 6 hr average SBP under a background of treatment (i.e. during Acute Challenge 2) is not zero.

2. INTRODUCTION

Anemia is a common complication of chronic kidney disease (CKD). The cause of anemia in this population is multi-factorial, including relative or absolute deficiency of erythropoietin (EPO), reduced iron availability related to chronic inflammation, and gastrointestinal blood loss. Current treatments for anemia associated with CKD include erythropoiesis-stimulating agents (ESAs) such as recombinant human (rh) EPO, supplemental iron therapy (intravenous and/or oral), and blood transfusions. However, each of these treatments has significant limitations:

- **ESAs**: In order to achieve target hemoglobin (Hgb) levels (which are lower than normal Hgb levels), treatment with ESAs markedly increases plasma EPO concentrations to supra-physiologic levels. Treatment with ESAs has also been associated with increased cancer-related morbidity and mortality and increased risk of major cardiovascular (CV) events (e.g., stroke, myocardial infarction and all-cause mortality) [FDA, 2011], as well as ESA-induced hypertension.
- **Iron**: Poor compliance is seen with oral iron therapy due to gastrointestinal intolerance while intravenous (IV) iron may have an increased risk of infection and/or iron overload as well as an increased risk for congestive heart failure [Agarwal, 2015].
- **Blood transfusions**: These are avoided when possible because of potential allo-immunization, which can preclude the possibility of receiving a kidney transplant in addition to a risk of infection.

Hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors (PHIs) are an emerging new class of agents under investigation for the treatment of anemia associated with CKD.

These molecules stimulate erythropoiesis through inhibition of HIF-prolyl hydroxylase domain 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].

Daprodustat (GSK1278863) is a small molecule, oral inhibiter of the HIF-PHD enzymes which may present several important advantages over other ESAs. It is an oral medication and does not require cold-chain storage as do some ESAs, thus increasing ease of use for patients. Moreover, data indicate that daprodustat can effectively raise Hgb concentrations with lower EPO levels than those observed after administration of ESAs [Provenzano, 2011]. Because of the increased CV risk associated with raising Hgb concentrations through large increases in EPO levels [Pfeffer, 2009], daprodustat has the potential to raise Hgb with less CV risk than other ESAs. CCI [redacted] CCI [redacted] CCI [redacted].

2.1. Study Rationale

Current labelling for all ESAs includes a contraindication in patients with uncontrolled hypertension, and increases in blood pressure (BP) and/or antihypertensive medication use has been observed in several clinical trials with ESAs. While the mechanism for this hypertensive effect is not known, hypertension has been consistently observed in approximately 25 to 30% of CKD patients initiating ESA therapy, and varies between an average of approximately 5 to 8 mmHg in systolic blood pressure (SBP), and 4 to 6 mmHg in diastolic blood pressure (DBP) reviewed in [Krapf, 2009]. Additionally, increases in BP are a well-recognized risk factor for CV events, in particular heart failure, ischemic stroke and myocardial infarction [Rodriguez, 2014]. Finally, treatment with ESAs has been associated with increased risk of major CV events including myocardial infarction, stroke and death.

Daprodustat has been shown to effectively raise and manage Hgb levels in subjects with CKD with lower levels of EPO than the ESAs. As the hypertensive effects of ESAs appear dose-related [Abraham, 1991], there is the potential that daprodustat can treat anemia in CKD patients without the hypertensive effects observed with the ESAs. This study will use two 24-hr Acute Challenges to compare the effect of daprodustat to rhEPO on BP as measured by ambulatory blood pressure monitoring (ABPM) following both a 2-week ESA washout period (Acute Challenge 1) and an 8-week Hgb-maintenance period (Acute Challenge 2).

Therefore, the purpose of this study is to compare the effects on blood pressure of daprodustat to epoetin alfa in hemodialysis-dependent (HD) subjects with anemia associated with CKD. The study will also assess various biomarkers associated with

blood pressure physiology that may provide insight into the mechanism(s) of the hypertensive effects associated with ESAs.

2.2. Brief Background

Daprodustat is a HIF-PHI currently being investigated as a treatment for anemia associated with CKD in both hemodialysis dependent (HD) and non-dialysis dependent (ND) subjects, with adequate safety and efficacy demonstrated in clinical trials of up to 24 weeks' duration. Both pre-clinical and clinical data show that daprodustat stimulates EPO production resulting in increased erythropoiesis and elevation in Hgb concentrations. These increases in Hgb are achieved with peak EPO levels substantially lower than those observed with ESAs [Holdstock, 2016]. Data from completed clinical and pre-clinical studies are provided in the Daprodustat Investigator Brochure (IB) and IB Supplement 01 and 02 [GlaxoSmithKline Document Number RM2008/00267/07, GlaxoSmithKline Document Number 2015N266524_00, GlaxoSmithKline Document Number 2015N266524_01].

3. OBJECTIVE(S) AND ENDPOINT(S)

Objectives	Endpoints
Primary	
• To compare the effect of daprodustat to epoetin alfa on blood pressure (BP) after Acute Challenge 2 (8 weeks of hemoglobin [Hgb] maintenance therapy)	• Average of systolic blood pressure (SBP) as measured by ambulatory blood pressure monitoring (ABPM) over 6-hr post-dosing after 8 weeks of Hgb maintenance therapy
Secondary	
• To compare the initial effect of daprodustat to epoetin alfa on BP after Acute Challenge 1 (2 weeks of erythropoiesis-stimulating agent [ESA] washout)	• Average of SBP, diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR) as measured by ABPM over 6-hr post-dosing at Day 1 • Area under the effect curve (AUEC) of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 1
• To compare the effect of daprodustat to epoetin alfa on BP after Acute Challenge 2	• Average of DBP, MAP, and HR as measured by ABPM over 6 hr post-dosing at Day 57. • AUEC of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 57.
• To estimate the initial effect of daprodustat on SBP, DBP, HR and MAP after Acute Challenge 1	• Change from pre-dose in SBP, DBP, HR and MAP at each timepoint at Day 1
• To characterize the pharmacokinetics of daprodustat	• Plasma concentrations of daprodustat and metabolites and derived pharmacokinetic parameters including maximum observed concentration (Cmax), time of occurrence of

Objectives	Endpoints
	Cmax (tmax), terminal phase half-life (t½) and area under concentration-time curve from time zero to 24 hours (AUC[0-24]) as appropriate
Safety	
• To assess the safety and tolerability of daprodustat	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) • Reasons for discontinuation of study treatment • Absolute values and changes from baseline over time in laboratory parameters, electrocardiograms (ECGs) and vital signs
Exploratory	
• To investigate the effect of daprodustat and epoetin alfa on vasoactive mediators of blood pressure	• Plasma concentrations and derived parameters including Cmax, tmax, t½ and AUC(0-24) as appropriate (to include erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II and metabolites, and noradrenalin)
• To summarize the effect of daprodustat and epoetin alfa on SBP after Acute Challenge 1 and Acute Challenge 2	• AUEC of SBP as measured by ABPM over 24-hr post-dosing
• To compare the effect of daprodustat to epoetin alfa on SBP after 8 weeks of Hgb maintenance therapy	• Change from Day 1 pre-dose SBP to Day 57 pre-dose
• To compare the effect of daprodustat to epoetin alfa on SBP at Cmax of each study treatment, as measured by ABPM	• Change in SBP from pre-dose on Day 1 to Cmax of the study treatment on Day 1 • Change in SBP from pre-dose on Day 1 to Cmax of the study treatment on Day 57 • Change in SBP from pre-dose on Day 57 to Cmax of the study treatment on Day 57
• To compare the effect of daprodustat to epoetin alfa on SBP at Cmax of the erythropoietin level, as measured by ABPM	• Change in SBP from pre-dose on Day 1 to Cmax of erythropoietin on Day 1 • Change in SBP from pre-dose on Day 1 to Cmax of erythropoietin on Day 57 • Change in SBP from pre-dose on Day 57 to Cmax of erythropoietin on Day 57

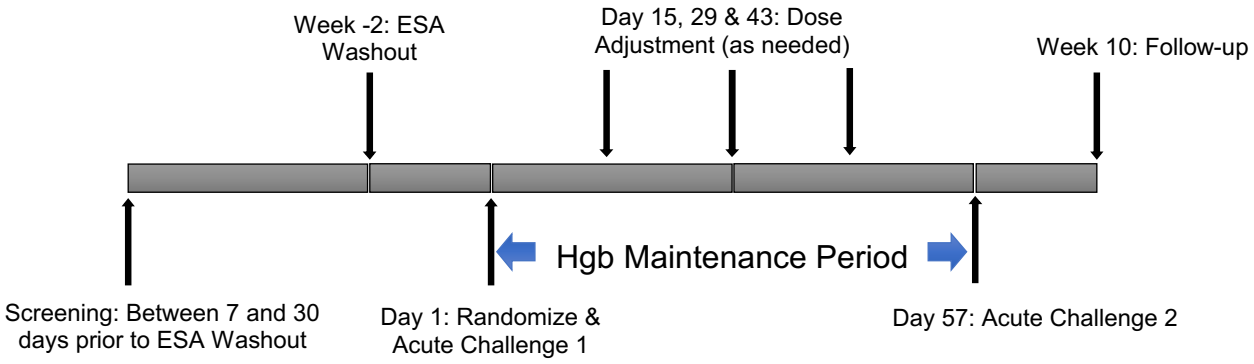
4. STUDY DESIGN

4.1. Overall Design

This study will be an open-label, randomized, parallel-group design in HD subjects with anemia associated with CKD. There will be a screening period, a 2-week ESA washout

period, an initial 24-hr Acute Challenge, an 8-week Hgb-maintenance period, a second 24-hr Acute Challenge and a follow-up visit 14±3 days after completing treatment. The study design is outlined in Figure 1.

Figure 1 Study Design



Subjects will be screened for eligibility within 7-30 days prior to the start of an ESA washout period.

Subjects that qualify for enrolment will be washed out of their ESA for 2 weeks; the start of the ESA washout should be timed to coincide as closely as possible with their next planned ESA dose. Subjects are enrolled once they enter the ESA washout period.

Following the 2-week ESA washout, subjects will be randomized 1:1 and stratified by prior ESA dose, based on the average weekly (for epoetins or darbepoetin) or monthly (for methoxy polyethylene glycol (PEG)-epoetin beta) dosing during the 12 weeks prior to ESA washout as follows:

- Low ESA dose: <100 U/kg/week epoetin alfa OR <0.5 µg/kg/week darbepoetin OR <0.6 µg/kg/week methoxy PEG-epoetin beta
- High ESA dose: ≥100 U/kg/week epoetin alfa OR ≥0.5 µg/kg/week darbepoetin OR ≥0.6 µg/kg/week methoxy PEG-epoetin beta

On Day 1, subjects will be randomized and undergo Acute Challenge 1, a single dose challenge to compare the acute effects on BP of the highest planned once-daily maintenance dose of daprodustat (24 mg) to the highest starting dose of epoetin alfa (100 U/kg). Subjects will have BP monitored for 24 hr using ABPM, and will have serial blood sampling to assess the pharmacokinetics of daprodustat and the time-course of various biomarkers. Acute Challenge 1 will be started promptly following completion of a mid-week dialysis session.

After completing Acute Challenge 1, all subjects will enter the 8-week Hgb maintenance period where doses of either daprodustat or epoetin alfa will be administered and adjusted, as needed, based on dosing guidance as described in Section 6.4.

On Day 57, following the 8-week Hgb maintenance period, subjects will undergo Acute Challenge 2; this challenge will, similar to Acute Challenge 1, involve assessing the effect of daprodustat and epoetin alfa on BP as assessed by 24-hr ABPM measurement. The subjects will be administered the same dose of either daprodustat or epoetin alfa that was given in Acute Challenge 1, and serial blood sampling will be performed to assess daprodustat pharmacokinetics, and to characterize the time course of various biomarkers. Acute Challenge 2 will be started following completion of a mid-week dialysis session.

Subjects will attend a follow-up visit 2 weeks after completion of Acute Challenge 2. Subjects will be asked to not reinitiate anemia treatment until after the follow-up visit unless deemed medically necessary.

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the QC criteria following Acute Challenge 2, one additional Acute Challenge may be made after a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed as detailed in Table 4.

The Acute Challenge 2 may be delayed one week if the subject's Hgb ≥11.5. See Section 7.5.1.1 for guidance.

4.2. Treatment Arms and Duration

The total duration of subject involvement is up to 16 weeks (Screening to Follow-up) as described below:

- Screening: Screening will occur at least one week prior to but not more than 30 days prior to washout.
- ESA Washout: Subjects that meet eligibility criteria will begin the 2-week ESA washout at Week -2.
    - This should be timed such that this day occurs on the date that would have been the next scheduled dose of ESA (or as close as practicable).
    - For subjects with a three-times weekly dialysis schedule, this day must not occur on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday).
    - For subjects with a four- to five-times weekly dialysis schedule, this day can be on any hemodialysis session of the week.
- Randomization & Acute Challenge 1 (Day 1): Subjects will be randomized 1:1 to two treatment arms as described in Table 1 and will begin a 24-hr Acute Challenge where the acute BP effects of daprodustat and epoetin alfa will be

evaluated. This challenge will be initiated promptly following the subject's dialysis session.

- Hgb Maintenance Period: Subjects randomized to daprodustat will start an 8-week Hgb maintenance period based on a starting dose and dose adjustment algorithm as described in Section 6.4.1. Subjects randomized to epoetin alfa will begin an 8-week Hgb maintenance period where Hgb will be managed in accordance with local labelling.

- Acute Challenge 2: At the end of the 8-week Hgb maintenance period, subjects will repeat the procedures of Acute Challenge 1 utilizing the same treatment administered in Acute Challenge 1. This challenge will be initiated promptly following the subject's dialysis session.

- Follow-up: A Follow-up visit will be scheduled to occur 14 ± 3 days after completing treatment.

Table 1 Treatment Arm Descriptions

Treatment Arm	Acute Challenge 1	Hgb Maintenance Phase	Acute Challenge 2
A	100 U/kg IV epoetin alfa	IV epoetin alfa according to label	100 U/kg IV epoetin alfa
B	24 mg daprodustat	QD daprodustat according to dose adjustment algorithm (Section 6.4.1)	24 mg daprodustat

4.3. Type and Number of Subjects

The study will enroll hemodialysis-dependent subjects with anemia associated with CKD currently treated with an ESA. Subjects with Hgb values of 8.5 to 11.5 g/dL, inclusive at both Screening and Day 1, are eligible, and the subjects must currently be on an ESA product.

Approximately 124 subjects will be screened to achieve approximately 62 randomized and 50 evaluable subjects for a total of 25 evaluable subjects per treatment group.

If subjects prematurely discontinue the study, additional replacement subjects may be randomized and assigned to the same treatment arm at the discretion of the Sponsor in consultation with the investigator.

4.4. Design Justification

This study will be an open-label, multi-center, randomized, parallel-group design in HD subjects with anemia associated with CKD. There will be a screening period, a 2-week ESA washout period, an initial 24-hour Acute Challenge, an 8-week Hgb-maintenance period, a second 24-hr Acute Challenge, and a follow-up visit 14 ± 3 days after completing treatment.

- This will be an open-label study since the primary endpoint (SBP by ABPM) is an objective measure and unlikely to be influenced by knowledge of the treatment received. Additionally, blinding of an injectable comparator and oral study drug is impractical in this case.

- A screening period will be used to assess Hgb stability in order to minimize the potential for enrolling patients with highly variable Hgb despite ESA treatment.

- In our previous daprodustat study experience in Phase 2b, HD-dependent subjects with anemia associated with CKD were administered placebo for 4 weeks, with a mean decrease between 0.61 and 0.72 g/dL in Hgb. No subject met either the Hgb stopping criterion or requiring rescue medication. Therefore, the risk of a subject meeting Hgb withdrawal criteria is considered low during this phase.

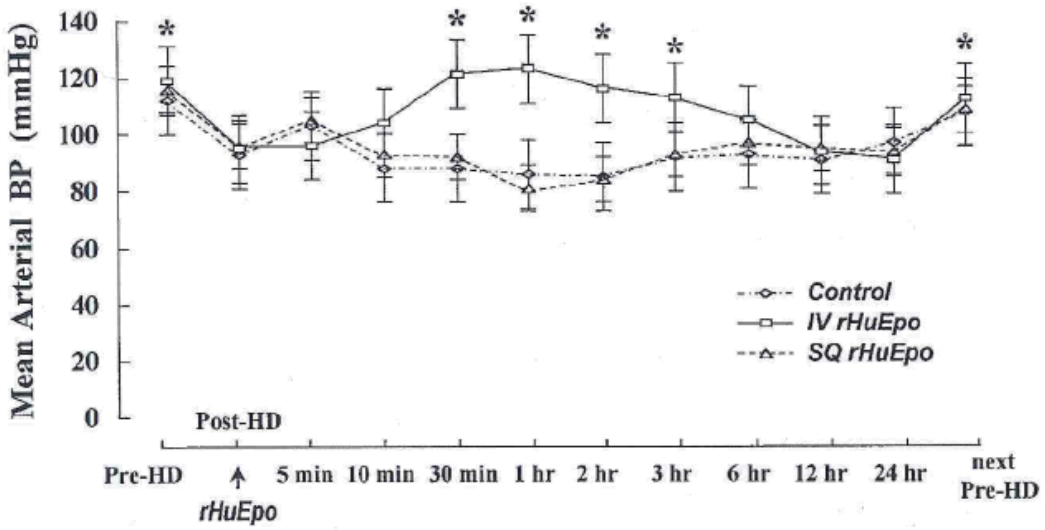
- The Acute Challenge days have been designed to be consistent with the methodology used in a published study [Kang, 1998] (See Figure 2). Although the goal of the current study is to attempt to replicate the blood pressure results from the IV administration of epoetin alfa in that publication, SBP will be the primary endpoint rather than MAP since SBP is the overall best predictor of future cardiovascular risk in a hypertensive population, and MAP is biased toward DBP [Agarwal, 2015].

- Acute Challenge 1 will assess whether there are any immediate effects of daprodustat or epoetin alfa on blood pressure. This assessment will also determine the ability to replicate the findings in the published study and will provide information for an interim analysis (See Section 9.3.2).

- The 8-week Hgb Maintenance Phase will replicate the management procedures planned for the Phase 3 studies as described in Section 6.4. The duration was chosen to be consistent with the timeframe in which the hypertensive effects of ESAs have been observed (i.e., 4 to 8 weeks) [Samtleben,1988].

- Acute Challenge 2 will again assess the effects of daprodustat or epoetin alfa on BP following 8 weeks of anemia treatment utilizing the same assessment as Acute Challenge 1. The purpose of Acute Challenge 2 is to assess any BP effects of ESA administration that may be potentiated (or possibly diminished) following long-term administration of an ESA.

Figure 2 Changes in MAP after administration of rhEPO (rHuEPO) and control



Values are mean ± SD; *P<0.05 vs. Post-HD [Kang, 1998]

4.5. Dose Justification

Daprodustat Treatment Group

- Acute Challenge 1 & 2: The dose to be used for this group will be the highest planned maintenance dose for a once-daily regimen (i.e., 24 mg) for treatment of anemia associated with CKD. In a previous GlaxoSmithKline (GSK) study (PHI112843), a single, oral dose of 150 mg daprodustat was administered to dialysis patients with no safety issues identified. For Acute Challenge 2, stringent Hgb stopping criteria and dose hold have been defined at Day 57 to minimize marked increases in Hgb.

- Hgb Maintenance Phase: Starting doses of daprodustat will be based on Hgb levels, as described in Section 6.4.1. These doses have been chosen to minimize the potential for subjects to meet Hgb stopping criteria during the initial dosing period prior to the first dose adjustment, and are based on modelling and simulation of the dose-Hgb response results from Phase 2 studies.

Epoetin alfa Treatment Group

- Acute Challenge 1 & 2: The dose to be used for this group will be the highest labelled starting dose for epoetin alfa (100 U/kg, IV) which is consistent with the epoetin alfa dose used in the published study where an acute increase in MAP with rhEPO was observed [Kang, 1998]. As subjects in this group will have been washed off ESA for 2 weeks prior to Acute Challenge 1 and only a single dose will be administered, the risk of a marked increase in Hgb levels is considered very low. For Acute Challenge 2, stringent Hgb-based stopping criteria and dose hold have been defined at Day 57 to minimize marked increases in Hgb (See Table 2).

- Hgb Maintenance Phase: The dosage of epoetin alfa during the 8-week Hgb

maintenance phase will be adjusted (if necessary) according to the local labelling in order to manage the subject's Hgb within the target range (10.0-11.0 g/dL). In a previous study (GSK Study PHI113633), HD subjects that had a 4-week ESA washout were initiated on a mean dose of rhEPO of 89 IU/kg/week (n=39), with the dose increased to a mean of 102 IU/kg/week 4 weeks later.

4.6. 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 Supplements, if applicable.

4.6.1. Risk Assessment

The potential risks of clinical significance including AEs of special interest (Section 7.3.1.4), and the mitigation strategies for this protocol taking into account the results of clinical and non-clinical studies with daprodustat, are outlined in Section 12.3.

4.6.2. Benefit Assessment

In clinical trials of up to 24 weeks in duration, in subjects with anemia associated with 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.

4.6.3. 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 subjects with anemia associated with 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 subjects (See Section 12.3). Given these precautions, as well as the potential benefit that daprodustat holds for the treatment of anemia associated with CKD compared to the current standard, the overall benefit risk balance is considered to be positive.

5. SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on daprodustat that may impact subject eligibility is provided in the Daprodustat Investigator's Brochure and IB Supplements, if applicable.

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

5.1. Hemoglobin Stability Criteria

Entry into the study requires a stable Hgb between **8.5 and 11.5 g/dL, inclusive**. This is confirmed from **two** Hgb values obtained at the Screening Visit and Washout Visit, with both values between 8.5 and 11.5 g/dL. These will be taken via a validated point-of-care device to measure Hgb (i.e., HemoCue) prior to the dialysis session of the day. The value at the Washout Visit must not have decreased by more than 1.0 g/dL from the Screening Visit value. For subjects with a three-times weekly dialysis schedule, Hgb values must not be obtained on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday). For subjects with a four- to five-times weekly dialysis schedule, Hgb values can be obtained on any hemodialysis session of the week.

5.2. Inclusion Criteria

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

AGE
1. ≥40 years of age, at the time of signing the informed consent.
TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2. **Hemoglobin:** Stable Hgb 8.5 to 11.5 g/dL inclusive (See Section 5.1).
3. **Dialysis frequency:** On hemodialysis (HD, hemofiltration or hemodiafiltration) three-to five-times weekly for at least 4 weeks prior to screening.
4. **Dialysis adequacy**: A single pool Kt/V_{urea} ≥1.2 based on a historical value obtained within the 3 months prior to screening in order to ensure the adequacy of dialysis. If Kt/V_{urea} is not available, then an average of the last 2 values of urea reduction ratio (URR) is at least 65%. **NOTE**: Only needs confirming at screening.
5. **ESA treatment:** Treated with an ESA (epoetins or their biosimilars, darbepoetin, or methoxy PEG-epoetin beta) for at least 4 weeks prior to screening.
6. **Iron replacement therapy**: Subjects may be on stable (≤50% change in overall dose and compliance of 80% of prescribed doses in the 4 weeks prior to and including the screening period) maintenance oral or IV (≤100 mg/week) iron supplementation. If subjects are on oral or IV iron, then doses must be stable for the 4 weeks prior to Washout.
7. **Weight:** Mid-week weight change between dialysis treatments <5% as assessed post-dialysis at the Screening and Washout visits.
8. **Antihypertensive Medication:** Meets the following criteria: • On at least 1 antihypertensive medication [excluding diuretics] **AND** • On that same medication and the same dose for at least 1 week prior to Washout

INFORMED CONSENT
9. Capable of giving signed informed consent as described in Section 10.2 which includes compliance with the requirements and restrictions listed in the consent form and in this protocol. 10. Willing and able to wear ABPM device for at least 25 hours on two separate sessions.

5.3. Exclusion Criteria

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

CKD-RELATED CRITERIA
1. **Dialysis modality**: Planned change from HD to peritoneal dialysis within the study time period, or on home dialysis. 2. **Renal transplant**: Planned kidney transplant within the 16 weeks following the Screening visit. 3. **High ESA dose**: An epoetin alfa dose of ≥360 U/kg/week IV or ≥250 U/kg/week subcutaneous (SC), or darbepoetin dose of ≥1.8 µg/kg/week IV or SC, or methoxy PEG-epoetin beta dose of ≥ 2.2 µg/kg/week within the 8 weeks prior to screening through Week -4. 4. **Mircera**: Planned or recorded administration of Mircera (methoxy PEG-epoetin beta) within the 4 weeks prior to the Washout visit.
CARDIOVASCULAR DISEASE-RELATED CRITERIA
5. **Myocardial infarction or acute coronary syndrome:** Within the 3 months prior to Washout. 6. **Stroke or transient ischemic attack:** Within the 3 months prior to Washout. 7. **Heart failure:** Chronic Class IV heart failure (HF), as defined by the New York Heart Association (NYHA) functional classification system diagnosed prior to Washout. 8. **QT interval** corrected for heart rate using Bazett's formula **(QTcB):** QTcB >500 msec, or QTcB >530 msec in subjects with Bundle Branch Block. There is no QTc exclusion for subjects with a predominantly paced rhythm. 9. **Current uncontrolled hypertension:** Resting post dialysis systolic blood pressure >160 mmHg; or diastolic blood pressure >100 mmHg at screening or uncontrolled hypertension as determined by the investigator. 10. **Atrial Fibrillation:** Presence of atrial fibrillation.
OTHER DISEASE-RELATED CRITERIA
11. **Inflammatory disease:** Active chronic inflammatory disease that could impact erythropoiesis (e.g., scleroderma, systemic lupus erythematosus, rheumatoid arthritis, celiac disease) diagnosed prior to Washout. 12. **Aplasias:** History of bone marrow aplasia or pure red cell aplasia. 13. **Other causes of anemia:** Pernicious anemia, thalassemia major, sickle cell disease or

myelodysplastic syndrome.

14. **Liver disease (any one of the following):**
    - Alanine transaminase (ALT) >2x upper limit of normal (ULN) (screening only)
    - Bilirubin >1.5xULN (screening only)

      NOTE: Isolated bilirubin >1.5xULN is acceptable to keep in the study if bilirubin is fractionated and direct bilirubin <35%.
    - Current unstable liver or biliary disease per investigator assessment, generally defined by the presence of ascites, encephalopathy, coagulopathy, hypoalbuminaemia, esophageal or gastric varices, persistent jaundice, or cirrhosis.

      NOTE: Stable chronic liver disease (including asymptomatic gallstones, chronic hepatitis B or C, or Gilbert's syndrome) are acceptable if subject otherwise meets entry criteria.
15. **Major surgery:** Major surgery (excluding vascular access surgery) within the 3 months prior to Washout or planned during the study.
16. **Transfusion:** Blood transfusion within the 8 weeks prior to Washout, or an anticipated need for blood transfusion during the study.
17. **Gastrointestinal (GI) bleeding:** Evidence of actively bleeding gastric, duodenal, or esophageal ulcer disease **OR** clinically significant GI bleeding within the 8 weeks prior to Washout.
18. **Acute Infection:** Clinical evidence of acute infection or history of infection requiring intravenous (IV) antibiotic therapy within the 8 weeks prior to Washout.
    NOTE: IV antibiotics as prophylaxis are allowed.
19. **Malignancy:** History of malignancy within the two years prior to screening through Day 1 or currently receiving treatment for cancer, or has a known complex kidney cyst (e.g., Bosniak Category IIF, III or IV) ≥3 cm.
    NOTE: ONLY exception is localized squamous cell or basal cell carcinoma of the skin that has been definitively treated more than 4 weeks prior to screening.
20. **Blood Pressure Measurement:** Subjects with an upper arm diameter which cannot be measured by oscillometer/ sphygmomanometer cuff **OR** for whom blood pressure cannot be measured in the opposite arm of current vascular access.

CONCOMITANT MEDICATIONS

21. **Severe allergic reactions:** History of severe allergic or anaphylactic reactions or hypersensitivity to excipients in the investigational product.
22. **Drugs and supplements:** Use of any prescription or non-prescription drugs or dietary supplements that are prohibited (See Section 6.11.3), from screening until Washout.
23. **Prior investigational product exposure**: The subject has participated in a clinical trial and has received an experimental investigational product within the 30 days prior to Day 1 or within 5 half lives of the investigational product prior to screening, whichever is longer.

GENERAL HEALTH RELATED CRITERIA
24. **Other conditions:** Any other condition, clinical or laboratory abnormality, or examination finding that the investigator considers would put the subject at unacceptable risk, which may affect study compliance or prevent understanding of the aims or investigational procedures or possible consequences of the study.
25. **Females ONLY:** Subject is pregnant [as confirmed by a positive serum human chorionic gonadotrophin (hCG) test for females of reproductive potential (FRP) only], subject is breastfeeding, or subject is of reproductive potential and does not agree to follow one of the contraceptive options listed in the List of Highly Effective Methods for Avoiding Pregnancy in Section 12.4.7.
DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
26. **Vitamin B_{12}:** At or below the lower limit of the reference range (may rescreen in a minimum of 8 weeks, following treatment).
27. **Folate:** <2.0 ng/mL (4.5 nmol/L) (may rescreen in a minimum of 4 weeks, following treatment).
28. **Ferritin:** <100 ng/mL
29. **Transferrin saturation (TSAT):** <20%.

5.4. Screening/Baseline/Run-in Failures

Screen failures are defined as subjects who consent to participate in the clinical trial but who never enter the washout period. In order to ensure transparent reporting of screen failure subjects, meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and respond to queries from Regulatory authorities, a minimal set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and Serious Adverse Events (see Section 7.3.1.6).

5.5. Withdrawal/Stopping Criteria

A subject 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 or administrative reasons. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records.

If a decision is made to withdraw a subject, he/she should return to the clinic as soon as possible to complete the Early Withdrawal assessments. The Follow-up visit should be performed 14 ± 3 days after last dose (see Time and Events Table Section 7.1).

In all cases, the reason for withdrawal from the study and date of the last dose of study treatment will be recorded in the eCRF.

5.5.1. Criteria for Permanent Discontinuation from Study Treatment and Early Withdrawal

Subjects must **permanently discontinue study treatment** and be withdrawn from the study for the following reasons:

- Meets Hgb stopping criteria (See Section 5.5.1.1)
- Receives a blood transfusion
- Receives a kidney transplant
- Becomes pregnant or intends to become pregnant during the study
- Active GI bleeding
- Diagnosis of cancer, with the exception of squamous cell or basal cell carcinoma
- Liver chemistry abnormalities exceeding the threshold criteria (See Section 5.5.3)
- Misses 2 consecutive dialysis sessions
- Use of prohibited medication (See Section 6.11.3)
- Myocardial infarction or acute coronary syndrome
- Stroke or transient ischemic attack
- New diagnosis of Class IV heart failure, as defined by the New York Heart Association (NYHA) functional classification system
- Active chronic inflammatory disease that could impact erythropoiesis
- Any new diagnosis of hematological disease including those affecting platelets, white or red blood cells, coagulation disorders, or any other cause of anemia of chronic disease other than renal disease

5.5.1.1. Hemoglobin 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 (Table 2) and recorded in the eCRF. The table below summarizes the Hgb values and corresponding action to be taken at each visit. If the subject meets the below condition(s), then IP must be permanently stopped.

Table 2 Hgb Stopping Criteria

Day 1 (Prior to Acute Challenge 1)

Hgb 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, withdraw subject from the study.	
≥7.5-<11.5	decrease of ≥2.0 in Hgb over 2 weeks	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently withdraw subject from the study.
≥11.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, avoid dosing and continue washout for one additional week. Progress with Acute Challenge 1 one week later if Hgb has decreased below 11.5; if not then withdraw subject from study.	

Days 15, 29, & 43

Hgb 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 subject from the study.	
≥7.5-<12.0	decrease of ≥2.0 in Hgb over 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 subject from the study.

Day 57 (Prior to Acute Challenge 2)

Hgb 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 subject from the study.	
≥7.5-<11.5	decrease of ≥2.0 in Hgb over 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 subject from the study.
≥11.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, HOLD the dose for 1 week and progress with Acute Challenge 2 one week later. If Hgb remains above 11.5 one week later, then withdraw subject from the study.	

5.5.2. Missed Clinic Visits/Lost to Follow-up

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

- The site must attempt to contact the subject and re-schedule the missed visit as soon as possible.
- The site must counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- In cases where the subject is deemed ‘lost to follow up’, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and if necessary a certified letter to the subject’s last known mailing address or local equivalent methods). These contact attempts should be documented in the subject’s medical record.

- Should the subject continue to be unreachable, only then will he/she be considered to have withdrawn from the study with a primary reason of "Lost to Follow-up".

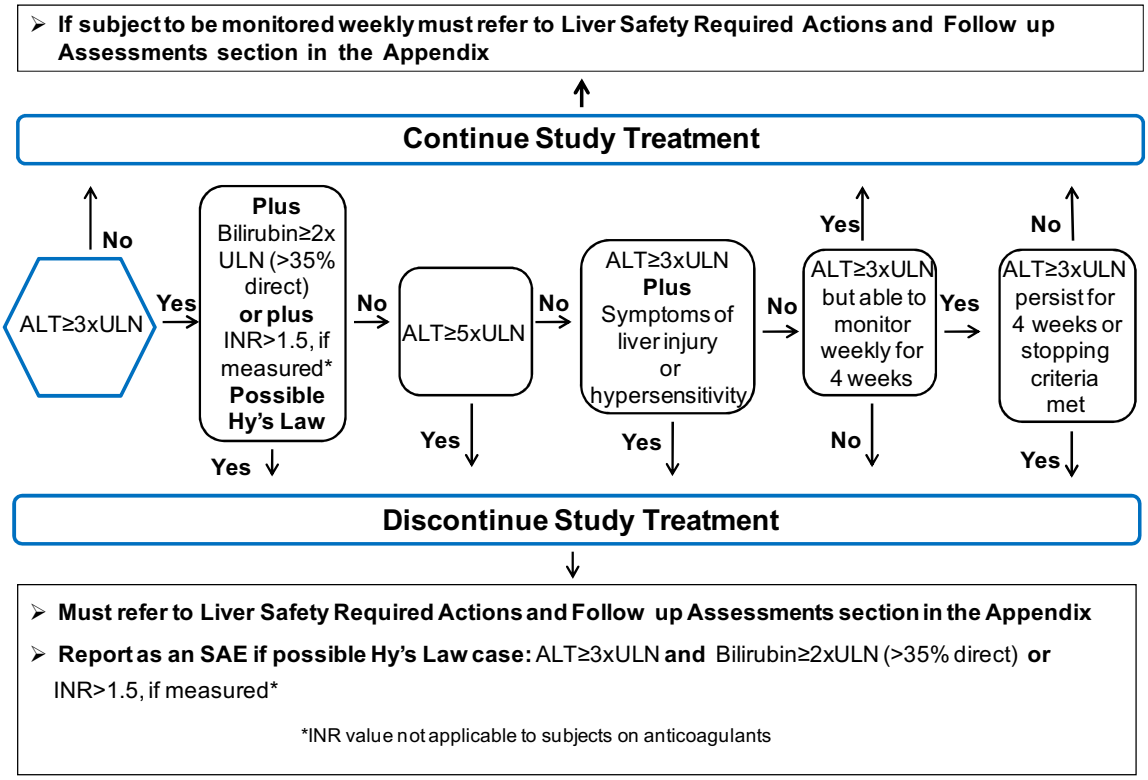
5.5.3. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure subject safety and evaluate liver event etiology (in alignment with the Food and Drug Administration [FDA] premarketing clinical liver safety guidance).

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

- a participant meets one of the conditions outlined in the algorithm below.
- when in the presence of abnormal liver chemistries not meeting protocol-specified stopping rules, the investigator believes study intervention discontinuation is in the best interest of the participant.

Phase II Liver Chemistry Stopping and Increased Monitoring Algorithm



Liver Safety Required Actions and Follow up Assessments Section can be found in Section 12.2.

5.5.3.1. Study Treatment Restart or Rechallenge

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

5.6. Subject and Study Completion

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

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

6. STUDY TREATMENT

6.1. Investigational Product and Other Study Treatment

The term 'study treatment' is used throughout the protocol to describe any combination of products received by the subject as per the protocol design. Study treatment may therefore refer to the individual study treatments or the combination of those study treatments.

Product name:	Daprodustat
Dosage form:	Tablet
Unit dose strength(s)/Dosage level(s):	1 mg, 2 mg, 4 mg, 6 mg, 8 mg, 10 mg tablet strengths/1 mg, 2 mg, 4 mg, 6 mg, 10 mg, 12 mg & 24 mg dosage levels
Route of Administration	Oral
Physical description:	1 mg, 2 mg & 4 mg tablets: 7.0 mm round, compound radius, white film coated tablets 6 mg, 8 mg & 10 mg tablets: 9.0 mm round, compound radius, white film coated tablets
Method for individualizing dosage:	See Section 6.4.1

For a description of epoetin alfa, please consult the local label.

All eligible subjects will discontinue their current ESA therapy and will undergo a 2-week ESA washout period. Following washout, subjects will be randomized to receive either daprodustat or epoetin alfa, on a dialysis day. For subjects with a three-time weekly dialysis schedule, randomization must not occur on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday). For subjects with a four to five times weekly dialysis schedule, randomization can be on any hemodialysis session of the week.

Subjects can take their tablets without regard to food. For all study visits, it is recommended for subjects not to eat a heavy meal before coming to the clinic in order to avoid lipemia of the blood sample collected for the analysis of transferrin, iron and total iron binding capacity (TIBC).

6.2. Treatment Assignment

Subjects will be stratified by prior ESA dose (as outlined in Section 4.1) and randomized 1:1 to receive open-label oral daprodustat or IV epoetin alfa. A central randomization approach will be used due to the small sample size and to protect against potential selection bias due to the open-label design. Once a randomization number has been assigned by an Interactive Voice/Web Response System (IVWRS) at time of Randomization, it must not be re-assigned.

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

6.3. Blinding

This will be an open-label study. However, there are no plans to generate any aggregated unblinded summaries during the conduct of the study.

6.4. Starting Dose and Dose Adjustment Algorithm

6.4.1. Subjects Randomized to Daprodustat

Subjects randomized to daprodustat will have doses adjusted, as required, to target Hgb within the range of 10.0-11.0 g/dL. Dose adjustments will be assigned **automatically** via the IVWRS based on the subject's Hgb value via onsite HemoCue assessment according to the following algorithms:

Day 1 (HemoCue Prior to Acute Challenge 1)

Hgb (g/dL)	Action or Dose
<7.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, withdraw subject from the study.
≥7.5 and <10.0	6 mg daprodustat
≥10.0 and <11.5	4 mg daprodustat
≥11.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, avoid dosing and continue washout for one additional week. If Hgb remains above 11.5 then withdraw subject from study.

The 8 week Hgb maintenance dosing will begin on Day 2, following Acute Challenge 1. Acute Challenge dosing is the same for all subjects on the daprodustat arm: 24 mg.

Days 15, 29, 43, & 57

The available dose steps for daprodustat are outlined below (highlighted boxes indicate starting doses). Dose adjustments will result in the daprodustat dose being increased or decreased by **one dose step**.

Hgb (g/dL)	Hgb change since previous study 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 drug and withdraw subject from the study.
≥7.5 to <9.5	Decreasing or No change[1]	Increase to the next higher dose step
≥7.5 to <9.5	Increasing[2]	Maintain dose

≥9.5 to ≤11.5	Any change	Maintain dose
>11.5 to <12.0	Increasing or No change	Decrease to the next lower dose step
	Decreasing	Maintain dose
≥12.0	Any change	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, HOLD dosing for 2 weeks until next study visit.[3]
≥7.5 to <12.0	increase of ≥1.3 in Hgb over 2 weeks[4]	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,
≥7.5 to <12.0	Increase of > 2.0 in Hgb over previous 4 weeks (Days 29, 43, 57[4] only)	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,

[1]No change is defined as an increase < 0.5 g/dL
[2]Increase is defined as an increase ≥ 0.5 g/dL
[3] If Hgb remains ≥12.0 at next visit, the subject should be withdrawn from the study. If the Hgb drops below 12.0, the subject should be restarted on the next lowest dose.
[4] Only applies at Day 57 if a repeat AC2 is necessary. The dose should be decreased for the 1 week between AC2 and Additional AC2.

6.4.2. Subjects Randomized to Epoetin Alfa

Subjects randomized to epoetin alfa will be administered according to local labelling and clinical practice guidelines to keep Hgb in the target range (10.0-11.0 g/dL). For additional guidance on Hgb-based stopping criteria please refer to Section 5.5.1.1.

6.5. Packaging and Labeling

Daprodustat tablets are packed in white, opaque high density polyethylene (HDPE) bottles with child-resistant closures. The contents of the label will be in accordance with all applicable regulatory requirements.

6.6. Preparation/Handling/Storage/Accountability

No special preparation of study treatment is required.

- Only subjects enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure environmentally controlled and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorized site staff.
- The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation and final disposition records).
- Further guidance and information for final disposition of unused study treatment are provided in the Study Reference Manual (SRM).

- 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.

6.7. Compliance with Study Treatment Administration

For subjects randomized to daprodustat, treatment dosing details including dates for dose increases/reductions, will be recorded in RAMOS NG.

For subjects randomized to daprodustat, for Acute Challenge 1 and 2, study site personnel will confirm compliance. When daprodustat is administered at home (Hgb maintenance period), subjects will be instructed to return all unused randomized treatment at each clinic visit. A record of the number of daprodustat tablets dispensed to and taken by each subject will be maintained and reconciled with randomized treatment and compliance records.

For subjects randomized to epoetin alfa, they will receive all study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study treatment and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment.

6.8. Treatment of Study Treatment 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 HD is very low and this is not an effective method 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 subject's clinical status. Additionally, subjects should be monitored closely for CV events, increased heart rate and hematologic abnormalities.

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

6.9. Treatment after the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because the indication being studied is not life threatening or seriously debilitating and other treatment options are available.

The investigator is responsible for ensuring that consideration has been given to the post-study care of the subject's medical condition.

6.10. Lifestyle and/or Dietary Restrictions

6.10.1. Meals and Dietary Restrictions

Daprodustat can be taken without regard to meals.

All subjects will be instructed to limit caffeine- or xanthine- containing products (e.g., coffee, tea, cola drinks, chocolate) for 24h prior to both Acute Challenge 1 and Acute Challenge 2.

6.11. Concomitant Medications and Non-Drug Therapies

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.

6.11.1. Weight and Antihypertensive Medication Changes

It is preferred that changes to subject weight and antihypertensive medication(s) are not made while the subject is part of this study, however, subjects should remain in the study regardless of any changes. All medication and dose changes should be recorded in the eCRF.

6.11.2. Permitted Medications and Non-Drug Therapies

Unless specified as a prohibited medication in Section 6.11.3, all concomitant medications should be considered permitted provided they are not contraindicated for the individual subject concerned.

6.11.3. Prohibited Medications and Non-Drug Therapies

Use of any of the following prescription drugs from Screening until 7 days after the last dose of randomized treatment is prohibited and will constitute a protocol violation.

- Strong inhibitors of CYP2C8 (e.g., gemfibrozil, high dose clopidogrel [300 mg])
- Strong inducers of CYP2C8 (e.g., rifampin/rifampicin)

6.11.4. Standard of Care

During the study, investigators are expected to monitor the subject'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.

6.11.5. Iron Protocol

Subjects must remain iron replete throughout the study. If ferritin and TSAT are collected per local clinical practice while enrolled, the following is recommended.

Iron therapy will be administered if ferritin is ≤100 ng/mL and/or TSAT is ≤20%. The investigator should choose the route of administration and dose of iron based on subject's iron status and local clinical practice.

All iron (excluding multivitamins) must be stopped and cannot be administered if:

- Ferritin >800 ng/mL **AND** TSAT >20% **OR**

- TSAT >40%

7. STUDY ASSESSMENTS AND PROCEDURES

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table (Section 7.1), are essential and required for study conduct.

- If assessments are scheduled for the same nominal time, then the assessments should occur in the following order:
    1. 12-lead ECG
    2. vital signs
    3. blood draws

  Note: The timing of the assessments should allow the blood draw to occur at the exact nominal time.
- The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment.
- The Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form.
- No more than 550 mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

Supplementary study conduct information is provided in the SRM. The SRM provides administrative and detailed technical information that does not impact subject safety.

7.1. Time and Events Table

Table 3 Study Procedures and Assessments

Procedure[1]	Screening	Washout	Treatment Period[2]					Follow-up[2]	
			Day 1	Day 15	Day 29	Day 43	Day 57	Early WD	Week 10
Informed Consent	X								
Entry Criteria	X								
Physical, Medical History, Demography	X								
HemoCue Hgb	X	X	X[4]	X	X	X	X[4]	X	X
Enrolment		X							
IVWRS			X	X	X	X			
Randomization			X						
Acute Challenge			X[4]				X[4]		
ABPM Assessment			X[4]				X[4]		
Dose Adjustment				X	X	X			
Females Only: Serum Pregnancy Test	X		X		X		X		X
Females Only: Estradiol & FSH (if required)[3]	X								
ECG	X		X[4]				X[4]		X
Vital signs & weight (Pre- & Post-dialysis)	X	X	X[4]		X		X[4]		X
Clinical Chemistry	X		X		X		X		X
Hematology	X		X		X		X		X
Folate and Vitamin B12	X								
Ferritin, transferrin, total iron, TSAT, UIBC	X								
Pharmacokinetic/Biomarker Assessments			X[4]				X[4]		
Hgb Maintenance Period[5]			←==========				==========→		
Adverse Events Assessment	X[6]	X	X[4]	X	X	X	X[4]		X
Review Concomitant Medications	X	X	X[4]	X	X	X	X[4]		X

1 All assessments should be done predialysis/predose except as noted.
2 Allowable time window ±2 days EXCEPT Follow-up Visit which is ±3 days.
3 As detailed in Inclusion Criteria.
4 Detailed timings for assessments on Acute Challenge Days are given in Table 4.
5 From the end of Acute Challenge 1 to the beginning of Acute Challenge 2.
6 Only SAEs assessed as related to study participation are collected at this visit. See Section 12.4 for additional details.

Table 4 Study Procedures and Assessments on Acute Challenge Days (Treatment Period Day 1 and Day 57)

Procedure	Pre-Dialysis	Post-Dialysis	Predose	Time (in hours relative to dosing)										
				0	0.5	1	2	3	4	6	8	12	16	24
HemoCue Hgb[1]			X											X
IVWRS			X											
ABPM Assessment[1, 3]			←==========→											
Administer Study Treatment[1]				X										
Serum Pregnancy Test[1]			X											
ECG[1]			X											X
Vital Signs and Weight[1]	X	X						X				X		X
Clinical Chemistry			X											
Hematology			X											
Pharmacokinetics[4]			X		X	X	X	X	X	X	X	X	X	X
Erythropoietin			X		X	X	X	X	X	X	X	X	X	X
Endothelin-1			X			X	X		X	X	X			X
Nitric Oxide			X			X	X		X	X	X			X
Asymmetric dimethylarginine			X			X	X		X	X	X			X
Renin			X			X	X		X	X	X			X
Angiotensin-II			X			X	X		X	X	X			X
Noradrenalin			X			X	X		X	X	X			X
Adverse Events Assessment[1]			X		X	X	X	X	X	X	X	X	X	X
Review Concomitant Medications[1]			X											X
Initiate Hgb maintenance dosing[2]														X

1 Procedures to be repeated if Acute Challenge 2 fails quality control criteria (For more detail refer to Section 7.5.1)
2 Applies to Acute Challenge 1 only
3 Timing of ABPM measurements does not correlate with specified time points in Table 4.
4 PK to be drawn from daprodustat subjects only.

7.2. Screening and Critical Baseline Assessments

Medical history, including cardiovascular disease and associated risk factors, (as detailed in the eCRF) will be assessed at screening.

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

Medical/medication/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5.

7.3. Safety

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

The definitions of an AE or SAE can be found in Section 12.4.

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

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

- Any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact.
- AEs will be collected from the start of washout until the follow-up contact (see Section 7.3.1.3), at the timepoints specified in the Time and Events Table (Section 7.1).
- Medical occurrences that begin prior to the start of washout but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF.
- All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Section 12.4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

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

7.3.1.2. Method of Detecting AEs and SAEs

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

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

7.3.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs, and AEs of Special Interest (as defined in Section 7.3.1.4) will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up (as defined in Section 5.5). Further information on follow-up procedures is given in Section 12.4.5.

7.3.1.4. Adverse Events of Special Interest

The investigator or site staff will be responsible for detecting, documenting and reporting any AEs of special interest or any event that may potentially be one of the categories listed below (using preferred terms):

- Death, myocardial infarction (MI), stroke, heart failure, thromboembolic events, thrombosis of vascular access
- Thrombosis and/or tissue ischemia secondary to excessive erythropoiesis
- Cardiomyopathy
- Pulmonary artery hypertension (see also Section 7.3.1.5)
- 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

The results of any investigation should be recorded in the relevant sections of the subject's eCRF.

7.3.1.5. Cardiovascular and Death Events

GSK has identified CV and death events of special interest for all clinical studies. Investigators will be required to fill out the specific CV event page of the eCRF for the CV AEs and SAEs or any event that may potentially be one of the categories listed in Section 12.4.3.

7.3.1.6. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to GSK of SAEs related to study treatment is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a product under clinical investigation are met.

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

Investigator safety reports are prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and GSK policy and are forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from GSK will file it with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

7.3.1.7. 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.

7.3.2. Pregnancy

Details of all pregnancies in female subjects and the outcome for the neonate, if applicable, will be collected after the start of dosing and until 14 days post-last dose. If a pregnancy is reported, then the investigator should inform GSK within 2 weeks of learning of the pregnancy and should follow the procedures outlined in Section 12.4.8.

7.3.3. Physical Exams

A complete physical examination will include, at a minimum, assessment of the cardiovascular, respiratory, gastrointestinal and neurological systems.

- Investigators should pay special attention to clinical signs related to previous serious illnesses.
- For height and weight measurements, the subject is recommended to wear indoor, daytime clothing with no shoes.

7.3.4. Vital Signs and Weight

Vital signs will be measured with the subject in a semi-supine position after 5 minutes rest and will include temperature, systolic and diastolic blood pressure and heart rate, and will be measured both pre- and post-dialysis.

Weight will be measured both pre- and post-dialysis and recorded as specified in the Time and Events Table (Section 7.1).

7.3.5. Electrocardiogram (ECG)

ECG measurements will be taken at the time points specified in the Time and Events Table (Section 7.1). Full 12-lead ECGs will be recorded with the subject in a supine position. Heart rate (HR), PR interval, QRS duration, and QT (uncorrected) interval will be measured. QTcB will be calculated (machine-read or manually).

At the Screening visit two additional ECGs are required if the initial ECG indicates prolonged QTc (see Section 5.3) using the automated or manually calculated QTcB value. The average QTcB value of all three ECGs will be used to determine eligibility. Additional details are provided in the SRM.

ECG data will be read locally.

All ECGs will be performed before measurement of vital signs and collection of blood samples for laboratory testing.

7.3.6. Clinical Safety Laboratory Assessments

All protocol required laboratory assessments, as defined in Table 5 must be conducted in accordance with the Laboratory Manual, and Protocol Time and Events Schedule. Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/center number, and visit date. Details for the preparation and shipment of samples will be provided by the laboratory and are detailed in the SRM. Reference ranges for all safety parameters will be provided to the site by the laboratory responsible for the assessments.

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

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

All study-required laboratory assessments will be performed by a central laboratory, apart from HemoCue Hgb. The results of each HemoCue test must be entered into the eCRF.

NOTE: Local laboratory results are only required in the event that the central laboratory results are not available in time for either a treatment and/or response evaluation to be performed. If a local sample is required, it is important that the sample for central

analysis is obtained at the same time. Additionally if the local laboratory results are used to make either a treatment or response evaluation, the results must be entered into the eCRF.

Hematology, clinical chemistry, and additional parameters to be tested are listed in Table 5.

Table 5 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	Parameters		
Hematology	Platelet Count	*RBC Indices:*	*WBC Count with Differential:*
	RBC Count	MCV	Neutrophils
	Hemoglobin	MCH	Lymphocytes
	Hematocrit	MCHC	Monocytes
	WBC Count (absolute)	RDW	Eosinophils
	Reticulocyte Count	CHr	Basophils
Clinical Chemistry	Potassium	AST (SGOT)	Total and direct/indirect bilirubin
	Sodium	ALT (SGPT)	Total Protein
	Glucose	Calcium (Albumin adjusted)	Alkaline Phosphatase
	Albumin	Phosphate	
Other Screening Tests	Serum hCG pregnancy test	Serum ferritin	Folate
	Estradiol[1]	Serum iron	Vitamin B12
	FSH[1]	Serum transferrin	UIBC
		TSAT	

NOTE: Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 12.2.
[1] As needed in women of non-child bearing potential only.
Abbreviations: ALT= Alanine transaminase; AST= Aspartate transaminase; CHr=Reticulocyte hemoglobin content; FSH=follicle stimulating hormone; MCH= Mean corpuscular hemoglobin; MCHC= Mean corpuscular hemoglobin concentration; MCV= Mean corpuscular volume; RBC= Red blood cell; RDW= Red blood cell distribution width; SGOT=Serum glutamic oxaloacetic transaminase; SGPT=Serum glutamic-pyruvic transaminase; TSAT = transferrin saturation; UIBC= unsaturated iron binding capacity; WBC= White blood cells;

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 etiology should be identified and the sponsor notified.

7.4. Pharmacokinetics

7.4.1. Blood Sample Collection

Blood samples for pharmacokinetic (PK) analysis of daprodustat and its metabolites (GSK2391220 [M2], GSK2531403 [M3], GSK2487818 [M4], GSK2506102 [M5], GSK2531398 [M6], and GSK2531401 [M13]) will be collected at the time points indicated in Section 7.1, Time and Events Table. The actual date and time of each blood

sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

Details of PK blood sample collection (including volume to be collected), processing, storage and shipping procedures are provided in the SRM.

If the subject is unable to provide a sufficient blood quantity per time point or there is significant concern for this by the subject or investigator, these serial blood draws may be omitted.

7.4.2. Sample Analysis

Plasma analysis will be performed under the control of Bioanalysis, Immunogenicity, and Biomarkers - In Vitro/In Vivo Translation Platform/Scinovo, GlaxoSmithKline, the details of which will be included in the SRM. Concentrations of daprodustat and its metabolites (GSK2391220 [M2], GSK2531403 [M3], GSK2487818 [M4], GSK2506102 [M5], GSK2531398 [M6], and GSK2531401 [M13]) will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

7.5. Biomarker(s)/Pharmacodynamic Markers

7.5.1. Ambulatory Blood Pressure Monitoring (ABPM)

Blood pressure and pulse will be measured during Acute Challenge 1 and 2 through the use of an ambulatory blood pressure monitoring device. Subjects will wear the ABPM device for two sessions during the study (i.e., Acute Challenge 1 and 2), occurring at Day 1 (randomization) and at Day 57. The ABPM device will be placed prior to Acute Challenge dosing, and will remain on the subject's arm until 24 hrs post-dosing.

ABPM measurements of systolic and diastolic blood pressure and heart rate will be automatically recorded every 15 minutes for the first 6 hrs post-dosing, and then every 20 minutes thereafter until 24 hrs post-dosing. The ABPM device will automatically calculate MAP.

The device planned for use in this study is the Mortara Ambulo 2400; a specification sheet for this device can be found in Section 12.6.

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the QC criteria following Acute Challenge 2, one additional Acute Challenge may be made in a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed as detailed in Table 4.

7.5.1.1. ABPM Delay Due to High Hgb

Day 1: If the HemoCue Hgb is confirmed to be ≥ 11.5 g/dL, then avoid dosing and continue washout for one additional week. No other assessments should be performed for that subject during the visit. When the subject returns 7 days later, re-draw the Hgb for HemoCue and assess. If confirmed to be < 11.5 g/dL then progress with the remainder of assessments and perform Acute Challenge 1. If the Hgb remains high, the subject must be withdrawn.

Day 57: If the HemoCue Hgb is confirmed to be ≥ 11.5 g/dL, then hold the dose(s) of IP for 1 week. No other assessments should be performed for that subject during the visit. When the subject returns 7 days later, re-draw the Hgb for HemoCue and assess. If confirmed to be < 11.5 g/dL then progress with the remainder of assessments and perform Acute Challenge 2. If the Hgb remains high, the subject must be withdrawn.

7.5.2. Blood Pressure Regulation Biomarkers

Blood samples will be collected during this study to investigate the mechanism of the effect of daprodustat on blood pressure. Biomarkers selected will explore the renin-angiotensin-aldosterone axis, the nitric oxide and endothelin axes, sodium retention and HIF signalling.

Biomarkers may include erythropoietin, nitric oxide (NO), asymmetric dimethyarginine (ADMA), renin, angiotensin-II (1-8) and metabolites (1-7 and 1-5), endothelin-1 (ET-1), and noradrenalin. Samples will be collected as specified in the Time and Events Table (Section 7.1). The timing of the collections may be adjusted on the basis of emerging pharmacokinetic or pharmacodynamic (PD) data from this study or other new information in order to ensure optimal evaluation of the PD endpoints.

If the subject is unable to provide a sufficient blood quantity per time point or there is significant concern for this by the subject or investigator, these serial blood draws may be omitted.

7.6. Genetics

Genetics are not evaluated in this study.

8. DATA MANAGEMENT

- For this study, subject data will be entered into GSK defined electronic case report forms (eCRFs), transmitted electronically to GSK or designee and combined with data provided from other sources in a validated data system.
- Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data.
- Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug.

- CRFs (including queries and audit trails) will be retained by GSK, and copies will be sent to the investigator to maintain as the investigator copy. Subject initials will not be collected or transmitted to GSK according to GSK policy.

9. STATISTICAL CONSIDERATIONS AND DATA ANALYSES

The primary endpoint for this study is average systolic blood pressure (SBP) over 6 hours of measurements taken at 15-minute intervals after the administration of study treatment for the Acute Challenge 2. The primary comparison of interest is daprodustat versus epoetin alfa for this challenge.

The comparison of daprodustat versus epoetin alfa on 6 hr average SBP during Acute Challenge 1 is a secondary endpoint.

9.1. Hypotheses

Null Hypothesis: The difference between 24 mg daprodustat versus 100 IU/kg epoetin alfa on 6 hr average SBP under a background of treatment (i.e., during Acute Challenge 2) is zero.

Alternative Hypothesis: The difference between 24 mg daprodustat versus 100 IU/kg epoetin alfa on 6 hr average SBP under a background of treatment (i.e. during Acute Challenge 2) is not zero.

9.2. Sample Size Considerations

9.2.1. Sample Size Assumptions

It is expected that 31 subjects will be randomized into each treatment arm. Assuming a 20% withdrawal rate during the Hgb Maintenance Period, there will be 25 subjects per treatment arm that will undertake Acute Challenge 2. The study will continue to recruit subjects until it is projected that 25 subjects per treatment arm complete and pass QC for Acute Challenge 2.

Assuming a 5% significance level and a true standard deviation (SD) of 16 mmHg for average SBP over 6 hours, a sample size of 25 subjects per treatment group will provide greater than 90% power to detect a -15 mmHg difference between treatment groups. Under these assumptions, statistical significance will be obtained if there is more than a 9.1 mmHg mean difference observed in favor of daprodustat.

Table 6 Power and Half-width of 95% CI for pair-wise comparisons, assuming a 16 mmHg SD for average SBP over 6 hours

Challenge	Expected Number of Subjects per Treatment Group	Power to detect 15 mm Hg difference	Half-Width of 95% CI[2]
Acute Challenge 1	31	95%	8.1 mmHg
Acute Challenge 2[1]	25	90%	9.1 mmHg

[1] Primary Comparison
[2] Minimal detectible effect

9.2.2. Sample Size Sensitivity

Changes to power and the half-width of the 95% confidence interval for Acute Challenge 2 due to varying assumptions on the SD for average SBP over 6 hr are explored in Table 7 below.

Table 7 Half-width of 95% CI for pair-wise comparisons, assuming 25 subjects per treatment group

Common SD for average SBP over 6 hours	Power to detect 15 mm Hg difference	Half-Width of 95% CI
14	96%	8.0 mmHg
16†	90%	9.1 mmHg
18	82%	10.2 mmHg
20	74%	11.4 mmHg

† Primary Comparison

9.2.3. Sample Size Re-estimation or Adjustment

Subjects that withdraw prior to the Acute Challenge 2 assessment will be replaced so that 25 subjects per treatment arm complete Acute Challenge 2.

9.3. Data Analysis Considerations

9.3.1. Analysis Populations

The primary population of interest is the intent to treat (ITT) population defined as all randomized subjects that received at least one dose of study treatment.

In addition, a per-protocol population may be investigated. Details will be provided in the Reporting and Analysis Plan (RAP) for the study.

9.3.2. Interim Analysis

No interim analyses are currently planned for this study.

9.4. Key Elements of Analysis Plan

9.4.1. Primary Analyses

The primary analysis of average SBP over 6 hr post-dose during Acute Challenge 2 will be an analysis of covariance (ANCOVA) with terms for treatment, prior ESA dose (low/high), and post-HD/pre-Acute Challenge 1 SBP, difference between post-HD/pre-Acute Challenge 2 SBP and post-HD/pre-Acute Challenge 1 SBP, and treatment by (difference in post-HD SBP between Acute Challenge 1 and 2) interaction. Note that the pre-challenge SBP may change over time between Acute Challenge 1 and Acute Challenge 2 as a result of the maintenance therapy, and thus the pre-challenge SBP at Acute Challenge 2 is not a true pre-treatment covariate. As a result, the interpretation of the effect of the Acute Challenge 2 may be confounded by the impact of treatment phase on the covariate. Therefore, if the interaction term in this model is significant at the 0.10 level, then the primary model will be ANCOVA with terms for treatment, prior ESA dose (low/high), and post-HD/pre-Acute Challenge 1 SBP. The primary model will provide a point estimate and two-sided 95% CI for the treatment effect and a p-value for the superiority assessment. Superiority will be established if the p-value is <0.05.

9.4.2. Secondary Analyses

Average SBP over 6 hr post-dose in Acute Challenge 1 will be analyzed using ANCOVA with terms for treatment, prior ESA dose (low/high), and post-HD/pre-Acute Challenge 1 SBP. Similar analyses will be performed for DBP, MAP, and HR.

ANCOVA using the primary model will be used for the analysis of average DBP, MAP, and HR over 6 hr post-dose in Acute Challenge 2 replacing the baseline SBP term with the analogous baseline measurement for DBP, MAP, and HR respectively.

AUECs of SBP, DBP, MAP, and HR post Acute Challenge 2 and 1 will be analyzed using ANCOVA with terms for treatment and prior ESA dose (low/high) with 95% CIs and p-values provided for the treatment effect.

9.4.3. Other Analyses

The difference in SBP between pre-dose in Acute Challenge 1 and pre-dose in Acute Challenge 2 will be summarized by treatment group. Similar summaries of DBP, MAP, and HR will be performed.

For each acute challenge, change from pre-challenge in SBP, DBP, MAP, and HR will be summarized by treatment group at each timepoint. In addition, for each acute challenge, SBP, DBP, MAP, and HR will be summarized by treatment group at each timepoint. Line graphs of this information will be provided by treatment group for each acute challenge.

For each acute challenge, the concentration of erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II and metabolites, and noradrenalin will be summarized by treatment group at each timepoint. Line graphs of this information will be provided by treatment group for each acute challenge.

Further statistical considerations will be addressed in the RAP.

10. STUDY GOVERNANCE CONSIDERATIONS

10.1. Posting of Information on Publicly Available Clinical Trial Registers

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

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

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

The study will be conducted in accordance with all applicable regulatory requirements, and with GSK policy.

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

- IRB/IEC review and favorable opinion/approval of the study protocol and amendments as applicable
- Obtaining signed informed consent
- Investigator reporting requirements (e.g., reporting of AEs/SAEs/protocol deviations to IRB/IEC)
- GSK will provide full details of the above procedures, either verbally, in writing, or both
- Signed informed consent must be obtained for each subject prior to participation in the study
- The IEC/IRB, and where applicable the regulatory authority, approve the clinical protocol and all optional assessments, including genetic research.
- Optional assessments (including those in a separate protocol and/or under separate informed consent) and the clinical protocol should be concurrently submitted for approval unless regulation requires separate submission.

- Approval of the optional assessments may occur after approval is granted for the clinical protocol where required by regulatory authorities. In this situation, written approval of the clinical protocol should state that approval of optional assessments is being deferred and the study, with the exception of the optional assessments, can be initiated.

10.3. Quality Control (Study Monitoring)

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

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

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

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

10.4. Quality Assurance

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

10.5. Study and Site Closure

- Upon completion or premature discontinuation of the study, the GSK monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK Standard Operating Procedures.
- GSK reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. For multicenter studies, this can occur at one or more or at all sites.

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

10.6. Records Retention

- Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere), in a safe and secure location.
- The records must be maintained to allow easy and timely retrieval, when needed (e.g., for a GSK audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.
- Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken.
- The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.
- GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, GSK standards/procedures, and/or institutional requirements.
- The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator is no longer associated with the site.

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

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

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

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

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

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

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12. APPENDICES

12.1. Appendix 1 – Abbreviations and Trademarks

Abbreviations

ABPM	Ambulatory blood pressure monitoring
ADMA	Asymmetric dimethyarginine
AE	Adverse event
ALT	Alanine transaminase
AMD	Age-Related Macular Degeneration
ANCOVA	Analysis of Covariance
AST	Aspartate transaminase
AUEC	Area under the effect curve
AUC (0-24)	Area under concentration-time curve from time zero to 24 hours
BP	Blood pressure
CHr	Reticulocyte hemoglobin content
CI	Confidence interval
CKD	Chronic kidney disease
Cmax	Maximum observed concentration
CPK	Creatine phosphokinase
CV	Cardiovascular
DBP	Diastolic blood pressure
dL	Deciliter
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
ECHO	Echocardiogram
eCRF	Electronic Case Report Form
EDW	Estimated Dry Weight
EPO	Erythropoietin
ESA	Erythropoiesis-stimulating agent
ET-1	Endothelin-1
FDA	Food and Drug Administration
FRP	Females of Reproductive Potential
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
G	Gram
GI	Gastrointestinal
GSK	GlaxoSmithKline
hCG	Human chorionic gonadotrophin
HD	Hemodialysis dependent
HDPE	High Density Polyethylene
HF	Heart Failure
Hgb	Hemoglobin
HIF	Hypoxia-inducible factor
HR	Hour/heart rate

IB	Investigator's Brochure
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
IU	International Unit
IV	Intravenous
IVWRS	Interactive Voice/Web Response System
KDIGO	Kidney Disease Improving Global Outcomes
KG	Kilogram
LDH	Lactate dehydrogenase
MAP	Mean Arterial Blood Pressure
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MG	Milligram
MI	Myocardial infarction
ML	Milliliter
mmHG	Millimeter of mercury
MSDS	Material Safety Data Sheet
ND	Non-dialysis dependent
NO	Nitric Oxide
NOAEL	No Observed Adverse Effect Level
NYHA	New York Heart Association
PASP	Pulmonary Artery Systolic Pressure
PCI	Percutaneous Coronary Intervention
PD	Pharmacodynamic
PEG	Polyethylene glycol
PGI_2	Prostaglandin I_2
PHD	Prolyl hydroxylase domain
PHI	Prolyl hydroxylase inhibitor
PK	Pharmacokinetic
proADM	Proadrenomedullin
PRS	Project Requirement Specification
PRVP	Peak Right Ventricular Pressure
PSRAE	Possible suicidality related adverse event
QC	Quality Control
QTc	QT interval corrected for heart rate
QTcB	QT interval corrected for heart rate using Bazett's formula
RAP	Reporting and Analysis Plan
RBC	Red blood cell
RDW	Red blood cell distribution width
rhEPO	Recombinant human erythropoietin
RNA	Ribonucleic acid

SAE	Serious adverse event
SBP	Systolic blood pressure
SC	Subcutaneous
SD	Standard deviation
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic-pyruvic transaminase
sPAP	Systolic Pulmonary Artery Pressure
SRM	Study Reference Manual
T1/2	Terminal phase half-life
TIBC	Total iron binding capacity
Tmax	Time of occurrence of Cmax
TSAT	Transferrin saturation
UG	Microgram
UIBC	Unsaturated iron binding capacity
ULN	Upper limit of normal
URR	Urea reduction ratio
WBC	White blood cells

Trademark Information

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HemoCue

12.2. Appendix 2: Liver Safety Required Actions and Follow up Assessments

The procedures listed below are to be followed if a subject meets the liver chemistry stopping criteria defined in Section 5.5.3:

- Immediately withdraw the subject from study treatment
- Notify the Medical Monitor within 24 hr of learning of the abnormality to confirm the subject's study treatment cessation and follow-up.
- Complete the "Safety Follow-Up Procedures" listed below.
- Complete the liver event case report forms. If the event also meets the criteria of an SAE (see Section 12.4), the SAE data collection tool will be completed separately with the relevant details.
- Upon completion of the safety follow-up withdraw the subject from the study unless further safety follow up is required
- Do not restart study treatment
- Refer to the Flow chart for a visual presentation of the procedures listed below.

Safety Follow-Up Procedures for subjects with ALT ≥3xULN:

- Monitor subjects weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.

Safety Follow-Up Procedures for subjects with ALT ≥3xULN and total bilirubin ≥2xULN (>35% direct bilirubin); or ALT ≥ 3xULN and INR[1] > 1.5:

- This event is considered an SAE (see Section 12.4) Serum bilirubin fractionation should be performed if testing is available. If fractionation is unavailable, urinary bilirubin is to be measured via dipstick (a measurement of direct bilirubin, which would suggest liver injury).
- Make every reasonable attempt to have subjects return to the clinic within 24 hr for repeat liver chemistries, additional testing, and close monitoring (with specialist or hepatology consultation recommended).
- Monitor subjects twice weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.

[1] INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants.

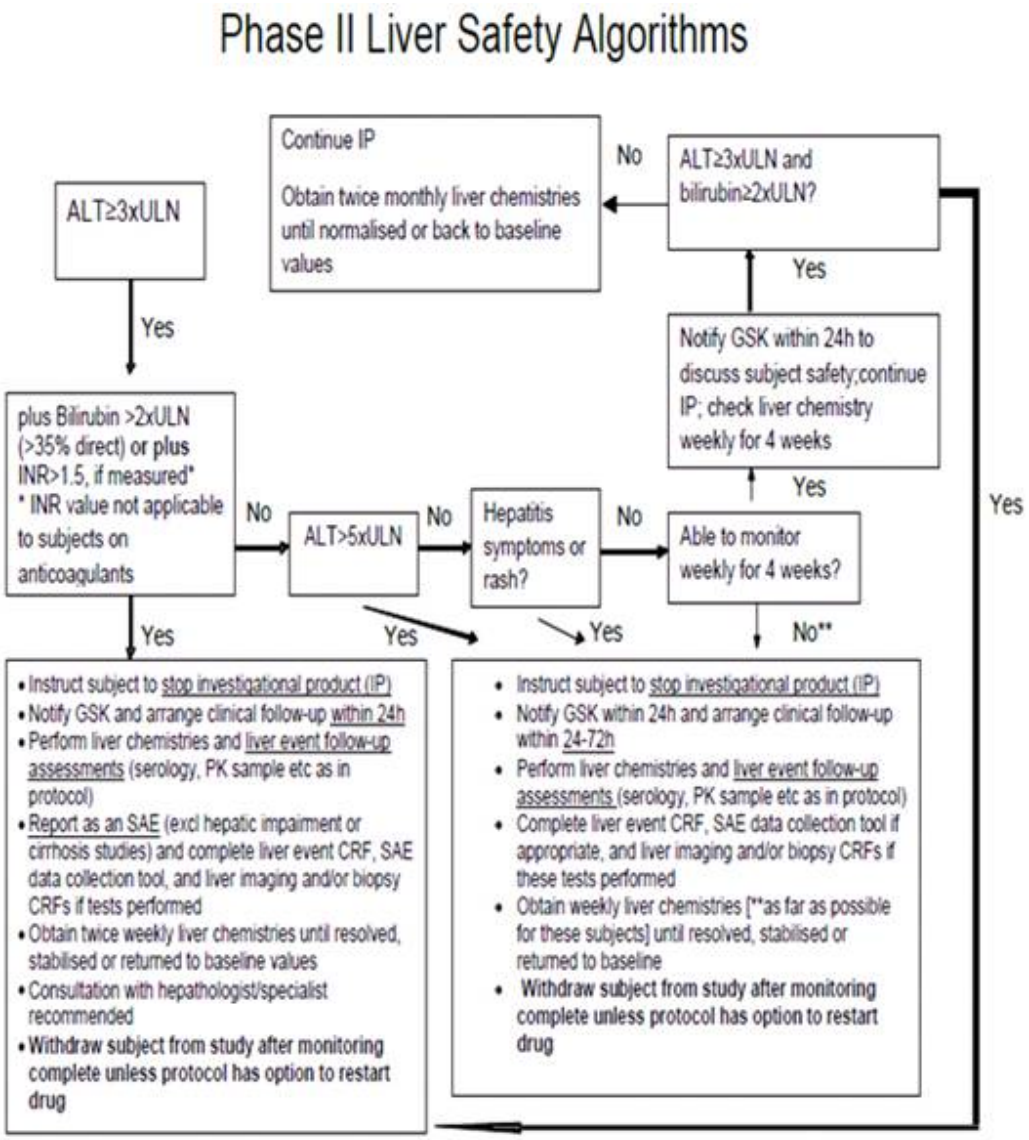
In addition, for <u>all</u> subjects with ALT ≥ 3xULN, every attempt must be made to also obtain the following:

- Viral hepatitis serology including:
  - Hepatitis A IgM antibody.
  - Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM).
  - Hepatitis C ribonucleic acid (RNA).
  - Cytomegalovirus IgM antibody.
  - Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing).
  - Hepatitis E IgM antibody.
- Blood sample for pharmacokinetic (PK) analysis, obtained within 24 h of last dose. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated <u>OR</u> a PK sample cannot be collected in the time period indicated above, **do not obtain a PK sample**. Instructions for sample handling and shipping are included in the SRM.
- Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
- Fractionate bilirubin, if total bilirubin ≥ 2xULN.
- Assess eosinophilia
- Record the appearance or worsening of clinical symptoms of hepatitis (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia) on the AE CRF.
- Record use of concomitant medications, acetaminophen, herbal remedies, other over the counter medications, or putative hepatotoxins on the Concomitant Medications CRF.
- Record alcohol use on the Liver Events CRF.

The following are required for subjects with ALT ≥ 3xULN **and** bilirubin ≥ 2xULN (>35% direct) but are optional for other abnormal liver chemistries:

- Anti-nuclear antibody, anti-smooth muscle antibody, and Type 1 anti-liver kidney microsomal antibodies.
  - Serum acetaminophen adduct high performance liquid chromatography (HPLC) assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009].
- Liver imaging (ultrasound, magnetic resonance, or computerized tomography) to evaluate liver disease.
- The Liver Imaging and/or Liver Biopsy CRFs are also to be completed if these tests are performed.

Refer to the diagram below for a visual presentation of the procedures listed above.



Additional charts are provided below for reference.

<table>
<tr><th colspan="2">Liver Chemistry Stopping Criteria – Liver Stopping Event</th></tr>
<tr><td>ALT-absolute</td><td>ALT ≥ 5xULN</td></tr>
<tr><td>ALT Increase</td><td>ALT ≥ 3xULN persists for ≥4 weeks</td></tr>
<tr><td>Bilirubin[1, 2]</td><td>ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin)</td></tr>
<tr><td>INR[2]</td><td>ALT ≥ 3xULN and INR>1.5, if INR measured</td></tr>
<tr><td>Cannot Monitor</td><td>ALT ≥ 3xULN and cannot be monitored weekly for 4 weeks</td></tr>
<tr><td>Symptomatic[3]</td><td>ALT ≥ 3xULN associated with symptoms (new or worsening) believed to be related to liver injury or hypersensitivity</td></tr>
<tr><th colspan="2">Required Actions and Follow up Assessments following ANY Liver Stopping Event</th></tr>
<tr><th>Actions</th><th>Follow Up Assessments</th></tr>
<tr><td>• Immediately discontinue study treatment Report the event to GSK within 24 hours
• Complete the liver event CRF and complete an SAE data collection tool if the event also meets the criteria for an SAE[2]
• Perform liver event follow up assessments
• Monitor the subject until liver chemistries resolve, stabilize, or return to within baseline (see MONITORING below)
• Do not restart/rechallenge subject with study treatment. 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

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</td><td>• Viral hepatitis serology[4]
• Blood sample for pharmacokinetic (PK) analysis, obtained within 24 hours after last dose[5]
• Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
• Fractionate bilirubin, if total bilirubin≥2xULN
• Obtain complete blood count with differential to assess eosinophilia
• Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE report form
• Record use of concomitant medications on the concomitant medications report form including acetaminophen, herbal remedies, other over the counter medications.
• Record alcohol use on the liver event alcohol intake case report form</td></tr>
</table>

baseline • A specialist or hepatology consultation is recommended **For All other criteria:** • 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	**For bilirubin or INR criteria:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG or gamma globulins). • Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009]). **NOTE: not required in China** • Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease; complete Liver Imaging and/or Liver Biopsy CRF forms.

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

References

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

12.3. Appendix 3: Daprodustat Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
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 5 • Hgb will be closely monitored throughout the dosing period as outlined in the Time and Events Table (Section 7.1) • Specific guidance for dose adjustment, dose interruption, or discontinuation of daprodustat based on achieved Hgb (including rate of change) is provided in Section 5.5.1.1 • Instream monitoring of safety data by an 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. Marketed rhEPO and its analogues have been associated with risks related to	• Specific eligibility criteria related to blood pressure, including exclusion of subjects with uncontrolled hypertension, are detailed in Section 6.2

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	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)]: ○ The majority (>90%) of subjects had baseline history of hypertension. ○ 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. ○ Although no clinically meaningful changes in blood pressure were observed, subjects in both treatment groups required increases in anti-HTN medications: ○ 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. ○ 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	• Blood pressure will be closely monitored throughout the dosing period as outlined in the Time and Events Table Section 7.1. • 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. In non-clinical studies conducted to date, not observed at tolerated doses when hemoglobin/hematocrit within normal range for species. 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 5.3 • Hgb will be closely monitored throughout the dosing period as outlined in the Time and Events Table Section 7.1 • Instream monitoring of safety data by an internal safety review team
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	• Suspected GI bleeding or significant symptoms consistent with erosion should be investigated diagnostically (i.e. endoscopic examination) as clinically warranted • Instream monitoring of safety data by an internal

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	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.	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 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 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	• Specific eligibility criteria related to personal history of malignancy or subjects with complex kidney cyst are outlined in Section 5.3. • Stopping criteria for subjects with treatment emergent malignancy are outlined in Section 5.5.1. • Instream monitoring of safety data by an internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	a safety concern for daprodustat.	
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 (up to 13-weeks duration 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. These hypoxia-induced PRVP changes fall within the range of PRVP differences noted among non-treated 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)	• Instream monitoring of safety data by an internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	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 commonly cited in the literature [Navaneethan, 2016]. Subjects with 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.	• Instream monitoring of safety data by an internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	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. 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 LVEF for 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 60 mg/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 up to 4 weeks duration, a dose-ordered increase in VEGF plasma concentrations was observed at doses ranging from 10 to 150 mg administered once daily and from 10 to 30 mg administered three times	• Instream monitoring of safety data by an internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	weekly. In studies up to 24 weeks duration at doses up to 25 mg, 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 with 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 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 an internal safety review team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Drug-drug interactions	Daprodustat is a substrate of CYP2C8: Co-administration of daprodustat with a strong CYP2C8 inhibitor 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 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. 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.	• Co-administration of daprodustat with strong CYP2C8 inhibitors (e.g., gemfibrozil) and inducers (e.g., rifampin/rifampicin) is not permitted as outlined in Section 6.11.3. • 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 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 6.11. • Hgb will be closely monitored throughout the dosing period as outlined in the Time and Events Table (Section 7.1) • Specific guidance for dose adjustment, dose interruption, or discontinuation of daprodustat based on achieved Hgb is provided in Section 5.5.1.1. • Instream monitoring of safety data by an internal safety review team.
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. However, the dose of FG-2216 (a PHI) used resulted in a	• Kidney function will be monitored throughout the dosing period as outlined in the Time and Events Table (Section 7.1). • 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
	significant erythropoietic response as reflected by ≥10% relative increases in hematocrit over the course of the study (Kraus, 2018; Hofherr, 2018). (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.	
Other		
rhEPO risks (Control)	See risks outlined in table for daprodustat for excessive erythropoiesis leading to thrombosis and/or tissue ischemia, death, MI, stroke, heart failure, thromboembolic events, thrombosis of vascular access, and for cancer-related mortality and tumor progression. Uncontrolled hypertension Pure red cell aplasia	• See mitigation strategies outlined in table for daprodustat for excessive erythropoiesis leading to thrombosis and/or tissue ischemia; risk of death, MI, stroke, heart failure, thromboembolic events, thrombosis of vascular access; and for increased cancer-related mortality and tumor progression. • Specific eligibility criteria related to blood

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		pressure management are outlined in Section 5. • Specific eligibility criteria related to personal history of pure red cell aplasia are outlined in Section 5.3

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

12.4.1. Definition of Adverse Events

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

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

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

leads to the procedure is an AE.

- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

12.4.2. Definition of Serious Adverse Events

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

Serious Adverse Event (SAE) is defined as any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

NOTE:

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

c. Requires hospitalization or prolongation of existing hospitalization

NOTE:

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

d. Results in disability/incapacity

NOTE:

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

e. Is a congenital anomaly/birth defect

f. Other situations:

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

g. Is associated with liver injury <u>and</u> impaired liver function defined as:

- ALT ≥3xULN and total bilirubin* ≥2xULN (>35% direct), **or**
- ALT ≥3xULN and International normalized ratio (INR)** >1.5.

* Serum bilirubin fractionation should be performed if testing is available; if unavailable, measure urinary bilirubin via dipstick. If fractionation is unavailable and ALT ≥3xULN and total bilirubin ≥2xULN, then the event is still to be reported as an SAE.

** INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants. If INR measurement is obtained, the value is to be recorded on the SAE form.

12.4.3. Definition of Cardiovascular Events

Cardiovascular Events (CV) Definition:

Investigators will be required to fill out the specific CV event page of the CRF for the following AEs and SAEs 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

12.4.4. Recording of AEs and SAEs

AEs and SAE Recording:

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event.
- The investigator will then record all relevant information regarding an AE/SAE in the CRF
- It is **not** acceptable for the investigator to send photocopies of the subject's medical records to GSK in lieu of completion of the GSK, AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.

12.4.5. Evaluating AEs and SAEs

Assessment of Intensity

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

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

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE.
- A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.

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

Follow-up of AEs and SAEs

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

12.4.6. Reporting of SAEs to GSK

SAE reporting to GSK via electronic data collection tool

- Primary mechanism for reporting SAEs to GSK will be the electronic data collection tool
- If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the Medical Monitor.
- Site will enter the serious adverse event data into the electronic system as soon as it becomes available.
- The investigator will be required to confirm review of the SAE causality by ticking

the 'reviewed' box at the bottom of the eCRF page within 72 hours of submission of the SAE.

- After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) will be taken off-line to prevent the entry of new data or changes to existing data
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, the site can report this information on a paper SAE form or to the Medical Monitor by telephone.
- Contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

12.4.7. Modified List of Highly Effective Methods for Avoiding Pregnancy in Females of Reproductive Potential (FRP)

This list does not apply to FRP with same sex partners, when this is their preferred and usual lifestyle or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

1. Contraceptive subdermal implant.
2. Intrauterine device or intrauterine system.
3. Combined estrogen and progestogen oral contraceptive [Trussell, 2011].
4. Injectable progestogen [Trussell, 2011]
5. Contraceptive vaginal ring [Trussell, 2011]
6. Percutaneous contraceptive patches [Trussell, 2011]
7. Male partner sterilization prior to the female subject's entry into the study, and this male is the sole partner for that subject [Trussell, 2011]. The documentation on male sterility can come from the site personnel's: review of subject's medical records, medical examination and/or semen analysis, or medical history interview provided by her or her partner.

This is an all inclusive list of those methods that meet the GSK definition of highly effective: having a failure rate of less than 1% per year when used consistently and, correctly and, when applicable, in accordance with the product label. For non-product methods (e.g. male sterility), the investigator determines what is consistent and correct use. The GSK definition is based on the definition provided by the ICH.

The investigator is responsible for ensuring that subjects understand how to properly use these methods of contraception.

References

Trussell J, Contraceptive Efficacy. In: Hatcher RA, Trussell J, Nelson AL, Cates W, Kowal D, and Policar M (editors). Contraceptive Technology: Twentieth Revised Edition. New York: Ardent Media, 2011.Table 26-1

12.4.8. Collection of Pregnancy Information

- Investigator will collect pregnancy information on any female subject, who becomes pregnant while participating in this study.
- Information will be recorded on the appropriate form and submitted to GSK within 2 weeks of learning of a subject's pregnancy.
- Subject will be followed to determine the outcome of the pregnancy. The investigator will collect follow up information on mother and infant, 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 Section 12.4. 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 subject who becomes pregnant while participating will discontinue study medication and be withdrawn from the study.

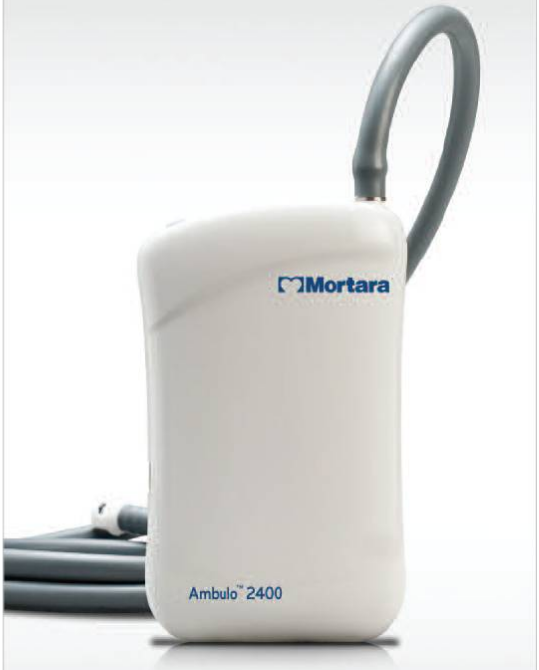
12.5. Appendix 5 - Country Specific Requirements

No country-specific requirements exist.

12.6. Appendix 6 – Ambulatory Blood Pressure Monitoring Device

Ambulo 2400

AMBULATORY BLOOD PRESSURE MONITORING SYSTEM



ABPM

PRODUCT FEATURES

- **24-hour Ambulatory Solution**
  24-hour ambulatory blood pressure monitoring system offering state-of-the-art innovation, accuracy, and reliability.
- **Highly Accurate**
  Validated to stringent clinical accuracy standards including the British Hypertension Society, AAMI SP-10, and the global ISO standard.
- **Trusted Technology**
  Patented oscillometric technology, along with powerful Microsoft® Windows®-based software, enables comprehensive setup, collection, analysis, and documentation of blood pressure data.
- **Actigraphy Option**
  Automatic determination of asleep/awake cycles based on patented technology reduces reliance on diaries, and enables synchronization of before/after studies in clinical review.
- **Full Disclosure**
  Detailed reporting of blood pressure measurements over the circadian rhythm, as well as all events and errors.
- **Impressively Comfortable**
  A compact, lightweight design, combined with gentle linear bleed technology, enables patients to go about their daily activities with comfort and minimal interruption.
- **Easy and Convenient**
  Quick, simple connection benefits clinicians and patients. Quiet and smooth operation increases patient compliance and aids in producing accurate measurement results.
- **Safe and Secure Data Transfer**
  Mortara Web Upload enables secure and efficient upload of ambulatory blood pressure data from virtually any location. Healthcare and clinical research professionals alike can share and expedite studies using this convenient data transfer tool.

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WARRANTY + SERVICE
Mortara Instrument is committed to the highest level of customer support. Please contact us for the program which best suits your needs.

AMBULO™ 2400 BP MONITORING SYSTEM

FEATURE	SPECIFICATION*
DIMENSIONS	4.7 x 2.7 x 1.2" (119 x 68 x 32 mm)
WEIGHT	9.0 oz (253 g) (with batteries)
MEASUREMENT PRINCIPLE	Patented oscillometric technique with linear deflation.
MEASUREMENT RANGES	Systolic: 60 to 280 mmHg Diastolic: 30 to 160 mmHg Pulse Rate: 30 to 180 bpm
MEASUREMENT ACCURACY	Blood Pressure: ±3 mmHg mean difference ±8 mmHg standard deviation Pulse Rate: ±3 bpm According to ANSI/AAMI SP-10:2002 & BHS (Grade A/A) and ISO 81060-2:2009
MEASUREMENT INTERVALS	Four adjustable intervals during 24-hour periods, each configurable to 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, or 120-minute measurements. Optional randomization factor up to 75% within intervals. Supports measurement over extended periods of time, typically 24 hours, and up to 7 days. Optional configuration support for Phase I clinical trials involving PK/PD dosing using discrete measurement time points based on protocol requirements. Measurements taken based on a sequence of time points until completed.
AVAILABLE CUFFS	Regular, large and small adult cuffs included. EasyWear™ cuff optional.
ACTIGRAPHY OPTION	Recording of 3-axis of motion via accelerometer for display and categorization of awake/asleep cycles via application software.
MEMORY	Solid-state Flash technology. Sufficient for 2,700 blood pressure measurements and 7 days of continuous actigraphy.
PC INTERFACE	USB
BATTERY POWER REQUIREMENTS	2 x 1.2V NiMH rechargeable batteries; 2.4V DC; Maximum current: 610mA. Sufficient for approximately 300 measurements using regular adult cuff.
SOFTWARE	Hypertension Diagnostics Suite requires PC running Microsoft Windows XP SP2, Windows Vista, or Windows 7 with a minimum graphics resolution of 1024 x 768.
MAXIMUM NOISE LEVEL	46 dBA during inflation; not measurable above ambient level during deflation

*Specifications subject to change without notice.

12.7. Appendix 7 – Protocol Changes

12.7.1. Amendment 1

Summary of Amendment Changes with Rationale

This protocol amendment applies to all centers where this study may be performed.

This protocol amendment was written to clarify specific biomarkers being drawn, modify time points of biomarkers, clarify safety laboratory studies, and clarify day/time of assessments. Additionally, a potential additional study visit has been added if necessary.

List of Specific Changes

Title Page
REVISED TEXT, where bolded text has been added

Authors:
PPD

Section 1 Protocol Synopsis for Study 205665
REVISED TEXT where bolded text has been added
The purpose of this study is to compare the effects on blood pressure of daprodustat to epoetin alfa in hemodialysis-dependent (HD) subjects with anemia associated with chronic kidney disease (CKD). The study will also assess various biomarkers associated with blood pressure physiology that may provide insight into the mechanism(s) of the hypertensive effects associated with **erythropoiesis stimulating agent(s) (**ESA**s).**

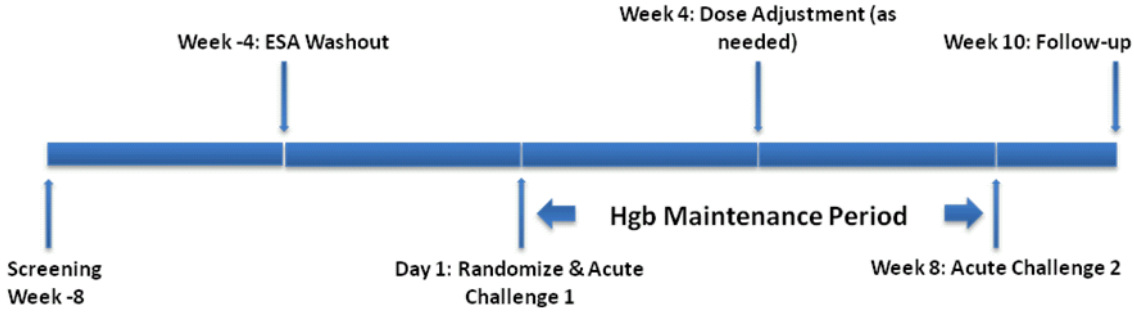
Exploratory Section of the Objective(s)/Endpoint(s) Table
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

Objectives	Endpoints
Exploratory	
• To investigate the effect of daprodustat on vasoactive mediators of blood pressure	• Plasma concentrations and derived parameters including Cmax, tmax, t½ and AUC(0-24) as appropriate (**to include** erythropoietin, endothelin-1, ~~proendothelin-1,~~ nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II, ~~aldosterone~~ and ~~MR-proadrenomedullin [proADM]~~ **noradrenalin**)
• Evaluate exposure-response relationships for daprodustat and: erythropoietin, endothelin-1, ~~proendothelin-1,~~ nitric oxide, asymmetric	• Further models (as appropriate) to describe the daprodustat exposure-response relationship with: erythropoietin, endothelin-1,

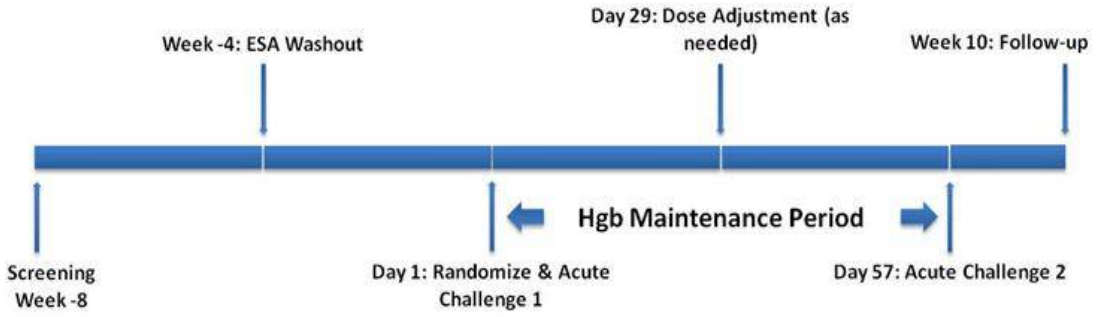
Objectives	Endpoints
dimethylarginine, renin, angiotensin-II, ~~aldosterone~~ and ~~MR-proADM~~ **noradrenalin** .	~~proendothelin-1,~~ nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II, ~~aldosterone~~ and ~~MR-proADM~~ **noradrenalin**.
• To summarize the effect of daprodustat and epoetin alfa on SBP after Acute Challenge 1 and Acute Challenge 2	• AUEC of SBP as measured by ABPM over 24-hr post-dosing
• To compare the effect of daprodustat to epoetin alfa on SBP after 8 weeks of Hgb maintenance therapy	• Change from Day 1 pre-dose SBP to Week 8 pre-dose

Section 1 Study Design

PREVIOUS IMAGE



REVISED IMAGE



REVISED TEXT where text in strikethrough has been removed and bolded text has been added.

Subjects will be screened for eligibility starting 4 weeks prior to the start of an ESA washout period (8 weeks prior to randomization/Day 1).

Subjects that qualify for enrolment will be washed out of their ESA for 4 weeks; the start of the ESA washout should be timed to coincide as closely as possible with their next planned ESA dose.

Following the 4-week ESA washout, subjects will be randomized 1:1 and stratified by prior ESA dose, based on the average weekly (for epoetins or darbepoetin) or monthly

(for methoxy polyethylene glycol (PEG)-epoetin beta) dosing during the 12 weeks prior to ESA washout (Week -4) as follows:

- Low ESA dose: <100 IU/kg/week epoetin alfa OR <0.5 μg/kg/week darbepoetin OR <0.6 μg/kg/week methoxy PEG-epoetin beta
- High ESA dose: ≥100 IU/kg/week epoetin alfa OR ≥0.5 μg/kg/week darbepoetin OR ≥0.6 μg/kg/week methoxy PEG-epoetin beta

On Day 1, subjects will be randomized and undergo Acute Challenge 1, a single dose challenge to compare the acute effects on BP of the highest planned once-daily maintenance dose of daprodustat (24 mg) to the highest starting dose of epoetin alfa (100 IU/kg). Subjects will have BP monitored for 24 hr using ABPM, and will have serial blood sampling to assess the pharmacokinetics of daprodustat and the time-course of various biomarkers. Acute Challenge 1 will be started immediately following completion of a mid-week dialysis session.

After completing Acute Challenge 1, all subjects will enter the 8-week Hgb maintenance period where doses of either daprodustat or epoetin alfa will be administered and adjusted, as needed.

On Day 57 ~~At Week 8~~, following the 8-week Hgb maintenance period, subjects will undergo Acute Challenge 2; this challenge will, similar to Acute Challenge 1, involve assessing the effect of daprodustat and epoetin alfa on SBP as assessed by 24-hr ABPM measurement. The subjects will be administered the same dose of either daprodustat or epoetin alfa that was given in Acute Challenge 1, and serial blood sampling will be performed to assess both daprodustat pharmacokinetics, and to characterize the time course of various biomarkers. Acute Challenge 2 will be started following completion of a mid-week dialysis session.

Subjects will be asked to not reinitiate anemia treatment until after the follow-up visit unless deemed medically necessary.

Subjects will attend a follow-up visit 2 weeks after **completion of** Acute Challenge 2.

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the QC criteria following Acute Challenge 2, one additional Acute Challenge may be made in a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed.

Section 1 Treatment Arms and Duration

REVISED TEXT where the bolded text has been added

- Acute Challenge 2 **(Day 57)** : At the end of the 8-week Hgb maintenance period subjects will repeat the procedures of Acute Challenge 1 utilizing the same treatment administered in Acute Challenge 1. This challenge will be initiated immediately following the subject's dialysis session.

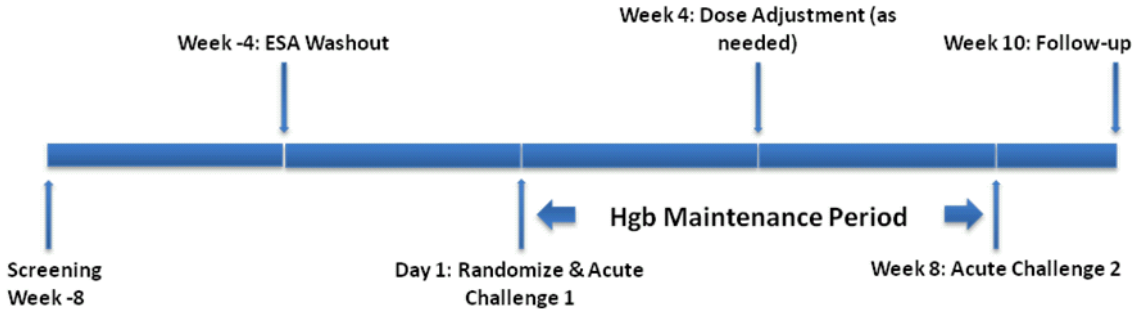
Section 3 OBJECTIVE(S) AND ENDPOINT(S)

REVISED TABLE where where text in strikethrough has been removed and bolded text has been added.

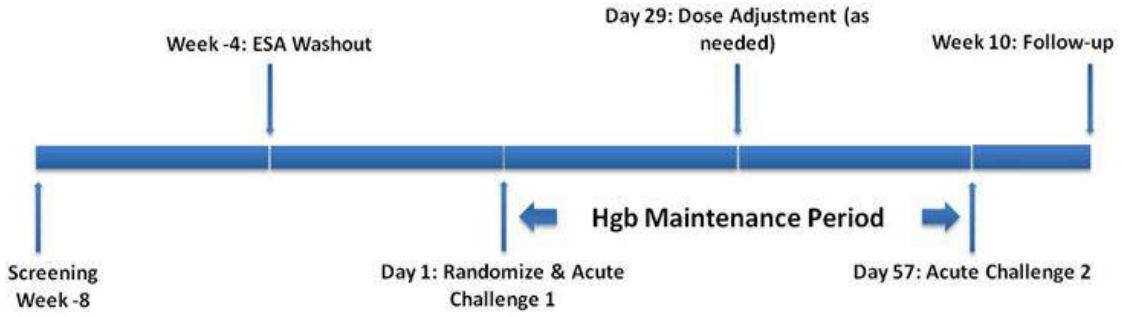
Exploratory	
• To investigate the effect of daprodustat on vasoactive mediators of blood pressure	• Plasma concentrations and derived parameters including Cmax, tmax, t½ and AUC(0-24) as appropriate (**to include** erythropoietin, endothelin-1, ~~proendothelin-1,~~ nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II, ~~aldosterone and MR-proadrenomedullin [proADM])~~ **and noradrenalin**)
• Evaluate exposure-response relationships for daprodustat and: erythropoietin, endothelin-1, ~~proendothelin-1,~~ nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II, ~~aldosterone and MR-proAdm~~ **and noradrenalin**.	• Further models (as appropriate) to describe the daprodustat exposure-response relationship with: erythropoietin, endothelin-1, ~~proendothelin-1,~~ nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II, ~~aldosterone and MR-proAdm~~ **and noradrenalin**.
• To summarize the effect of daprodustat and epoetin alfa on SBP after Acute Challenge 1 and Acute Challenge 2	• AUEC of SBP as measured by ABPM over 24-hr post-dosing
• To compare the effect of daprodustat to epoetin alfa on SBP after 8 weeks of Hgb maintenance therapy	• Change from Day 1 pre-dose SBP to Week 8 pre-dose

Section 4.1 Overall Design

PREVIOUS FIGURE 1



REVISED FIGURE 1



REVISED TEXT where text in strikethrough has been removed and bolded text has been added

Subjects will be screened for eligibility starting 4 weeks prior to the start of an ESA washout period (8 weeks prior to randomization/Day 1).

Subjects that qualify for enrolment will be washed out of their ESA for 4 weeks; the start of the ESA washout should be timed to coincide as closely as possible with their next planned ESA dose.

Following the 4-week ESA washout, subjects will be randomized 1:1 and stratified by prior ESA dose, based on the average weekly (for epoetins or darbepoetin) or monthly (for methoxy polyethylene glycol (PEG)-epoetin beta) dosing during the 12 weeks prior to ESA washout (Week -4) as follows:

- Low ESA dose: <100 IU/kg/week epoetin alfa OR <0.5 µg/kg/week darbepoetin OR <0.6 µg/kg/week methoxy PEG-epoetin beta
- High ESA dose: ≥100 IU/kg/week epoetin alfa OR ≥0.5 µg/kg/week darbepoetin OR ≥0.6 µg/kg/week methoxy PEG-epoetin beta

On Day 1, subjects will be randomized and undergo Acute Challenge 1, a single dose challenge to compare the acute effects on BP of the highest planned once-daily maintenance dose of daprodustat (24 mg) to the highest starting dose of epoetin alfa (100 IU/kg). Subjects will have BP monitored for 24 hr using ABPM, and will have serial blood sampling to assess the pharmacokinetics of daprodustat and the time-course of various biomarkers. Acute Challenge 1 will be started immediately following completion of a mid-week dialysis session.

After completing Acute Challenge 1, all subjects will enter the 8-week Hgb maintenance period where doses of either daprodustat or epoetin alfa will be administered and adjusted, as needed.

On Day 57 ~~At Week 8~~, following the 8-week Hgb maintenance period, subjects will undergo Acute Challenge 2; this challenge will, similar to Acute Challenge 1, involve assessing the effect of daprodustat and epoetin alfa on SBP as assessed by 24-hr ABPM measurement. The subjects will be administered the same dose of either daprodustat or epoetin alfa that was given in Acute Challenge 1, and serial blood sampling will be performed to assess both daprodustat pharmacokinetics, and to characterize the time

course of various biomarkers. Acute Challenge 2 will be started following completion of a mid-week dialysis session.

Subjects will be asked to not reinitiate anemia treatment until after the follow-up visit unless deemed medically necessary.

Subjects will attend a follow-up visit 2 weeks after **completion of** Acute Challenge 2.

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the QC criteria following Acute Challenge 2, one additional Acute Challenge may be made in a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed as detailed in Table 4.

Section 5.1 Hemoglobin Stability Criteria
REVISED TEXT, where the text in strikethrough has been removed

Entry into the study requires a stable Hgb between **9.0 and 11.5 g/dL, inclusive**. This is confirmed from an average of **three** Hgb values obtained during the screening period at Weeks -8, -6 and -4 via a validated point-of-care device to measure Hgb (i.e., HemoCue) as outlined in Figure 3. ~~Calculations to determine eligibility will be performed automatically by an Interactive Voice/Web Response System (IVWRS).~~ For subjects with a three-times weekly dialysis schedule, Hgb values must not be obtained on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday). For subjects with a four- to five-times weekly dialysis schedule, Hgb values can be obtained on any hemodialysis session of the week.

Section 5.3 Exclusion Criteria
REVISED TEXT, where the text in strikethrough has been removed and bolded text has been added

4. Mircera: **Planned or recorded** administration of Mircera (methoxy PEG-epoetin beta) within the 4 weeks prior to **start of washout** ~~screening through~~ **at** Week -4.

Section 5.5.1.1 Hemoglobin Stopping Criteria

REVISED TABLE 2 where text in strikethrough has been removed and bolded text has been added

Table 8 Hgb Stopping Criteria

Week -2 & Day 1 (Prior to Acute Challenge 1)

Hgb 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, withdraw subject from the study.	
≥7.5-<11.5	decrease of ≥2.0 in Hgb over 2 weeks	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently withdraw subject from the study.
	increase of ≥1.0 in Hgb over 2 weeks	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, withdraw subject from the study.
≥11.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently withdraw subject from the study.	

Days 15, 29, &43 ~~Weeks 2, 4 & 6~~

Hgb 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 subject from the study.	
≥7.5-<12.0	decrease of ≥2.0 in Hgb over 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 subject from the study.
	increase of ≥1.0 in Hgb over 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 subject from the study.
≥12.0	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If cofirmed, permanently discontinue study treatment and withdraw subject from the study.	

Day 57 ~~Week 8~~ (Prior to Acute Challenge 2)

Hgb 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 subject from the study.	
≥7.5-<11.0	decrease of ≥2.0 in Hgb over 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 subject from the study.
	increase of ≥1.0 in Hgb over 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 subject from the study.
≥11.0	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 subject from the study.	

Section 6.4.1 Subjects Randomized to Daprodustat

REVISED TEXT where text in strikethrough has been removed and bolded text has been added

Subjects randomized to daprodustat will have doses adjusted, as required, to target Hgb within the range of 10.0-11.0 g/dL. Dose adjustments will be assigned **automatically** via the IVWRS based on the subject's Hgb value via onsite HemoCue assessment according to the following algorithms:

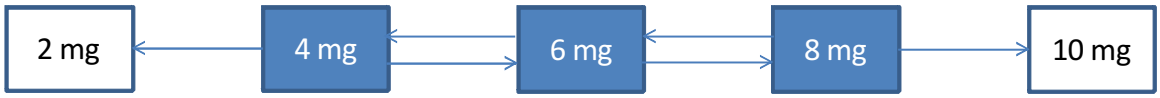
Day **1** ~~2~~

Hgb (g/dL)	Action or Dose
<7.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, withdraw subject from the study.
≥7.5 and <9.0	8 mg daprodustat
≥9 and <10.0	6 mg daprodustat
≥10.0 and <11.5	4 mg daprodustat
≥11.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, withdraw subject from the study.

The 8 week Hgb maintenance dosing will begin on Day 2, following Acute Challenge 1.

Day 29 ~~Week 4~~

The available dose steps for daprodustat are outlined below (highlighted boxes indicate starting doses). Dose adjustments will result in the daprodustat dose being increased or decreased by **one dose step**.



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 drug and withdraw subject from the study.
≥7.5 to <9.5	Decreasing or No change	Increase to the next higher dose step
≥7.5 to <9.5	Increasing	Maintain dose
≥9.5 to ≤11.5	Any change	Maintain dose
>11.5 to <12.0	Increasing or No change	Decrease to the next lower dose step
	Decreasing	Maintain dose
≥12.0	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.

Section 6.7 Compliance with Study Treatment Administration
REVISED TEXT where text in strikethrough has been removed and bolded text has been added.

Randomized treatment start and stop dates and dosing details, including dates for randomized treatment interruptions and/or dose increases/reductions, will be recorded in ~~the eCRF~~ **RAMOS NG**.

Section 7 STUDY ASSESSMENTS AND PROCEDURES
REVISED TEXT where test in strikethrough has been removed and bolded text has been added

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table (Section 7.1), are essential and required for study conduct.

- If assessments are scheduled for the same nominal time, then the assessments should occur in the following order:
    1. 12-lead ECG
    2. vital signs
    3. blood draws

  Note: The timing of the assessments should allow the blood draw to occur at the exact nominal time.
- The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment.
- The Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent Form.
- No more than **550** ~~500~~ mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

Supplementary study conduct information is provided in the SRM. The SRM provides administrative and detailed technical information that does not impact subject safety.

Section 7.1 Time and Events Table

PREVIOUS TABLE 3

Table 3 Study Procedures and Assessments

Procedure[1]	Screening		ESA Washout		Treatment Period[2]					Follow-up[2]	
	Week -8	Week -6	Week -4	Week -2	Day 1	Week 2	Week 4	Week 6	Week 8	Early WD	Week 10
Informed Consent	X										
Entry Criteria	X										
Physical, Medical History, Demography	X										
HemoCue Hgb	X	X	X	X	X[4]	X	X	X	X[4]	X	
IVWRS Call	X	X	X	X	X	X	X	X	X	X	X
Randomization					X						
Acute Challenge					X[4]				X[4]		
ABPM Assessment					X[4]				X[4]		
Initiation of epoetin alfa/daprodustat dosing					X						
Dose Adjustment							X				
Females Only: Serum Pregnancy Test		X			X		X		X	X	X
Females Only: Estradiol & FSH (if required)[3]		X									
ECG	X				X[4]				X[4]	X	X
Vital signs & weight (Pre- & Post-dialysis)	X	X	X		X[4]		X		X[4]	X	X
Clinical Chemistry		X			X		X		X	X	X
Hematology		X			X		X		X	X	X
Folate	X										
Ferritin, transferrin, total iron, unsaturated iron binding capacity (UIBC)	X		X								
Pharmacokinetic/Biomarker Assessments					X[4]				X[4]		
Hgb Maintenance Period[5]					←==========				==========→		
Blood draw for PGx					X						
Adverse Events Assessment	X[6]	X[6]	X[6]	X[6]	X[4]	X	X	X	X[4]	X	X
Review Concomitant Medications	X		X	X	X[4]	X	X	X	X[4]	X	X

1 All assessments should be done predialysis/predose except as noted.
2 Allowable time window ±2 days EXCEPT Follow-up Visit which is ±3 days.
3 As detailed in Inclusion Criteria.
4 Detailed timings for assessments on Acute Challenge Days are given in Table 4.
5 From the end of Acute Challenge 1 to the beginning of Acute Challenge 2.
6 Only SAEs assessed as related to study participation are collected at this visit. See Section 12.4 for additional details.

REVISED TABLE 3

Table 3 Study Procedures and Assessments

Procedure[1]	Screening		ESA Washout		Treatment Period[2]					Follow-up[2]	
	Week -8	Week -6	Week -4	Week -2	Day 1	Day 15	Day 29	Day 43	Day 57	Early WD	Week 10
Informed Consent	X										
Entry Criteria	X										
Physical, Medical History, Demography	X										
HemoCue Hgb	X	X	X	X	X[4]	X	X	X	X[4]	X	
IVWRS					X		X				
Randomization					X						
Acute Challenge					X[4]				X[4]		
ABPM Assessment					X[4]				X[4]		
Dose Adjustment							X				
Females Only: Serum Pregnancy Test		X			X		X		X	X	X
Females Only: Estradiol & FSH (if required)[3]		X									
ECG	X				X[4]				X[4]	X	X
Vital signs & weight (Pre- & Post-dialysis)	X	X	X		X[4]		X		X[4]	X	X
Clinical Chemistry		X			X		X		X	X	X
Hematology		X			X		X		X	X	X
Folate and Vitamin B12	X										
Ferritin, transferrin, total iron,TSAT, UIBC	X		X								
Pharmacokinetic/Biomarker Assessments					X[4]				X[4]		
Hgb Maintenance Period[5]					←==========				==========→		
Adverse Events Assessment	X[6]	X[6]	X[6]	X[6]	X[4]	X	X	X	X[4]	X	X
Review Concomitant Medications	X		X	X	X[4]	X	X	X	X[4]	X	X

1 All assessments should be done predialysis/predose except as noted.

2 Allowable time window ±2 days EXCEPT Follow-up Visit which is ±3 days.

3 As detailed in Inclusion Criteria.

4 Detailed timings for assessments on Acute Challenge Days are given in Table 4.

5 From the end of Acute Challenge 1 to the beginning of Acute Challenge 2.

6 Only SAEs assessed as related to study participation are collected at this visit. See Section 12.4 for additional details

.

Section 7.1 Time and Events Table

PREVIOUS TABLE 4

Table 4 Study Procedures and Assessments on Acute Challenge Days (Treatment Period Day 1 and Week 8 Visit)

Procedure	Predose	Time (in hours relative to dosing)										
		0	0.5	1	2	3	4	6	8	12	16	24
ABPM Assessment	←==========→											
Administer Study Treatment		X										
HemoCue Hgb	X											X
ECG	X											X
Vitals	X					X				X		X
Pharmacokinetic/Biomarker Assessments	X		X	X	X	X	X	X	X	X	X	X
Adverse Events Assessment	X		X	X	X	X	X	X	X	X	X	X
Review Concomitant Medications	X											X

REVISED TABLE 4

Table 4 Study Procedures and Assessments on Acute Challenge Days (Treatment Period Day 1 and Day 57)

Procedure	Pre-Dialysis	Post - Dialysis	Predose	Time (in hours relative to dosing)										
				0	0.5	1	2	3	4	6	8	12	16	24
HemoCue Hgb[1]			X											X
IVWRS			X											
ABPM Assessment[1]		X	←==========→											
Administer Study Treatment[1]				X										
Serum Pregnancy Test[1]			X											
ECG[1]			X											X
Vital Signs and Weight[1]	X	X						X				X		X
Clinical Chemistry			X											
Hematology			X											
Pharmacokinetics			X		X	X	X	X	X	X	X	X	X	X
Erythropoietin			X		X	X	X	X	X	X	X	X	X	X
Endothelin-1			X			X	X		X	X	X			X
Nitric Oxide			X			X	X		X	X	X			X
Asymmetric dimethylarginine			X			X	X		X	X	X			X
Renin			X			X	X		X	X	X			X
Angiotensin-II			X			X	X		X	X	X			X
Noradrenalin			X			X	X		X	X	X			X
Adverse Events Assessment[1]			X		X	X	X	X	X	X	X	X	X	X
Review Concomitant Medications[1]			X											X
Initiate Hgb maintenance dosing[2]														X

1 Procedures to be repeated if Acute Challenge 2 fails quality control criteria
2 Applies to Acute Challenge 1 only

Section 7.3.6 Clinical Safety Laboratory Assessments

PREVIOUS TABLE 5

Table 5 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	Parameters		
Hematology	Platelet Count	*RBC Indices:*	*WBC Count with Differential:*
	RBC Count	MCV	Neutrophils
	Hemoglobin	MCH	Lymphocytes
	Hematocrit	MCHC	Monocytes
	WBC Count (absolute)	RDW	Eosinophils
	Reticulocyte Count	CHr	Basophils
Clinical Chemistry	Potassium	AST (SGOT)	Total and direct/indirect bilirubin
	Sodium	ALT (SGPT)	Total Protein
	Glucose	Calcium (Albumin adjusted)	Alkaline Phosphatase
	Albumin	Phosphate	
Other laboratory Tests	Erythropoietin	Vascular Endothelial Growth Factor	Hepcidin
	Serum ferritin	Serum iron	Serum transferrin
	Transferrin saturation (% saturation)	Unbound iron binding capacity	Folate
	Vitamin B_{12}		
Other Screening Tests[1]	FSH	Estradiol	Serum hCG Pregnancy test

NOTE: Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 12.2.

[1] As needed in women of non-child bearing potential only.

Abrreviations: ALT= Alanine transaminase; AST= Aspartate transaminase; CHr=Reticulocyte haemoglobin content; FSH=follicle stimulating hormone; MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular hemoglobin concentration; MCV= Mean corpuscular volume; RBC= Red blood cell; RDW= Red blood cell distribution width; SGOT=Serum glutamic oxaloacetic transaminase; SGPT=Serum glutamic-pyruvic transaminase; WBC= White blood cells;

REVISED TABLE 5

Table 5 Protocol Required Safety Laboratory Assessments

Laboratory Assessments	Parameters		
Hematology	Platelet Count	*RBC Indices:*	*WBC Count with Differential:*
	RBC Count	MCV	Neutrophils
	Hemoglobin	MCH	Lymphocytes
	Hematocrit	MCHC	Monocytes
	WBC Count (absolute)	RDW	Eosinophils
	Reticulocyte Count	CHr	Basophils
Clinical Chemistry	Potassium	AST (SGOT)	Total and direct/indirect bilirubin
	Sodium	ALT (SGPT)	Total Protein
	Glucose	Calcium (Albumin adjusted)	Alkaline Phosphatase
	Albumin	Phosphate	
Other Screening Tests	Serum hCG pregnancy test	Serum ferritin	Folate
	Estradiol	Serum iron	Vitamin B12
	FSH	Serum transferrin	UIBC
		TSAT	

NOTE: Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 12.2.

[1] As needed in women of non-child bearing potential only.

Abrreviations: ALT= Alanine transaminase; AST= Aspartate transaminase; CHr=Reticulocyte haemoglobin content; FSH=follicle stimulating hormone; MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular hemoglobin concentration; MCV= Mean corpuscular volume; RBC= Red blood cell; RDW= Red blood cell distribution width; SGOT=Serum glutamic oxaloacetic transaminase; SGPT=Serum glutamic-pyruvic transaminase; TSAT = transferrin saturation;; UIBC = unsaturated iron binding capacity; WBC= White blood cells;

Section 7.5.1 Ambulatory Blood Pressure Monitoring (ABPM)

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

Blood pressure and pulse will be measured during Acute Challenge 1 and 2 through the use of an ambulatory blood pressure monitoring device. Subjects will wear the ABPM device for two sessions during the study (i.e., Acute Challenge 1 and 2), occurring at Day 1 (randomization) and at **Day 57** ~~Week 8~~. The ABPM device will be placed 1 hr prior to completion of the dialysis session, and will remain on the subject's arm until 24 hr post-dosing.

ABPM measurements of systolic and diastolic blood pressure and pulse will be **manually recorded at one hour prior to completion of the dialysis session,** automatically recorded every 15 minutes for the first 6 hrs post-dosing, and then every 20 minutes thereafter until 24 hrs post-dosing. The ABPM device will automatically calculate MAP.

The device planned for use in this study is the Mortara Ambulo 2400; a specification sheet for this device can be found in Section 12.6.

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the QC criteria following Acute Challenge 2, one additional Acute Challenge may be made in a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed as detailed in Table 4.

Section 7.5.2 Blood Pressure Regulation Biomarkers
REVISED TEXT where text in strikethrough has been removed and bolded text has been added

Blood samples will be collected during this study to investigate the mechanism of the effect of daprodustat on blood pressure. Biomarkers selected will explore the renin-angiotensin-aldosterone axis, the nitric oxide and endothelin axes, sodium retention and HIF signalling.

Biomarkers may include **erythropoietin**, nitric oxide (NO), asymmetric dimethyarginine (ADMA), renin, angiotensin-II, ~~aldosterone,~~ endothelin-1 (ET-1), ~~proendothelin-1, prostaglandin E_2 (PGE_2), prostaglandin I_2 (PGI_2),~~**and** noradrenalin, ~~epinephrine, and MR-proadrenomedullin.~~

Samples will be collected as specified in the Time and Events Table (Section 7.1). The timing of the collections may be adjusted on the basis of emerging pharmacokinetic or pharmacodynamic (PD) data from this study or other new information in order to ensure optimal evaluation of the PD endpoints.

Section 7.6 GENETICS
Summary of changes:

All genetic research has been removed from this study.

PREVIOUS TEXT:

Information regarding genetic research is included in Appendix 4.

(Note: Appendix 4 has been deleted due to removal of genetic testing. This may be referenced at the end of Appendix 7, within the eliminated genetics Section 12.4)

REVISED TEXT:

Genetics are not evaluated in this study.

Section 9.4.3 Other analyses
REVISED TEXT where text in strikethrough has been removed and bolded text has been added

The difference in SBP between pre-dose in Acute Challenge 1 and pre-dose in Acute Challenge 2 will be summarized by treatment group. Similar summaries of DBP and MAP will be performed.

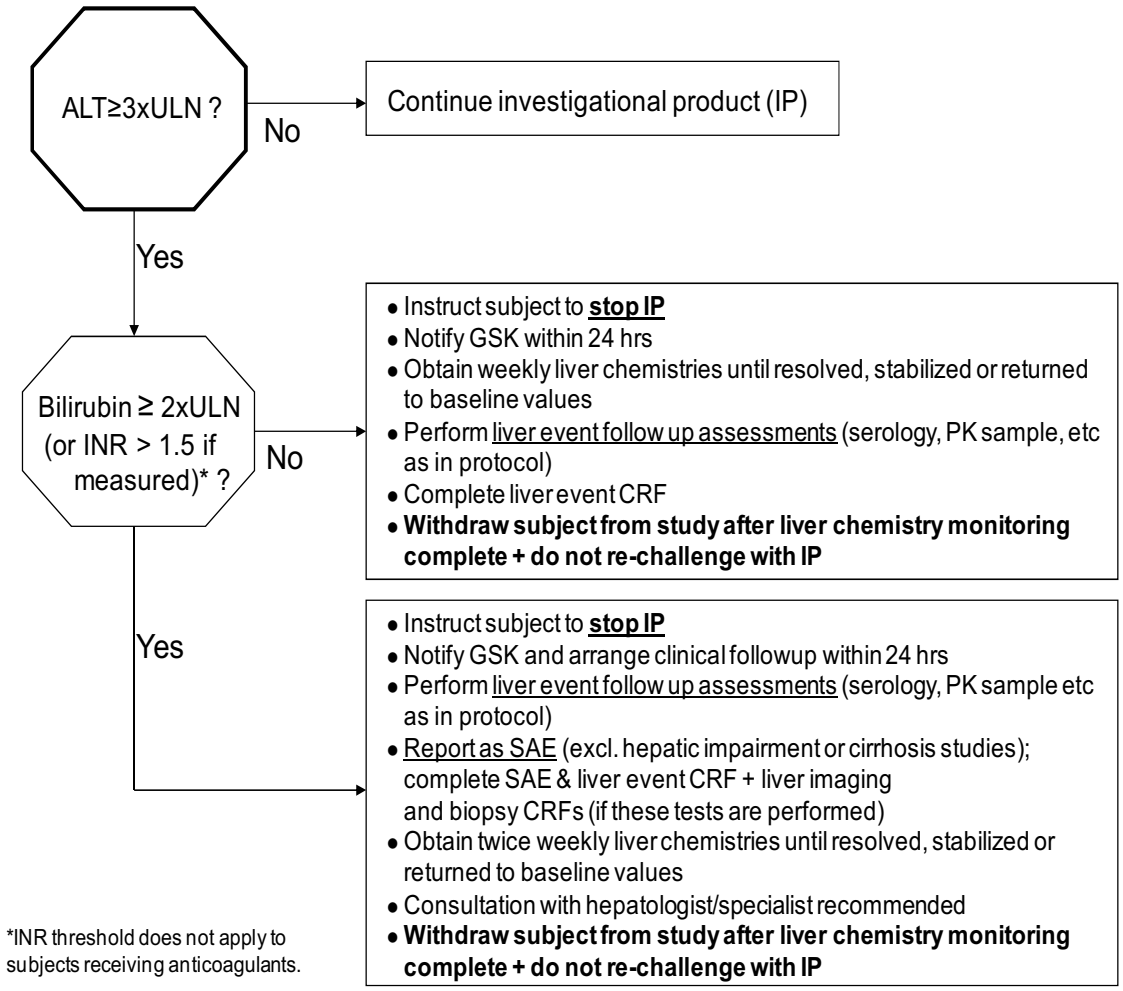
For each acute challenge, change from pre-challenge in SBP, DBP, MAP, and pulse will be summarized by treatment group at each timepoint. In addition, for each acute challenge, SBP, DBP, MAP, and pulse will be summarized by treatment group at each timepoint. Line graphs of this information will be provided by treatment group for each acute challenge.

For each acute challenge, the **concentration of** ~~PK parameters for~~ erythropoietin, endothelin-1~~, proendothelin-1,~~ nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II~~, aldosterone, adrenomedullin and MR-proadrenomedullin~~ **and noradrenalin** will be summarized by treatment group at each timepoint. Line graphs of this information will be provided by treatment group for each acute challenge.

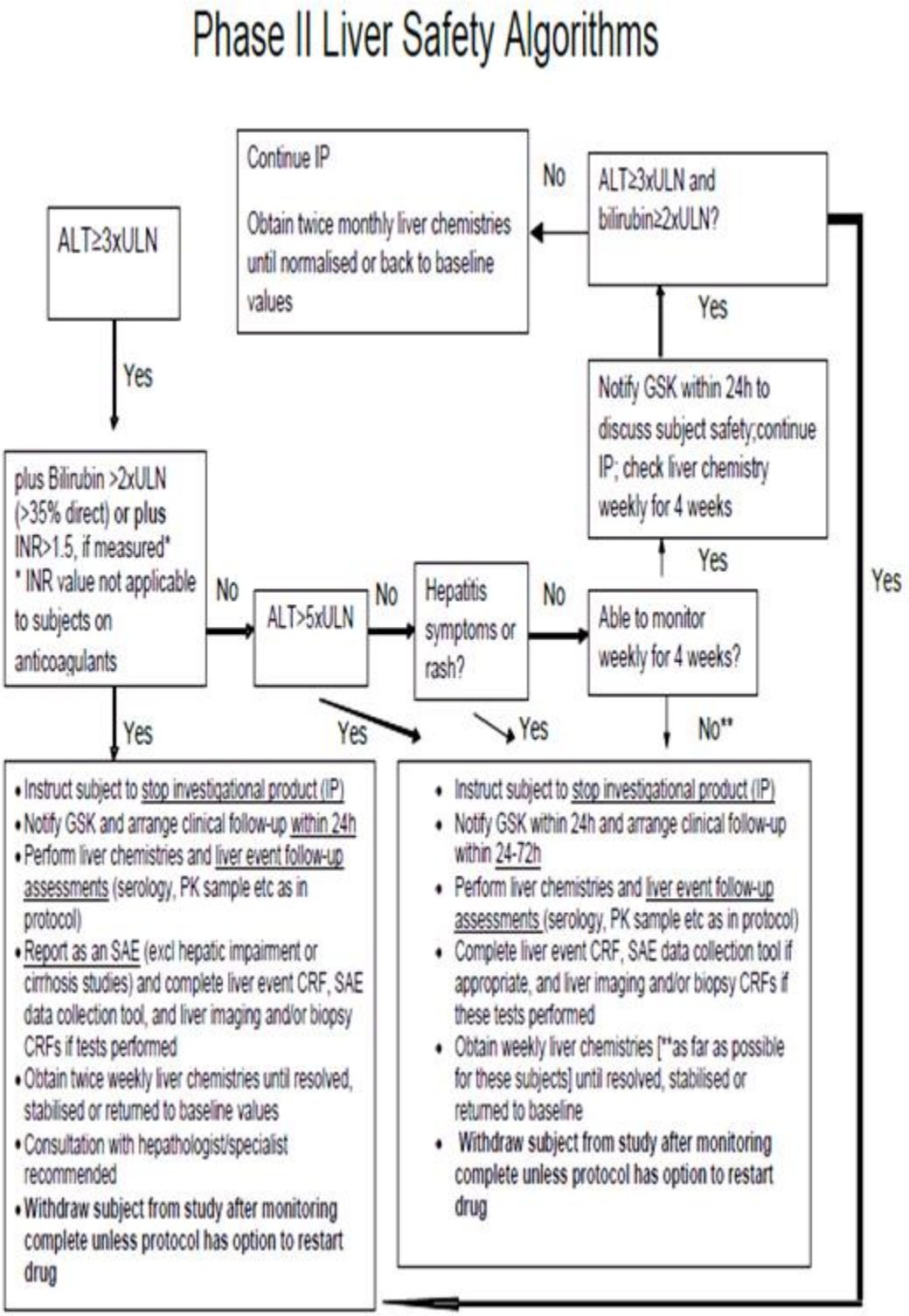
Further statistical considerations will be addressed in the RAP.

Section 12.2 Appendix 2 – Liver Safety Required Actions and Follow Up Assessments

PREVIOUS DIAGRAM



REVISED DIAGRAM



ADDED CHARTS

The following two charts and corresponding text were added to Appendix 2 in their entirety.

Additional charts are provided below for reference.

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 e**vent to GSK **within 24 hours** • Complete the liver event **CRF and complete** an SAE data collection tool if the event also meets the criteria for an SAE[2] • Perform liver event follow up assessments • Monitor the subject until liver chemistries resolve, stabilize, or return to within baseline (see **MONITORING** below) • **Do not restart/rechallenge** subject with study treatment. **If restart/rechallenge not allowed per protocol or not granted**, permanently dis**continue study treatment and may contin**ue subject in the study for any protocol specified follow up assessments **MONITORING:** **<u>For bilirubin or INR criteria:</u>** • Repeat liver chemistries (include **ALT, AST,**	• Viral hepatitis serology[4] • Blood sample for pharmacokinetic (PK) analysis, obtained within 24 hours after last dose[5] • Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). • Fractionate bilirubin, if total bilirubin≥2xULN • Obtain complete blood count with differential to assess eosinophilia • Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE report form • Record use of concomitant medications on the concomitant medications report form including acetaminophen, herbal remedies, other over the counter medications. • Record alcohol use on the liver event

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 **For All other criteria:** • 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	alcohol intake case report form **For bilirubin or INR criteria:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG or gamma globulins). • Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James 2009]). **NOTE: not required in China** • Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease; complete Liver Imaging and/or Liver Biopsy CRF forms.

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

References

James LP, Letzig L, Simpson PM, Capparelli E, Roberts DW, Hinson JA, Davern TJ, Lee WM. Pharmacokinetics of Acetaminophen-Adduct in Adults with Acetaminophen Overdose and Acute Liver Failure. Drug Metab Dispos 2009; 37:1779-1784.

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

Section 12.4 Appendix 4 – Genetic Research

Summary of changes:

Due to genetic research being removed from this study, this section was deleted in its entirety. No revised text was created.

PREVIOUS TEXT:

Genetics – Background

Naturally occurring genetic variation may contribute to inter-individual variability in response to medicines, as well as an individual's risk of developing specific diseases. Genetic factors associated with disease characteristics may also be associated with response to therapy, and could help to explain some clinical study outcomes. For example, genetic variants associated with age-related macular degeneration (AMD) are reported to account for much of the risk for the condition with certain variants reported to influence treatment response [Chen, 2012]. Thus, knowledge of the genetic etiology of disease may better inform understanding of disease and the development of medicines. Additionally, genetic variability may impact the pharmacokinetics (absorption, distribution, metabolism, and elimination), or pharmacodynamics (relationship between concentration and pharmacologic effects or the time course of pharmacologic effects) of a specific medicine and/or clinical outcomes (efficacy and/or safety) observed in a clinical study.

Genetic Research Objectives and Analyses

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

- Response to medicine, including any treatment regimens under investigation in this study;
- Anemia associated with chronic kidney disease
- susceptibility, severity, and progression and related conditions

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

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

Study Population

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

Study Assessments and Procedures

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

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

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

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

for the purpose stated in this protocol and in the informed consent form. Samples may be used as part of the development of a companion diagnostic to support the GSK medicinal product.

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

Informed Consent

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

Subject Withdrawal from Study

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

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

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

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

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

Screen and Baseline Failures

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

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

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

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

Section 12.5, Section 12.6, and Section 12.7
Summary of changes:

Appendix 5, Appendix 6, and Appendix 7 re-numbered due to removal of Appendix 4.

12.7.2. Amendment 2

Summary of Amendment Changes with Rationale

This protocol amendment applies to all centers where this study may be performed.

This protocol amendment was written to clarify the descriptive statistics and include them in the objectives and endpoints table(s) as well as remove one blood pressure measurement. Additional minor changes were made for clarity of study procedures.

List of Specific Changes

Section 1 Protocol Synopsis for Study 205665
Objective(s)/Endpoint(s) Table
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

Objectives	Endpoints
Primary	
• To compare the effect of daprodustat to epoetin alfa on blood pressure (BP) after Acute Challenge 2 (8 weeks of hemoglobin [Hgb] maintenance therapy)	• Average of systolic blood pressure (SBP) as measured by ambulatory blood pressure monitoring (ABPM) over 6-hr post-dosing after 8 weeks of Hgb maintenance therapy
Secondary	
• To compare the initial effect of daprodustat to epoetin alfa on BP after Acute Challenge 1 (4 weeks of erythropoiesis-stimulating agent [ESA]	• Average of SBP, **diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR)** as measured by ABPM

Objectives	Endpoints
washout)	over 6-hr post-dosing at Day 1 • ~~Average of diastolic blood pressure (DBP) as measured by ABPM over 6-hr post-dosing at Day 1~~ • Area under the effect curve (AUEC) of SBP, **DBP, MAP, and HR** as measured by ABPM over 24-hr post-dosing at Day 1
• To compare the effect of daprodustat to epoetin alfa on BP after Acute Challenge 2	• Average of DBP, **MAP, and HR** as measured by ABPM over 6 hr post-dosing at ~~Week 8~~ Day 57. • AUEC of SBP**, DBP, MAP, and HR** as measured by ABPM over 24-hr post-dosing at ~~Week 8~~ Day 57.
• To estimate the initial effect of daprodustat on SBP, DBP, ~~pulse~~ **HR and MAP** ~~mean arterial blood pressure (MAP)~~ after Acute Challenge 1	• Change from pre-dose in SBP, DBP, ~~pulse~~ **HR** and MAP at each timepoint at Day 1
• To characterize the pharmacokinetics of daprodustat	• Plasma concentrations of daprodustat **and metabolites** and derived pharmacokinetic parameters including maximum observed concentration (Cmax), time of occurrence of Cmax (tmax), terminal phase half-life (t½) and area under concentration-time curve from time zero to 24 hours (AUC[0-24]) as appropriate
• To assess the safety and tolerability of daprodustat	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) • Reasons for discontinuation of study treatment • Absolute values and changes from baseline over time in laboratory parameters, electrocardiograms (ECGs) and vital signs
Exploratory	
• To investigate the effect of daprodustat on vasoactive mediators of blood pressure	• Plasma concentrations and derived parameters including Cmax, tmax, t½ and AUC(0-24) as appropriate (to include erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II **and metabolites**, and noradrenalin)
• Evaluate exposure-response relationships for daprodustat and: erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II **(and metabolites)**, and noradrenalin	• Further models (as appropriate) to describe the daprodustat exposure-response relationship with: erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II **(and metabolites)**, and noradrenalin.

Objectives	Endpoints
• To summarize the effect of daprodustat and epoetin alfa on SBP after Acute Challenge 1 and Acute Challenge 2	• AUEC of SBP as measured by ABPM over 24-hr post-dosing
• To compare the effect of daprodustat to epoetin alfa on SBP after 8 weeks of Hgb maintenance therapy	• Change from Day 1 pre-dose SBP to ~~Week 8~~ Day 57 pre-dose

Section 1 Protocol Synopsis for Study 205665

Study Design

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

Subjects will be screened for eligibility starting 4 weeks prior to the start of an ESA washout period (8 weeks prior to randomization/Day 1).

Subjects that qualify for enrolment will be washed out of their ESA for 4 weeks; the start of the ESA washout should be timed to coincide as closely as possible with their next planned ESA dose.

Following the 4-week ESA washout, subjects will be randomized 1:1 and stratified by prior ESA dose, based on the average weekly (for epoetins or darbepoetin) or monthly (for methoxy polyethylene glycol (PEG)-epoetin beta) dosing during the 12 weeks prior to ESA washout (Week -4) as follows:

- Low ESA dose: <100 IU/kg/week epoetin alfa OR <0.5 µg/kg/week darbepoetin OR <0.6 µg/kg/week methoxy PEG-epoetin beta
- High ESA dose: ≥100 IU/kg/week epoetin alfa OR ≥0.5 µg/kg/week darbepoetin OR ≥0.6 µg/kg/week methoxy PEG-epoetin beta

On Day 1, subjects will be randomized and undergo Acute Challenge 1, a single dose challenge to compare the acute effects on BP of the highest planned once-daily maintenance dose of daprodustat (24 mg) to the highest starting dose of epoetin alfa (100 IU/kg). Subjects will have BP monitored for 24 hr using ABPM, and will have serial blood sampling to assess the pharmacokinetics of daprodustat and the time-course of various biomarkers. Acute Challenge 1 will be started ~~immediately~~ **promptly** following completion of a mid-week dialysis session.

After completing Acute Challenge 1, all subjects will enter the 8-week Hgb maintenance period where doses of either daprodustat or epoetin alfa will be administered and adjusted, as needed.

On Day 57, following the 8-week Hgb maintenance period, subjects will undergo Acute Challenge 2; this challenge will, similar to Acute Challenge 1, involve assessing the effect of daprodustat and epoetin alfa on SBP as assessed by 24-hr ABPM measurement. The subjects will be administered the same dose of either daprodustat or epoetin alfa that was given in Acute Challenge 1, and serial blood sampling will be performed to assess

both daprodustat pharmacokinetics, and to characterize the time course of various biomarkers. Acute Challenge 2 will be started following completion of a mid-week dialysis session.

Subjects will be asked to not reinitiate anemia treatment until after the follow-up visit unless deemed medically necessary.

Subjects will attend a follow-up visit 2 weeks after completion of Acute Challenge 2.

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the QC criteria following Acute Challenge 2, one additional Acute Challenge may be made in a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed.

Section 1 Protocol Synopsis for Study 205665

Treatment Arms and Duration

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

The total duration of subject involvement is up to 18 weeks (Screening to Follow-up) as described below:

- Screening: Screening will be for at least 4 weeks prior to starting the ESA washout.

- ESA Washout: Subjects that meet eligibility criteria will begin the 4-week ESA washout at Week -4.

    - This should be timed such that this day occurs on the date that would have been the next scheduled dose of ESA (or as close as practicable).

    - For subjects with a three-times weekly dialysis schedule, this day must not occur on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday).

    - For subjects with a four- to five-times weekly dialysis schedule, this day can be on any hemodialysis session of the week.

- Randomization & Acute Challenge 1 (Day 1): Subjects will be randomized 1:1 to two treatment arms and will begin a 24-hr Acute Challenge where the acute BP effects of daprodustat and epoetin alfa will be evaluated. This challenge will be initiated ~~immediately~~ **promptly** following the subject's dialysis session.

- Hgb Maintenance Period: Subjects randomized to daprodustat will start an 8-week Hgb maintenance period based on a starting dose and dose adjustment algorithm. Subjects randomized to epoetin alfa will begin an 8-week Hgb maintenance period where Hgb will be managed in accordance with local labelling.

- Acute Challenge 2 (Day 57): At the end of the 8-week Hgb maintenance period subjects will repeat the procedures of Acute Challenge 1 utilizing the same treatment administered in Acute Challenge 1. This challenge will be initiated ~~immediately~~ **promptly** following the subject's dialysis session.

- Follow-up: A Follow-up visit will be scheduled to occur 14 ± 3 days after completing treatment.

Section 1 Protocol Synopsis
Analysis
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

The primary endpoint for this study is average systolic blood pressure (SBP) over 6 hours of measurements taken at 15-minute intervals after the administration of study treatment for the Acute Challenge 2. The primary comparison of interest is daprodustat versus epoetin alfa for this challenge.

The primary analysis of average SBP over 6 hr post-dose during Acute Challenge 2 will be an analysis of covariance (ANCOVA) with terms for treatment, and post-HD/pre-Acute Challenge 1 SBP, difference between post-HD/pre-Acute Challenge 2 SBP and post-HD/pre-Acute Challenge 1 SBP, and treatment by (difference in post-HD SBP between Acute Challenge 1 and 2) interaction. Note that the pre-challenge SBP may change over time between Acute Challenge 1 and Acute Challenge 2 as a result of the maintenance therapy, and thus the pre-challenge SBP at Acute Challenge 2 is not a true pre-treatment covariate. As a result, the interpretation of the effect of the Acute Challenge 2 may be confounded by the impact of treatment phase on the covariate. Therefore, if the interaction term in this model is significant at the 0.10 level, then the primary model will be ANCOVA with terms for treatment and post-HD/pre-Acute Challenge 1 SBP. The primary model will provide a point estimate and two-sided 95% CI for the treatment effect and a p-value for the superiority assessment. Superiority will be established if the p-value is <0.05.

The comparison of daprodustat versus epoetin alfa on 6 hr average SBP during Acute Challenge 1 is a secondary endpoint.

Average SBP over 6 hr post-dose in Acute Challenge 1 will be analyzed using ANCOVA with terms for treatment and post-HD/pre-Acute Challenge 1 SBP. Similar analyses will be performed for DBP, MAP, and ~~pulse~~ **HR**.

ANCOVA using the primary model will be used for the analysis of average DBP, MAP, and ~~pulse~~ **HR** over 6 hr post-dose in Acute Challenge 2 replacing the baseline SBP term with the analogous baseline measurement for DBP, MAP, and ~~pulse~~ **HR**, respectively.

AUECs of SBP, DBP, ~~and~~ MAP**, and HR** post Acute Challenge 2 and 1 will be analyzed using ANCOVA with terms for treatment with 95% CIs and p-values provided for the treatment effect.

Null Hypothesis: The difference between 24 mg daprodustat versus 100 IU/kg epoetin alfa on 6 hr average SBP under a background of treatment (i.e., during Acute Challenge 2) is zero.

Alternative Hypothesis: The difference between 24 mg daprodustat versus 100 IU/kg epoetin alfa on 6 hr average SBP under a background of treatment (i.e. during Acute Challenge 2) is not zero.

An interim analysis will be conducted after approximately 24 subjects have completed Acute Challenge 2.

If the mean change in SBP from pre-Acute Challenge 1 to the 6-hour mean following both Acute Challenge 1 and Acute Challenge 2 is less than 5 mmHg in the epoetin alfa group, then the study will be stopped. However, if a clinically meaningful difference between the two treatment groups in mean change in SBP from pre-Acute Challenge 1 to the 6-hour mean following Acute Challenge 1 (i.e., >5 mmHg) is observed, then the study will be continued in order to further characterize the effects of daprodustat on blood pressure.

Simulations show that under assumptions of a true mean change in SBP of 0 mmHg and SD of 18 mmHg for both treatment groups at Acute Challenge 1 and the epoetin alfa group at Acute Challenge 2, this stopping guideline will result in stopping the trial >99% of the time. Under the assumptions of a true mean change in SBP of 8 mmHg and SD of 18 mmHg for both treatment groups at Acute Challenge 1 and the epoetin alfa group at Acute Challenge 2, this rule will result in stopping the trial <1% of the time.

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.

Section 3 OBJECTIVE(S) AND ENDPOINT(S)

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

Objectives	Endpoints
Primary	
• To compare the effect of daprodustat to epoetin alfa on blood pressure (BP) after Acute Challenge 2 (8 weeks of hemoglobin [Hgb] maintenance therapy)	• Average of systolic blood pressure (SBP) as measured by ambulatory blood pressure monitoring (ABPM) over 6-hr post-dosing after 8 weeks of Hgb maintenance therapy
Secondary	
• To compare the initial effect of daprodustat to epoetin alfa on BP after Acute Challenge 1 (4 weeks of erythropoiesis-stimulating agent [ESA] washout)	• Average of SBP**, diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR)** as measured by ABPM over 6-hr post-dosing at Day 1 • ~~Average of diastolic blood pressure (DBP) as measured by ABPM over 6-hr post-dosing at Day 1~~ • Area under the effect curve (AUEC) of SBP, **DBP, MAP, and HR** as measured by ABPM over 24-hr post-dosing at Day 1
• To compare the effect of daprodustat to epoetin alfa on BP after Acute Challenge 2	• Average of DBP, **MAP, and HR** as measured by ABPM over 6 hr post-dosing at ~~Week 8~~ Day 57. • AUEC of SBP**, DBP, MAP, and HR** as measured by ABPM over 24-hr post-dosing at ~~Week 8~~ Day 57.
• To estimate the initial effect of daprodustat on SBP, DBP, ~~pulse~~ **HR and MAP** ~~mean arterial blood pressure (MAP)~~ after Acute Challenge 1	• Change from pre-dose in SBP, DBP, ~~pulse~~ **HR** and MAP at each timepoint at Day 1
• To characterize the pharmacokinetics of daprodustat	• Plasma concentrations of daprodustat **and metabolites** and derived pharmacokinetic parameters including maximum observed concentration (Cmax), time of occurrence of Cmax (tmax), terminal phase half-life (t½) and area under concentration-time curve from time zero to 24 hours (AUC[0-24]) as appropriate
• To assess the safety and tolerability of daprodustat	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) • Reasons for discontinuation of study treatment • Absolute values and changes from baseline over time in laboratory parameters, electrocardiograms (ECGs) and vital signs
Exploratory	
• To investigate the effect of daprodustat on vasoactive mediators of blood pressure	• Plasma concentrations and derived parameters including Cmax, tmax, t½ and AUC(0-24) as appropriate (to include erythropoietin, endothelin-1, nitric oxide,

Objectives	Endpoints
	asymmetric dimethylarginine, renin, angiotensin-II **and metabolites**, and noradrenalin)
• Evaluate exposure-response relationships for daprodustat and: erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II **(and metabolites)**, and noradrenalin	• Further models (as appropriate) to describe the daprodustat exposure-response relationship with: erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II **(and metabolites)**, and noradrenalin.
• To summarize the effect of daprodustat and epoetin alfa on SBP after Acute Challenge 1 and Acute Challenge 2	• AUEC of SBP as measured by ABPM over 24-hr post-dosing
• To compare the effect of daprodustat to epoetin alfa on SBP after 8 weeks of Hgb maintenance therapy	• Change from Day 1 pre-dose SBP to ~~Week 8~~ Day 57 pre-dose

Section 4.1 Overall Design
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

Subjects will be screened for eligibility starting 4 weeks prior to the start of an ESA washout period (8 weeks prior to randomization/Day 1).

Subjects that qualify for enrolment will be washed out of their ESA for 4 weeks; the start of the ESA washout should be timed to coincide as closely as possible with their next planned ESA dose.

Following the 4-week ESA washout, subjects will be randomized 1:1 and stratified by prior ESA dose, based on the average weekly (for epoetins or darbepoetin) or monthly (for methoxy polyethylene glycol (PEG)-epoetin beta) dosing during the 12 weeks prior to ESA washout (Week -4) as follows:

- Low ESA dose: <100 IU/kg/week epoetin alfa OR <0.5 µg/kg/week darbepoetin OR <0.6 µg/kg/week methoxy PEG-epoetin beta
- High ESA dose: ≥100 IU/kg/week epoetin alfa OR ≥0.5 µg/kg/week darbepoetin OR ≥0.6 µg/kg/week methoxy PEG-epoetin beta

On Day 1, subjects will be randomized and undergo Acute Challenge 1, a single dose challenge to compare the acute effects on BP of the highest planned once-daily maintenance dose of daprodustat (24 mg) to the highest starting dose of epoetin alfa (100 IU/kg). Subjects will have BP monitored for 24 hr using ABPM, and will have serial blood sampling to assess the pharmacokinetics of daprodustat and the time-course of various biomarkers. Acute Challenge 1 will be started ~~immediately~~ **promptly** following completion of a mid-week dialysis session.

After completing Acute Challenge 1, all subjects will enter the 8-week Hgb maintenance period where doses of either daprodustat or epoetin alfa will be administered and adjusted, as needed, based on dosing guidance as described in Section 6.4.

On Day 57, following the 8-week Hgb maintenance period, subjects will undergo Acute Challenge 2; this challenge will, similar to Acute Challenge 1, involve assessing the effect of daprodustat and epoetin alfa on SBP as assessed by 24-hr ABPM measurement. The subjects will be administered the same dose of either daprodustat or epoetin alfa that was given in Acute Challenge 1, and serial blood sampling will be performed to assess both daprodustat pharmacokinetics, and to characterize the time course of various biomarkers. Acute Challenge 2 will be started following completion of a mid-week dialysis session.

Subjects will be asked to not reinitiate anemia treatment until after the follow-up visit unless deemed medically necessary.

Subjects will attend a follow-up visit 2 weeks after completion of Acute Challenge 2.

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the QC criteria following Acute Challenge 2, one additional Acute Challenge may be made in a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed as detailed in Table 4.

Section 4.2 Treatment Arms and Duration

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

The total duration of subject involvement is up to 18 weeks (Screening to Follow-up) as described below:

- Screening: Screening will be for at least 4 weeks prior to starting the ESA washout.
- ESA Washout: Subjects that meet eligibility criteria will begin the 4-week ESA washout at Week 4.
    - This should be timed such that this day occurs on the date that would have been the next scheduled dose of ESA (or as close as practicable).
    - For subjects with a three-times weekly dialysis schedule, this day must not occur on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday).
    - For subjects with a four- to five-times weekly dialysis schedule, this day can be on any hemodialysis session of the week.

- Randomization & Acute Challenge 1 (Day 1): Subjects will be randomized 1:1 to two treatment arms as described in Table 1 and will begin a 24-hr Acute Challenge where the acute BP effects of daprodustat and epoetin alfa will be evaluated. This challenge will be initiated ~~immediately~~ **promptly** following the subject's dialysis session.

- Hgb Maintenance Period: Subjects randomized to daprodustat will start an 8-week Hgb maintenance period based on a starting dose and dose adjustment algorithm as described in Section 6.4.1. Subjects randomized to epoetin alfa will begin an 8-week Hgb maintenance period where Hgb will be managed in accordance with local labelling.

- Acute Challenge 2: At the end of the 8-week Hgb maintenance period, subjects will repeat the procedures of Acute Challenge 1 utilizing the same treatment administered in Acute Challenge 1. This challenge will be initiated ~~immediately~~ **promptly** following the subject's dialysis session.

- Follow-up: A Follow-up visit will be scheduled to occur 14 ± 3 days after completing treatment.

Section 4.4. Design Justification
REVISED TEXT, where text in strikethrough has been removed.

This study will be an open-label, multi-center, randomized, parallel-group design in HD subjects with anemia associated with CKD. There will be a 4-week screening period, a 4-week ESA washout period, an 8-week Hgb-maintenance period including two 24-hr acute challenges and a follow-up visit 14 ± 3 days after completing treatment.

It is preferred that no changes are made to estimated dry weight (EDW) and antihypertensive medications while the subject is participating in this study. ~~However, if changes in EDW and/or antihypertensive medications are necessary, these must be documented in the electronic case report form (eCRF) along with the reasons.~~ Subjects will remain in the study regardless of any changes.

(Note: No changes made to the bullet points within this section)

Section 6.7 Compliance with Study Treatment Administration
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

For subjects randomized **to daprodustat**, treatment ~~start and stop dates and~~ dosing details including dates for ~~randomized treatment interruptions and/or~~ dose increases/reductions, will be recorded in RAMOS NG.

For subjects randomized to daprodustat, for Acute Challenge 1 and 2, study site personnel will confirm compliance. When daprodustat is administered at home (Hgb maintenance period), subjects will be instructed to return all unused randomized treatment at each clinic visit. A record of the number of daprodustat tablets dispensed to and taken by each subject will be maintained and reconciled with randomized treatment and compliance records.

For subjects randomized to epoetin alfa, they will receive all study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study treatment and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment.

Section 6.10.1 Meals and Dietary Restrictions
REVISED TEXT, where bolded text has been added

Daprodustat can be taken without regard to meals.

All subjects will be instructed to limit caffeine- or xanthine- containing products (e.g., coffee, tea, cola drinks, chocolate) for 24h prior to both Acute Challenge 1 and Acute Challenge 2. Should the Additional Acute Challenge 2 be necessary, the subject does not need to limit the products listed above.

Section 6.11 Concomitant Medications and Non-Drug Therapies
REVISED TEXT, where text in strikethrough has been removed.

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~~, while additional details will be recorded for certain medications (e.g., anti-hypertensive medications).~~

Section 6.11.1 Estimated Dry Weight and Antihypertensive Medication Changes
REVISED TEXT, where text in strikethrough has been removed.

It is preferred that changes to EDW and antihypertensive medication(s) are not made while the subject is part of this study. ~~If changes in EDW and/or antihypertensive medications are necessary, these changes as well as the reason(s) for the change must be documented in the eCRF.~~ Subjects should remain in the study regardless of any changes.

Section 7.1 Time and Events Table
Table 3 Study Procedures and Assessments

REVISED TEXT, where text in strikethrough has been removed.

(Note: Changes are difficult to see on table. Superscript 6 has been removed from Adverse Events Assessment in columns Weeks – 4 and Week – 2)

Procedure[1]	Screening		ESA Washout		Treatment Period[2]					Follow-up[2]	
	Week -8	Week -6	Week -4	Week -2	Day 1	Day 15	Day 29	Day 43	Day 57	Early WD	Week 10
Informed Consent	X										
Entry Criteria	X										
Physical, Medical History, Demography	X										
HemoCue Hgb	X	X	X	X	X[4]	X	X	X	X[4]	X	
IVWRS					X		X				
Randomization					X						
Acute Challenge					X[4]				X[4]		
ABPM Assessment					X[4]				X[4]		
Dose Adjustment							X				
Females Only: Serum Pregnancy Test		X			X		X		X	X	X
Females Only: Estradiol & FSH (if required)[3]		X									
ECG	X				X[4]				X[4]	X	X
Vital signs & weight (Pre- & Post-dialysis)	X	X	X		X[4]		X		X[4]	X	X
Clinical Chemistry		X			X		X		X	X	X
Hematology		X			X		X		X	X	X
Folate and Vitamin B12	X										
Ferritin, transferrin, total iron,, TSAT, UIBC	X		X								
Pharmacokinetic/Biomarker Assessments					X[4]				X[4]		
Hgb Maintenance Period[5]					←==========				==========→		
Adverse Events Assessment	X[6]	X[6]	X[6]	X[6]	X[4]	X	X	X	X[4]	X	X
Review Concomitant Medications	X		X	X	X[4]	X	X	X	X[4]	X	X

1 All assessments should be done predialysis/predose except as noted.

2 Allowable time window ±2 days EXCEPT Follow-up Visit which is ±3 days.

3 As detailed in Inclusion Criteria.

4 Detailed timings for assessments on Acute Challenge Days are given in Table 4.

5 From the end of Acute Challenge 1 to the beginning of Acute Challenge 2.

6 Only SAEs assessed as related to study participation are collected at this visit. See Section 12.4 for additional details.

Table 4 Study Procedures and Assessments on Acute Challenge Days (Treatment Period Day 1 and Day 57)

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.
(Note: The box for ABPM Assessment in the Post-Dialysis column has been grayed out as well as the X removed)

Procedure	Pre-Dialysis	Post - Dialysis	Predose	Time (in hours relative to dosing)										
				0	0.5	1	2	3	4	6	8	12	16	24
HemoCue Hgb[1]			X											X
IVWRS			X											
ABPM Assessment[1, 3]		~~X~~	←==========→											
Administer Study Treatment[1]				X										
Serum Pregnancy Test[1]			X											
ECG[1]			X											X
Vital Signs and Weight[1]	X	X						X				X		X
Clinical Chemistry			X											
Hematology			X											
Pharmacokinetics[4]			X		X	X	X	X	X	X	X	X	X	X
Erythropoietin			X		X	X	X	X	X	X	X	X	X	X
Endothelin-1			X			X	X		X	X	X			X
Nitric Oxide			X			X	X		X	X	X			X
Asymmetric dimethylarginine			X			X	X		X	X	X			X
Renin			X			X	X		X	X	X			X
Angiotensin-II			X			X	X		X	X	X			X
Noradrenalin			X			X	X		X	X	X			X
Adverse Events Assessment[1]			X		X	X	X	X	X	X	X	X	X	X
Review Concomitant Medications[1]			X											X
Initiate Hgb maintenance dosing[2]														X

1 Procedures to be repeated if Acute Challenge 2 fails quality control criteria
2 Applies to Acute Challenge 1 only
3**. Timing of ABPM measurements does not correlate with specified time points in Table 4.**
4. PK to be drawn from daprodustat subjects only.

Section 7.3.1.1 Time Period and Frequency for Collecting AE and SAE information
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

- Any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact.
- AEs will be collected from the start of **washout (Week – 4)** ~~study treatment (Day 1)~~ until the follow-up contact (see Section 7.3.1.3), at the timepoints specified in the Time and Events Table (Section 7.1).
- Medical occurrences that begin prior to the start of **washout** ~~study treatment~~ but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF.
- All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Section 12.4.
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

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

Section 7.3.1.4 Adverse Events of Special Interest
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

The investigator or site staff will be responsible for detecting, documenting and reporting any AEs of special interest or any event that may potentially be one of the categories listed below (using preferred terms):

- Death, myocardial infarction (MI), stroke, **heart failure, thromboembolic events,** ~~venous thromboembolism,~~ thrombosis of vascular access
- Thrombosis and/or tissue ischemia secondary to excessive erythropoiesis
- Cardiomyopathy
- Pulmonary artery hypertension (see also Section 7.3.1.5)
- Cancer-related mortality and tumor progression and recurrence
- Esophageal and gastric erosions
- Proliferative retinopathy, macular edema, choroidal neovascularization

- Exacerbation of rheumatoid arthritis

The results of any investigation should be recorded in the relevant sections of the subject's eCRF.

Section 7.3.5 Electrocardiogram (ECG)
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

ECG measurements will be taken at the time points specified in the Time and Events Table (Section 7.1). Full 12-lead ECGs will be recorded with the subject in a supine position. Heart rate (HR), PR interval, QRS duration, and QT (uncorrected) interval will be measured. QTcB will be calculated (machine-read or manually).

At the Screening visit (~~Week –4~~ **Week -8**) two additional ECGs are required if the initial ECG indicates prolonged QTc (see Section 5.3) using the automated or manually calculated QTcB value. The average QTcB value of all three ECGs will be used to determine eligibility. Additional details are provided in the SRM.

ECG data will be read locally.

All ECGs will be performed before measurement of vital signs and collection of blood samples for laboratory testing.

Section 7.5.1 Ambulatory Blood Pressure Monitoring (ABPM)
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

Blood pressure and pulse will be measured during Acute Challenge 1 and 2 through the use of an ambulatory blood pressure monitoring device. Subjects will wear the ABPM device for two sessions during the study (i.e., Acute Challenge 1 and 2), occurring at Day 1 (randomization) and at Day 57. The ABPM device will be placed ~~1 hr prior to completion of the dialysis session~~ **prior to Acute Challenge dosing**, and will remain on the subject's arm until 24 hr**s** post-dosing.

ABPM measurements of systolic and diastolic blood pressure and ~~pulse~~ **heart rate** will be ~~manually recorded at one hour prior to completion of the dialysis session,~~ automatically recorded every 15 minutes for the first 6 hrs post-dosing, and then every 20 minutes thereafter until 24 hrs post-dosing. The ABPM device will automatically calculate MAP.

The device planned for use in this study is the Mortara Ambulo 2400; a specification sheet for this device can be found in Section 12.6.

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the

QC criteria following Acute Challenge 2, one additional Acute Challenge may be made in a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed as detailed in Table 4.

Section 7.5.2 Blood Pressure Regulation Biomarkers
REVISED TEXT, where bolded text has been added.

Blood samples will be collected during this study to investigate the mechanism of the effect of daprodustat on blood pressure. Biomarkers selected will explore the renin-angiotensin-aldosterone axis, the nitric oxide and endothelin axes, sodium retention and HIF signalling.

Biomarkers may include erythropoietin, nitric oxide (NO), asymmetric dimethyarginine (ADMA), renin, angiotensin-II **(1-8) and metabolites (1-7 and 1-5)**, endothelin-1 (ET-1), and noradrenalin. Samples will be collected as specified in the Time and Events Table (Section 7.1). The timing of the collections may be adjusted on the basis of emerging pharmacokinetic or pharmacodynamic (PD) data from this study or other new information in order to ensure optimal evaluation of the PD endpoints.

Section 9.4.2 Secondary Analyses
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

Average SBP over 6 hr post-dose in Acute Challenge 1 will be analyzed using ANCOVA with terms for treatment and post-HD/pre-Acute Challenge 1 SBP. Similar analyses will be performed for DBP, MAP, and ~~pulse~~ **HR**.

ANCOVA using the primary model will be used for the analysis of average DBP, MAP, and ~~pulse~~ **HR** over 6 hr post-dose in Acute Challenge 2 replacing the baseline SBP term with the analogous baseline measurement for DBP, MAP, and ~~pulse~~ **HR**, respectively.

AUECs of SBP, DBP, ~~and~~ MAP**, and HR** post Acute Challenge 2 and 1 will be analyzed using ANCOVA with terms for treatment with 95% CIs and p-values provided for the treatment effect.

Section 9.4.3 Other Analyses
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

The difference in SBP between pre-dose in Acute Challenge 1 and pre-dose in Acute Challenge 2 will be summarized by treatment group. Similar summaries of DBP**,** ~~and~~ MAP**, and HR** will be performed.

For each acute challenge, change from pre-challenge in SBP, DBP, MAP, and ~~pulse~~ **HR** will be summarized by treatment group at each timepoint. In addition, for each acute challenge, SBP, DBP, MAP, and ~~pulse~~ **HR** will be summarized by treatment group at each timepoint. Line graphs of this information will be provided by treatment group for each acute challenge.

For each acute challenge, the concentration of erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II **and metabolites**, and noradrenalin will be summarized by treatment group at each timepoint. Line graphs of this information will be provided by treatment group for each acute challenge.

Further statistical considerations will be addressed in the RAP.

Section 12.3 Appendix 3: Daprodustat Risk Assessment

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Excessive erythropoiesis (polycythemia) leading to thrombosis and/or tissue ischemia	In animal studies, excessive erythropoiesis attributed to daprodustat was associated with vascular congestion, microthrombi, and tissue ischemia in a number of organs. Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat. 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.	• Specific eligibility criteria related to requirements for entry Hgb are detailed in Section 5 • Hgb will be closely monitored throughout the dosing period as outlined in the Time and Events Table (Section 7.1) • Specific guidance for discontinuation of daprodustat based on achieved Hgb is provided in Section 5.5.1.1 • Monitoring of emerging safety data by an internal GSK Safety Review Team
Death, MI, stroke, **heart failure** ~~congestive HF~~, **thromboembolic events** ~~venous thromboembolism~~, thrombosis of vascular access at Hgb levels which are within the normal range (i.e. not polycythemic conditions)	Marketed rhEPO/ESAs have been associated with an increased risk for death and serious cardiovascular events when used in patients with anemia of CKD. **Not observed at tolerated doses when hemoglobin/hematocrit within normal range for species.** **The clinical data received to date are insufficient to conclude or refute this risk.** ~~Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat.~~	• Specific eligibility criteria related to CV risk are outlined in Section 5.3 • Hgb will be closely monitored throughout the dosing period as outlined in the Time and Events Table Section 7.1 • Monitoring of emerging safety data by an internal GSK Safety Review Team
Esophageal and gastric erosions	In animal studies, undesirable GI effects including emesis, abnormal feces and/or decreased food consumption/body	• Suspected GI bleeding or significant symptoms consistent with erosion should

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	weight loss and stomach erosions/ ulcers with hemorrhage were observed with daprodustat. In ~~rodents~~ **rats** stomach erosions were observed with intravenous and oral administration of daprodustat. **Stomach erosions/ulcers also reported in rats with marketed rhEPOs/ESAs.** ~~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. Following review of clinical data received to date, GI erosions have not been identified as a safety concern for daprodustat.	be investigated diagnostically (i.e. endoscopic examination) as clinically warranted • Monitoring of emerging safety data by an internal GSK Safety Review Team
Cancer-related mortality and tumor progression and recurrence	Marketed reEPOs 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 VEGF while significant EPO increases were observed. **There were no daprodustat-related neoplastic findings in a 2-year rat oral carcinogenicity study (lifetime study).** **In clinical studies conducted to date, administration of daprodustat has been associated with:** **<u>Once daily administration:</u>**	• Specific eligibility criteria related to personal history of malignancy or subjects with complex kidney cyst are outlined in Section 5.3. • Stopping criteria for subjects with treatment emergent malignancy are outlined in Section 5.5.1. • 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
	• **In studies up to 4 weeks duration, a dose-ordered increase in VEGF plasma concentrations at doses ranging from 10 to 150 mg.** • **In studies up to 24 weeks duration at doses up to 25mg, changes in VEGF plasma concentration were variable but similar relative to control.** • **Systemic EPO concentrations within the physiologic range.** **<u>Three times weekly administration:</u>** • **In studies up to 4 weeks duration at doses of 10 to 30 mg:** ○ **Dose dependent increases in plasma VEGF and EPO concentrations were observed.** ○ **Pre-dose concentrations of EPO and VEGF were near or below baseline indicating no accumulation of EPO or VEGF after three times weekly dosing.** ~~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.~~ Following review of clinical data received to date, this has not been identified 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 (up to 13-weeks duration in mice and dog, up to 26-weeks in rat, 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. These hypoxia-induced PRVP changes fall within the range of PRVP differences noted among non-treated 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 has no clinically significant effect on echocardiographically estimated systolic pulmonary artery pressure (sPAP) 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 sPAP in subjects not on dialysis. In hemodialysis subjects, mean absolute change from baseline in sPAP was similar for both treatment groups; however, there was a numeric imbalance (Daprodustat: 8 [7%]; Control 0) in subjects reaching the sPAP 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	• 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
	inconsistency in timing of ECHOs relative to dialysis day. Additionally, 2 of 3 subjects with resolution of sPAP on safety follow-up ECHOs had confounding conditions that could contribute to resolution other than discontinuation of study treatment; and there was no dose relationship for subjects meeting the sPAP PCI criterion. Overall, there is insufficient evidence to conclude a relationship to treatment with daprodustat. 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. **With lifetime exposure to daprodustat in a 2-year rat oral carcinogenicity study, an exacerbation of rat spontaneous, progressive cardiomyopathy (PCM)(focal myofiber degeneration/necrosis with inflammatory infiltrates) was observed at doses of 0.8 mg/kg/day and above, although total incidence and severity distribution within any daprodustat-group were within historical control ranges. This is consistent with an equivocal threshold for exacerbation of spontaneous, progressive cardiomyopathy at 0.8 mg/kg/day which is also the threshold dose for observing increased Hct values in individual rats.** ~~Small increases in cardiac troponin in 6 month rat study were consistent with the background finding of spontaneous rodent cardiomyopathy. There were no elevations observed in cardiac~~	• 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
	~~troponin in 9 month monkey study with daprodustat.~~ 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 LVEF for daprodustat. 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 Administration of 60 mg/kg daprodustat to mice caused minimal increases in circulating VEGF while significant EPO increases were observed. **Aside from congestion of retinal vessels and optic disc hyperemia secondary to markedly increased red cell mass, there were no ocular abnormalities observed in non-clinical studies.** ~~No ocular abnormalities 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 up to 4 weeks duration, a dose-ordered increase in VEGF plasma concentrations was observed at doses ranging from 10 to 150 mg **administered once daily and from 10 to 30mg administered three times weekly**. In studies	~~• Suspected proliferative retinopathy, macular edema, choroidal neovascularization or symptoms consistent with these events should be investigated by ophthalmologic consultation as clinically warranted.~~ • 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
	up to 24 weeks duration at doses up to 25 mg, 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 with daprodustat. 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 seen in non-clinical studies conducted to date for daprodustat. Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat.	• Monitoring of emerging safety data by an internal GSK Safety Review Team
Drug-drug interactions	Co-administration of daprodustat with a strong CYP2C8 inhibitor increased the Cmax and AUC of daprodustat, 4- and 19-fold, respectively, while co-administration of a weak inhibitor increased the Cmax and AUC of daprodustat by 1.3- and 1.5-fold, respectively. **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.**	• Co-administration of daprodustat with strong CYP2C8 inhibitors (e.g., gemfibrozil) and inducers (e.g., rifampin/rifampicin) is not permitted as outlined in Section 6.11.3. • Co-administration of daprodustat with moderate CYP2C8 inhibitors (i.e., clopidogrel, teriflunomide, deferasirox)

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	Although 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 approximately 10-14 days of dosing with rifampin (Brodie et al, 2013 and Ohnhaus et al, 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.** Population PK analysis from completed Phase 2 studies suggests that co-administration of daprodustat with a moderate CYP2C8 inhibitor, leads to a ~ 2-fold increase in AUC, with no clinically-significant increase in the measured Hgb response. Daprodustat is an inhibitor of CYP2C8 *in vitro,* with an IC_{50} value of 21 µM. Population PK analysis from completed Phase 2 studies suggests that co-administration of daprodustat with clopidogrel (a moderate CYP2C8 inhibitor) leads to a ~ 2-fold increase in AUC, with no clinically-significant increase in the measured Hgb response Co-administration of daprodustat with potent BCRP inhibitors	should be performed with caution.Specific guidance on the management of potential drug-drug interactions and concomitant medications is provided in Section 6.11. • Hgb will be closely monitored throughout the dosing period as outlined in the Time and Events Table (Section 7.1) • Specific guidance for discontinuation of daprodustat based on achieved Hgb is provided in Section 5.5.1.1. • 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
	has the potential to increase exposure of daprodustat. Use of BCRP inhibitors (mostly weak) was found to result in a small change in metabolite exposure (20% increase in AUC). Daprodustat is an inhibitor of OATP1B1/1B3 *in vitro,* with IC_{50} values of 6 µM and 11 µM, respectively. A clinical drug interaction study between 25 mg daprodustat with either a CYP2C8 substrate or an OATP1B1/1B3 substrate showed that there is no PK interaction at this dose of daprodustat.	
Other		
rhEPO risks (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** ~~venous thromboembolism~~, thrombosis of vascular access, and for 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; death, MI, stroke, **heart failure, thromboembolic events** ~~venous thromboembolism~~, thrombosis of vascular access; and for cancer-related mortality and tumor progression. • Specific eligibility criteria related to blood pressure management are outlined in Section 5. • Specific eligibility criteria related to personal history of pure red cell aplasia are outlined in Section 5.3

12.7.3. Amendment 3

Summary of Amendment Changes with Rationale

This protocol amendment applies to all centers where this study may be performed.

This protocol amendment was written to streamline recruitment of subjects into the study and maintain appropriate Hgb levels while in the study. This includes removing study visits, altering entry requirements as well as stopping criteria, changing dosing and dose adjustments, and restructuring Hgb parameters.

List of Specific Changes

Title Page
A Short Title has been added:

Short Title: **A**nemia **S**tudies in **C**KD: **E**rythropoiesis via a **N**ovel PHI **D**aprodustat – **B**lood **P**ressure (**ASCEND-BP**)

Medical Monitor Page
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

Role	Name	Day Time Phone Number and email address	After-hours (Cell) Number	Fax Number
Primary Medical Monitor	PPD , MD, **PhD**	Tel: PPD	PPD	PPD
Secondary Medical Monitor	PPD ~~MD, PhD~~ **, MD, FACC**	Tel: PPD	PPD	PPD
Tertiary Medical Monitor	PPD MD	Tel: PPD	PPD	PPD
Site and SAE Contact Address:	GlaxoSmithKline 1250 S. Collegeville Road, UP4400 Collegeville, PA 19426			

Regulatory Agency Identifying Number(s): IND Number: 101,29**1**

Section 1 Protocol Synopsis for Study 205665
Objective(s)/Endpoint(s) Table
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added. (Within secondary endpoints, 4 weeks changed to 2 weeks)

Objectives	Endpoints
Primary	
• To compare the effect of daprodustat to epoetin alfa on blood pressure (BP) after Acute Challenge 2 (8 weeks of hemoglobin [Hgb] maintenance therapy)	• Average of systolic blood pressure (SBP) as measured by ambulatory blood pressure monitoring (ABPM) over 6-hr post-dosing after 8 weeks of Hgb maintenance therapy
Secondary	
• To compare the initial effect of daprodustat to epoetin alfa on BP after Acute Challenge 1 (4-**2** weeks of erythropoiesis-stimulating agent [ESA] washout)	• Average of SBP, diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR) as measured by ABPM over 6-hr post-dosing at Day 1 • Area under the effect curve (AUEC) of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 1
• To compare the effect of daprodustat to epoetin alfa on BP after Acute Challenge 2	• Average of DBP, MAP, and HR as measured by ABPM over 6 hr post-dosing at Day 57. • AUEC of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 57.
• To estimate the initial effect of daprodustat on SBP, DBP, HR and MAP after Acute Challenge 1	• Change from pre-dose in SBP, DBP, HR, and MAP at each timepoint at Day 1
• To characterize the pharmacokinetics of daprodustat	• Plasma concentrations of daprodustat and metabolites and derived pharmacokinetic parameters including maximum observed concentration (Cmax), time of occurrence of Cmax (tmax), terminal phase half-life (t½) and area under concentration-time curve from time zero to 24 hours (AUC[0-24]) as appropriate
• To assess the safety and tolerability of daprodustat	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) • Reasons for discontinuation of study treatment • Absolute values and changes from baseline over time in laboratory parameters, electrocardiograms (ECGs) and vital signs
Exploratory	
• To investigate the effect of daprodustat on vasoactive mediators of blood pressure	• Plasma concentrations and derived parameters including Cmax, tmax, t½ and AUC(0-24) as appropriate (to include erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II and metabolites, and noradrenalin)
• Evaluate exposure-response relationships for daprodustat and: erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin,	• Further models (as appropriate) to describe the daprodustat exposure-response relationship with: erythropoietin, endothelin-1, nitric oxide,

Objectives	Endpoints
angiotensin-II (and metabolites), and noradrenalin	asymmetric dimethylarginine, renin, angiotensin-II (and metabolites), and noradrenalin.
• To summarize the effect of daprodustat and epoetin alfa on SBP after Acute Challenge 1 and Acute Challenge 2	• AUEC of SBP as measured by ABPM over 24-hr post-dosing
• To compare the effect of daprodustat to epoetin alfa on SBP after 8 weeks of Hgb maintenance therapy	• Change from Day 1 pre-dose SBP to Day 57 pre-dose

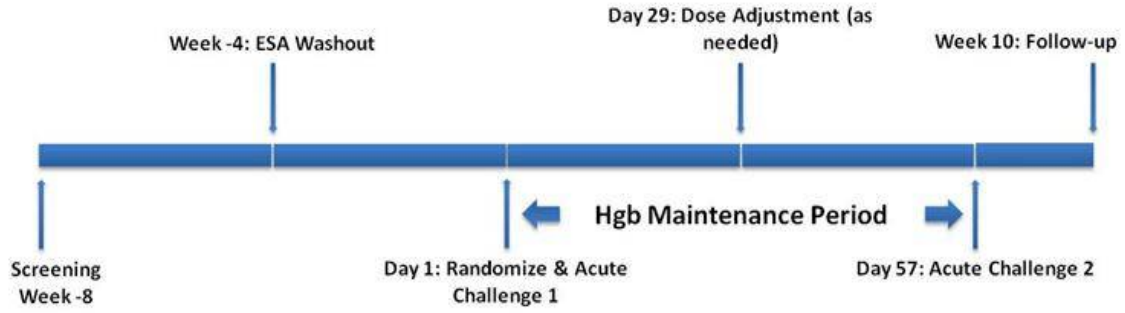
Section 1 Overall Design

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

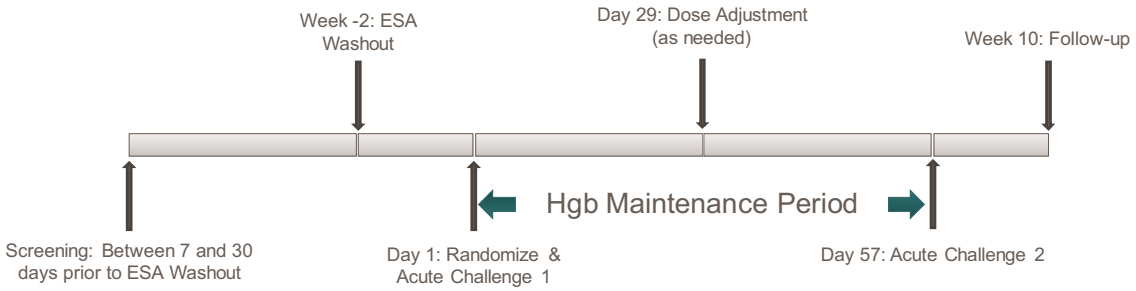
This study will be an open-label, randomized, parallel-group design in HD subjects with anemia associated with CKD. There will be a ~~4-week~~ screening period, a **4 2**-week ESA washout period, **an initial 24-hour acute challenge**, an 8-week Hgb-maintenance period, ~~two~~ **a second** 24-hr acute challenge~~s~~ and a follow-up visit 14±3 days after completing treatment. The study design is outlined below:

Section 1 Study Design

PREVIOUS IMAGE



REVISED IMAGE



REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

Subjects will be screened for eligibility ~~starting 4 weeks~~ **within 7 - 30 days** prior to the start of ~~an~~ **the** ESA washout period ~~(8 weeks prior to randomization/Day 1)~~.

Subjects that qualify for enrolment will be washed out of their ESA for 4 **2** weeks; the start of the ESA washout should be timed to coincide as closely as possible with their next planned ESA dose. **Subjects are enrolled once they enter the ESA washout period.**

Following the 4 **2**-week ESA washout, subjects will be randomized 1:1 and stratified by prior ESA dose, based on the average weekly (for epoetins or darbepoetin) or monthly (for methoxy polyethylene glycol (PEG)-epoetin beta) dosing during the 12 weeks prior to ESA washout ~~(Week -4)~~ as follows:

- Low ESA dose: <100 IU/kg/week epoetin alfa OR <0.5 µg/kg/week darbepoetin OR <0.6 µg/kg/week methoxy PEG-epoetin beta
- High ESA dose: ≥100 IU/kg/week epoetin alfa OR ≥0.5 µg/kg/week darbepoetin OR ≥0.6 µg/kg/week methoxy PEG-epoetin beta

On Day 1, subjects will be randomized and undergo Acute Challenge 1, a single dose challenge to compare the acute effects on BP of the highest planned once-daily maintenance dose of daprodustat (24 mg) to the highest starting dose of epoetin alfa (100 IU/kg). Subjects will have BP monitored for 24 hr using ABPM, and will have serial blood sampling to assess the pharmacokinetics of daprodustat and the time-course of various biomarkers. Acute Challenge 1 will be started promptly following completion of a mid-week dialysis session.

After completing Acute Challenge 1, all subjects will enter the 8-week Hgb maintenance period where doses of either daprodustat or epoetin alfa will be administered and adjusted, as needed.

On Day 57, following the 8-week Hgb maintenance period, subjects will undergo Acute Challenge 2; this challenge will, similar to Acute Challenge 1, involve assessing the effect of daprodustat and epoetin alfa on ~~SBP~~ **BP** as assessed by 24-hr ABPM measurement. The subjects will be administered the same dose of either daprodustat or epoetin alfa that was given in Acute Challenge 1, and serial blood sampling will be performed to assess ~~both~~ daprodustat pharmacokinetics~~,~~ and to characterize the time course of various biomarkers. Acute Challenge 2 will be started following completion of a mid-week dialysis session.

~~Subjects will be asked to not reinitiate anemia treatment until after the follow-up visit unless deemed medically necessary.~~

Subjects will attend a follow-up visit 2 weeks after completion of Acute Challenge 2. **Subjects will be asked to not reinitiate anemia treatment until after the follow-up visit unless deemed medically necessary**

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the QC criteria following Acute Challenge 2, one additional Acute Challenge may be made ~~in~~ **after** a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed.

The Acute Challenge 2 may be delayed one week if the subject's Hgb ≥11.5.

Section 1 Treatment Arms and Duration

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

The total **maximum** duration of subject involvement is up to ~~18~~ **1**6 weeks (Screening to Follow-up) as described below:

- Screening: Screening will **occur at least one week prior to but not more than 30 days prior to washout.** ~~Be for at least 4 weeks prior to starting the ESA washout~~

- ESA Washout: Subjects that meet eligibility criteria will begin the **4 2**-week ESA washout ~~at Week -4~~.

    - This should be timed such that this day occurs on the date that would have been the next scheduled dose of ESA (or as close as practicable).

    - For subjects with a three-times weekly dialysis schedule, this day must not occur on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday).

    - For subjects with a four- to five-times weekly dialysis schedule, this day can be on any hemodialysis session of the week.

- Randomization & Acute Challenge 1 (Day 1): Subjects will be randomized 1:1 to two treatment arms and will begin a 24-hr Acute Challenge where the acute BP effects of daprodustat and epoetin alfa will be evaluated. This challenge will be initiated promptly following the subject's dialysis session.

- Hgb Maintenance Period: Subjects randomized to daprodustat will start an 8-week Hgb maintenance period based on a starting dose and dose adjustment algorithm. ~~(See Section 6.4)~~ Subjects randomized to epoetin alfa will begin an 8-week Hgb maintenance period where Hgb will be managed in accordance with local labelling.

- Acute Challenge 2 (Day 57): At the end of the 8-week Hgb maintenance period subjects will repeat the procedures of Acute Challenge 1 utilizing the same

treatment **dose** administered in Acute Challenge 1. This challenge will be initiated promptly following the subject's dialysis session.

- Follow-up: A Follow-up visit will be scheduled to occur 14 ± 3 days after completing treatment.

Section 1 Type and Number of Subjects
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

- The study will enroll hemodialysis-dependent subjects with anemia associated with CKD currently treated with an ESA. Subjects with Hgb values of ~~9.0~~ **8.5** - 11.5 g/dL, inclusive **at both Screening and Day 1**, are eligible, and the subjects must **currently be on an** ~~have received the same~~ ESA product. ~~with total weekly doses that varied by no more than 50% during the 4 weeks prior to the Screening visit.~~
- Approximately 124 subjects will be screened to achieve approximately 62 randomized and 50 evaluable subjects for a total of 25 evaluable subjects per treatment group.
- If subjects prematurely discontinue the study, additional replacement subjects may be randomized and assigned to the same treatment arm at the discretion of the Sponsor in consultation with the investigator

Section 1 Analysis
This section was bulleted for clarity only. No text was changed.

Section 2 Introduction
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

After the bulleted section:
Hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors (PHIs) are an emerging new class of agents under investigation for the treatment of anemia associated with CKD. These molecules stimulate erythropoiesis through inhibition of HIF-prolyl hydroxylase domain 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, ~~the~~ PHD enzymes are inhibited, resulting in the accumulation of unhydroxylated HIFα subunits, which dimerize with HIFβ subunits to ~~effect~~ **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].

Daprodustat (GSK1278863) is a small molecule, oral inhibiter of the HIF-PHD enzymes which may present several important advantages over other ESAs. It is an oral medication and does not require cold-chain storage as do some ESAs, thus increasing ease of use for patients. Moreover, data indicate that daprodustat can effectively raise

Hgb concentrations with lower EPO levels than those observed after administration of ESAs [Provenzano, 2011]. Because of the increased CV risk associated with raising Hgb concentrations through large increases in EPO levels [Pfeffer, 2009], daprodustat has the potential to raise Hgb with less CV risk than other ESAs. CCI [redacted]
CCI [redacted]
CCI [redacted]
CCI [redacted].

Section 2.1 Study Rationale
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

Current labelling for all ESAs includes a contraindication in patients with uncontrolled hypertension, and increases in blood pressure (BP) and/or antihypertensive medication use has been observed in several clinical trials with ESAs. While the mechanism for this hypertensive effect is not known, hypertension has been consistently observed in approximately 25 to 30% of CKD patients initiating ESA therapy, and varies between an average of approximately 5 to 8 mmHg in systolic blood pressure (SBP), and 4 to 6 mmHg in diastolic blood pressure (DBP) reviewed in [Krapf, 2009]. Additionally, increases in BP are a well-recognized risk factor for CV events, in particular heart failure, ischemic stroke and myocardial infarction [Rodriguez, 2014]. Finally, treatment with ESAs has been associated with increased risk of major CV events including myocardial infarction, stroke and death.

Daprodustat has been shown to effectively raise and manage Hgb levels in subjects with CKD with lower levels of EPO than the ESAs. As the hypertensive effects of ESAs appear dose-related [Abraham, 1991], there is the potential that daprodustat can treat anemia in CKD patients without the hypertensive effects observed with the ESAs. This study will use two 24-hr Acute Challenges to compare the effect of daprodustat to rhEPO on BP as measured by ambulatory blood pressure monitoring (ABPM) following both a 4 **2**-week ESA washout period (Acute Challenge 1) and an 8-week Hgb-maintenance period (Acute Challenge 2).

~~The~~ **Therefore, the** purpose of this study is to compare the effects on blood pressure of daprodustat to epoetin alfa in hemodialysis-dependent (HD) subjects with anemia associated with CKD. The study will also assess various biomarkers associated with blood pressure physiology that may provide insight into the mechanism(s) of the hypertensive effects associated with ESAs.

Section 2.2 Brief Background
A reference was added here. (Holdstock 2016)

Section 3 Objectives and Endpoints
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added. (Within secondary endpoints, 4 weeks changed to 2 weeks)

Objectives	Endpoints
Primary	
• To compare the effect of daprodustat to epoetin alfa on blood pressure (BP) after Acute Challenge 2 (8 weeks of hemoglobin [Hgb] maintenance therapy)	• Average of systolic blood pressure (SBP) as measured by ambulatory blood pressure monitoring (ABPM) over 6-hr post-dosing after 8 weeks of Hgb maintenance therapy
Secondary	
• To compare the initial effect of daprodustat to epoetin alfa on BP after Acute Challenge 1 (4-**2** weeks of erythropoiesis-stimulating agent [ESA] washout)	• Average of SBP, diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR) as measured by ABPM over 6-hr post-dosing at Day 1 • Area under the effect curve (AUEC) of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 1
• To compare the effect of daprodustat to epoetin alfa on BP after Acute Challenge 2	• Average of DBP, MAP, and HR as measured by ABPM over 6 hr post-dosing at Day 57. • AUEC of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 57.
• To estimate the initial effect of daprodustat on SBP, DBP, HR and MAP after Acute Challenge 1	• Change from pre-dose in SBP, DBP, HR, and MAP at each timepoint at Day 1
• To characterize the pharmacokinetics of daprodustat	• Plasma concentrations of daprodustat and metabolites and derived pharmacokinetic parameters including maximum observed concentration (Cmax), time of occurrence of Cmax (tmax), terminal phase half-life (t½) and area under concentration-time curve from time zero to 24 hours (AUC[0-24]) as appropriate
• To assess the safety and tolerability of daprodustat	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) • Reasons for discontinuation of study treatment • Absolute values and changes from baseline over time in laboratory parameters, electrocardiograms (ECGs) and vital signs
Exploratory	
• To investigate the effect of daprodustat on vasoactive mediators of blood pressure	• Plasma concentrations and derived parameters including Cmax, tmax, t½ and AUC(0-24) as appropriate (to include erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II and metabolites, and noradrenalin)
• Evaluate exposure-response relationships for daprodustat and: erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin,	• Further models (as appropriate) to describe the daprodustat exposure-response relationship with: erythropoietin, endothelin-1, nitric oxide,

Objectives	Endpoints
angiotensin-II (and metabolites), and noradrenalin	asymmetric dimethylarginine, renin, angiotensin-II (and metabolites), and noradrenalin.
• To summarize the effect of daprodustat and epoetin alfa on SBP after Acute Challenge 1 and Acute Challenge 2	• AUEC of SBP as measured by ABPM over 24-hr post-dosing
• To compare the effect of daprodustat to epoetin alfa on SBP after 8 weeks of Hgb maintenance therapy	• Change from Day 1 pre-dose SBP to Day 57 pre-dose

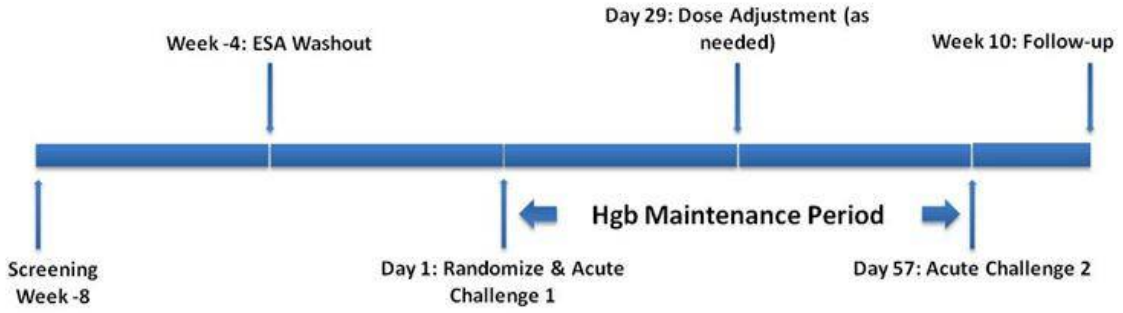
Section 4.1 Overall Design

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

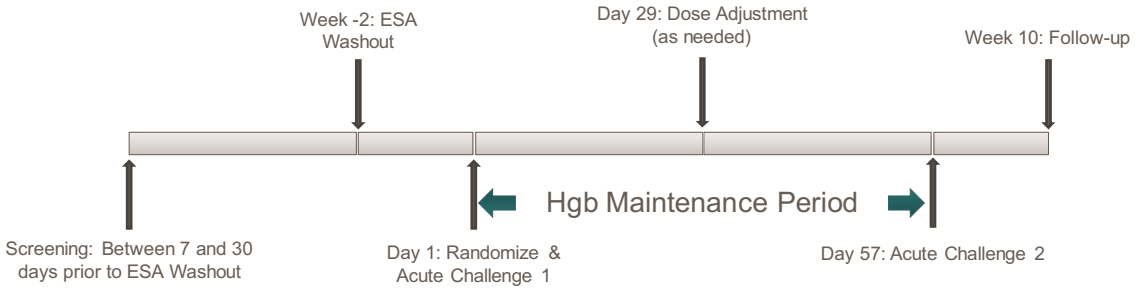
This study will be an open-label, randomized, parallel-group design in HD subjects with anemia associated with CKD. There will be a ~~4-week~~ screening period, a 4 **2**-week ESA washout period, **an initial 24-hour Acute Challenge**, an 8-week Hgb-maintenance period, ~~two~~ **a second** 24-hr Acute Challenge~~s~~ and a follow-up visit 14±3 days after completing treatment. The study design is outlined in Figure 1.

Figure 1

PREVIOUS IMAGE



REVISED IMAGE



Subjects will be screened for eligibility ~~starting 4 weeks~~ **within 7 - 30 days** prior to the start of ~~an~~ **the** ESA washout period ~~(8 weeks prior to randomization/Day 1)~~.

Subjects that qualify for enrolment will be washed out of their ESA for ~~4~~ **2** weeks; the start of the ESA washout should be timed to coincide as closely as possible with their next planned ESA dose. **Subjects are enrolled once they enter the ESA washout period.**

Following the ~~4~~ **2**-week ESA washout, subjects will be randomized 1:1 and stratified by prior ESA dose, based on the average weekly (for epoetins or darbepoetin) or monthly (for methoxy polyethylene glycol (PEG)-epoetin beta) dosing during the 12 weeks prior to ESA washout ~~(Week -4)~~ as follows:

- Low ESA dose: <100 IU/kg/week epoetin alfa OR <0.5 µg/kg/week darbepoetin OR <0.6 µg/kg/week methoxy PEG-epoetin beta
- High ESA dose: ≥100 IU/kg/week epoetin alfa OR ≥0.5 µg/kg/week darbepoetin OR ≥0.6 µg/kg/week methoxy PEG-epoetin beta

On Day 1, subjects will be randomized and undergo Acute Challenge 1, a single dose challenge to compare the acute effects on BP of the highest planned once-daily maintenance dose of daprodustat (24 mg) to the highest starting dose of epoetin alfa (100 IU/kg). Subjects will have BP monitored for 24 hr using ABPM, and will have serial blood sampling to assess the pharmacokinetics of daprodustat and the time-course of various biomarkers. Acute Challenge 1 will be started promptly following completion of a mid-week dialysis session.

After completing Acute Challenge 1, all subjects will enter the 8-week Hgb maintenance period where doses of either daprodustat or epoetin alfa will be administered and adjusted, as needed.

On Day 57, following the 8-week Hgb maintenance period, subjects will undergo Acute Challenge 2; this challenge will, similar to Acute Challenge 1, involve assessing the effect of daprodustat and epoetin alfa on ~~SBP~~ **BP** as assessed by 24-hr ABPM measurement. The subjects will be administered the same dose of either daprodustat or epoetin alfa that was given in Acute Challenge 1, and serial blood sampling will be performed to assess ~~both~~ daprodustat pharmacokinetics~~,~~ and to characterize the time course of various biomarkers. Acute Challenge 2 will be started following completion of a mid-week dialysis session.

~~Subjects will be asked to not reinitiate anemia treatment until after the follow-up visit unless deemed medically necessary.~~

Subjects will attend a follow-up visit 2 weeks after completion of Acute Challenge 2. **Subjects will be asked to not reinitiate anemia treatment until after the follow-up visit unless deemed medically necessary**

After both Acute Challenge 1 and 2, the 24-hr ABPM results will be checked for data quality as defined in the Project Requirement Specification (PRS). If the ABPM fails the QC criteria following Acute Challenge 2, one additional Acute Challenge may be made ~~in~~ **after** a subsequent, mid-week dialysis visit 1 week later if the subject agrees. At that

visit, the subject will receive the same study treatment for the repeat Acute Challenge 2 as was administered on Day 57 as a single additional dose. No additional clinical chemistry, hematology, pharmacokinetic or biomarker sampling will be done, however all other procedures will be performed as detailed in Table 4.

The Acute Challenge 2 may be delayed one week if the subject's Hgb ≥11.5. See Section 7.5.1.1 for guidance.

Section 4.2 Treatment Arms and Duration

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

The total duration of subject involvement is up to ~~18~~ **1**6 weeks (Screening to Follow-up) as described below:

- Screening: Screening will **occur at least one week prior to but not more than 30 days prior to washout.** ~~Be for at least 4 weeks prior to starting the ESA washout~~

- ESA Washout: Subjects that meet eligibility criteria will begin the **4 2**-week ESA washout ~~at Week -4~~.

    - This should be timed such that this day occurs on the date that would have been the next scheduled dose of ESA (or as close as practicable).

    - For subjects with a three-times weekly dialysis schedule, this day must not occur on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday).

    - For subjects with a four- to five-times weekly dialysis schedule, this day can be on any hemodialysis session of the week.

- Randomization & Acute Challenge 1 (Day 1): Subjects will be randomized 1:1 to two treatment arms and will begin a 24-hr Acute Challenge where the acute BP effects of daprodustat and epoetin alfa will be evaluated. This challenge will be initiated promptly following the subject's dialysis session.

- Hgb Maintenance Period: Subjects randomized to daprodustat will start an 8-week Hgb maintenance period based on a starting dose and dose adjustment algorithm. (See Section 6.4) Subjects randomized to epoetin alfa will begin an 8-week Hgb maintenance period where Hgb will be managed in accordance with local labelling.

- Acute Challenge 2 (Day 57): At the end of the 8-week Hgb maintenance period subjects will repeat the procedures of Acute Challenge 1 utilizing the same treatment **dose** administered in Acute Challenge 1. This challenge will be initiated promptly following the subject's dialysis session.

- Follow-up: A Follow-up visit will be scheduled to occur 14 ± 3 days after completing treatment.

Section 4.3 Type and Number of Subjects
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

The study will enroll hemodialysis-dependent subjects with anemia associated with CKD currently treated with an ESA. Subjects with Hgb values of ~~9.0~~ **8.5** to 11.5 g/dL, inclusive **at both Screening and Day 1**, are eligible, and the subjects must ~~have received the~~ **currently be on an** ~~same~~ ESA product. ~~with total weekly doses that varied by no more than 50% during the 4 weeks prior to the Screening visit.~~

Approximately 124 subjects will be screened to achieve approximately 62 randomized and 50 evaluable subjects for a total of 25 evaluable subjects per treatment group.

If subjects prematurely discontinue the study, additional replacement subjects may be randomized and assigned to the same treatment arm at the discretion of the Sponsor in consultation with the investigator.

Section 4.4 Design Justification
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added. Note: no change was made to Figure 2 at the end of this section.

This study will be an open-label, multi-center, randomized, parallel-group design in HD subjects with anemia associated with CKD. There will be a ~~4-week~~ screening period, a ~~4~~ **2**-week ESA washout period, an 8-week Hgb-maintenance period including two 24-hr acute challenges and a follow-up visit 14 ± 3 days after completing treatment.

~~It is preferred that no changes are made to estimated dry weight (EDW) and antihypertensive medications while the subject is participating in this study. Subjects will remain in the study regardless of any changes.~~

- This will be an open-label study since the primary endpoint (SBP by ABPM) is an objective measure and unlikely to be influenced by knowledge of the treatment received. Additionally, an open label design will facilitate the conduct of the interim analysis. **Lastly, blinding of an injectable comparator and oral study drug is impractical in this case.**

- A ~~4-week~~ screening period will be used to assess Hgb stability in order to minimize the potential for enrolling patients with highly variable Hgb despite ESA treatment.

- ~~A 4-week ESA washout will be used as this is the timeframe which was used in a published study which demonstrated an acute increase in MAP following IV administration of epoetin alfa as measured using ABPM over a 24-hr period starting from the end of the dialysis session following a single dose of epoetin alfa(both subcutaneous and intravenous) [Kang, 1998]. The results of the~~

~~published MAP assessments are demonstrated in Figure 2. Of note, in~~ **In** ~~a~~ **our** previous **daprodustat** study ~~(PHI113633)~~**experience in Phase 2b**, HD-dependent subjects with anemia associated with CKD were administered placebo for 4 weeks, with a mean decrease ~~of~~ **between 0.61 and** 0.72 g/dL in Hgb. No subject met either the Hgb stopping criterion or requiring rescue medication. Therefore, the risk of a subject meeting Hgb withdrawal criteria is considered low during this phase.

- The Acute Challenge days have been designed to be consistent with the methodology used in a published study [Kang, 1998] **(See Figure 2)**. Although the goal of the current study is to attempt to replicate the blood pressure results from the IV administration of epoetin alfa in ~~the~~ **that** publication ~~by. [Kang, 1998], in order to compare the response to daprodustat,~~SBP will be the primary endpoint rather than MAP since SBP is the overall best predictor of future cardiovascular risk in a hypertensive population, and MAP is biased toward DBP [Agarwal, 2015].

- Acute Challenge 1 will assess whether there are any immediate effects of daprodustat or epoetin alfa on blood pressure. This assessment will also determine the ability to replicate the findings in the published study and will provide information for an interim analysis (See Section 9.3.2).

- The 8-week Hgb Maintenance Phase will replicate the management procedures planned for the Phase 3 studies as described in Section 6.4. The duration was chosen to be consistent with the timeframe in which the hypertensive effects of ESAs have been observed (i.e., 4 to 8 weeks) [Samtleben, 1988].

- Acute Challenge 2 will again assess the effects of daprodustat or epoetin alfa on BP following 8 weeks of anemia treatment utilizing the same assessment as Acute Challenge 1. The purpose of Acute Challenge 2 is to assess any BP effects of ESA administration that may be potentiated (or possibly diminished) following long-term administration of an ESA.

Section 4.5 Dose Justification

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

Daprodustat Treatment Group

- Acute Challenge 1 & 2: The dose to be used for this group will be the highest planned maintenance dose for a once-daily regimen (i.e., 24 mg) for treatment of anemia associated with CKD. In a previous GlaxoSmithKline (GSK) study (PHI112843), a single, oral dose of 150 mg daprodustat was administered to dialysis patients with no safety issues identified. For Acute Challenge 2, stringent Hgb stopping criteria **and dose hold** have been defined at ~~Week 8~~ **Day 57** to minimize marked increases in Hgb.

- Hgb Maintenance Phase: Starting doses of daprodustat will be based on Hgb levels,

as described in Section 6.4.1. These doses have been chosen to minimize the potential for subjects to meet Hgb stopping criteria during the initial ~~4-week~~ dosing period prior to the first dose adjustment, and are based on modelling and simulation of the dose-Hgb response results from Phase 2 studies. ~~Additionally, Hgb levels will be assessed at 2 and 6 weeks after start of the maintenance period with specific Hgb stopping criteria (See Table 2).~~

Epoetin alfa Treatment Group

- Acute Challenge 1 & 2: The dose to be used for this group will be the highest labelled starting dose for epoetin alfa (100 U/kg, IV) which is consistent with the epoetin alfa dose used in the published study where an acute increase in MAP with rhEPO was observed [Kang, 1998]. As subjects in this group will have been washed off ESA for ~~4~~ **2** weeks prior to Acute Challenge 1 and only a single dose will be administered, the risk of a marked increase in Hgb levels is considered very low. For Acute Challenge 2, stringent Hgb-based stopping criteria **and dose hold** have been defined at ~~Week 8~~ **Day 57** to minimize marked increases in Hgb (See Table 2).

- Hgb Maintenance Phase: The dosage of epoetin alfa during the 8-week Hgb maintenance phase will be adjusted (if necessary) according to the local labelling in order to manage the subject's Hgb within the target range (10.0-11.0 g/dL). In a previous study (GSK Study PHI113633), HD subjects that had a ~~similar~~ 4-week ESA washout were initiated on a mean dose of rhEPO of 89 IU/kg/week (n=39), with the dose increased to a mean of 102 IU/kg/week 4 weeks later.

Section 5.1 Hemoglobin Stability Criteria

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added. Note: Figure 3 has been removed from the end of this section. There is no revised figure to insert. As some values were already in bold, they are underlined below to indicate addition of this text.

Entry into the study requires a stable Hgb between **~~9.0~~ 8.5 and 11.5 g/dL, inclusive**. This is confirmed from ~~an average of~~ **~~three~~ two** Hgb values obtained ~~during~~ **at** the **S**screening **Visit and Washout Visit, with both values between 8.5 and 11.5 g/dL.** ~~period at Weeks -8, -6 and -4~~ **These will be taken** via a validated point-of-care device to measure Hgb (i.e., HemoCue) **prior to the dialysis session of the day**. ~~as outlined in Figure 3.~~ **The value at the Washout Visit must not have decreased by more than 1.0 g/dL from the Screening Visit value.** For subjects with a three-times weekly dialysis schedule, Hgb values must not be obtained on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday). For subjects with a four- to five-times weekly dialysis schedule, Hgb values can be obtained on any hemodialysis session of the week.

Section 5.2 Inclusion Criteria

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added. Already bolded text has been underlined to show addition.

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

AGE
1. ≥40 years of age, at the time of signing the informed consent.
TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2**. Hemoglobin:** Stable Hgb ~~9.0~~ **8.5** to 11.5 g/dL inclusive (See Section 5.1). 3. **Dialysis frequency:** On hemodialysis (HD, hemofiltration or hemodiafiltration) three-to five-times weekly for at least 4 weeks prior to screening. 4**. Dialysis adequacy**: A single pool Kt/V_{urea} ≥1.2 based on a historical value obtained within the 3 months prior to screening in order to ensure the adequacy of dialysis. If Kt/V_{urea} is not available, then an average of the last 2 values of urea reduction ratio (URR) is at least 65%. **NOTE**: Only needs confirming at screening. 5**. ESA ~~dose~~ treatment:** Treated with ~~the same~~ **an** ESA (epoetins or their biosimilars, darbepoetin, or methoxy PEG-epoetin beta) ~~with total weekly dose varying by no more than 50% during the~~ **for at least** 4 weeks prior to screening. **6. Iron replacement therapy**: Subjects may be on stable maintenance oral or IV (≤100 mg/week) iron supplementation. If subjects are on oral or IV iron, then doses must be stable for the 4 weeks prior to ~~screening through Day 1~~**Washout**. **7. Estimated Dry Weight (EDW):** Mid-week average weight gain between dialysis treatments <5% as assessed pre- and post-dialysis from screening to **Washout**~~Day 1.~~ 8. **Antihypertensive Medication:** Meets the following criteria: • On at least ~~2~~ **1** ~~different~~ antihypertensive medication~~s of different classes~~ **[excluding diuretics]** **AND** • On **that same medication and the same dose for at least 1 week prior to Washout** ~~a stable dose and number of hypertensive medications for the 4 weeks prior to screening through Day 1.~~
INFORMED CONSENT
9. Capable of giving signed informed consent as described in Section 10.2 which includes compliance with the requirements and restrictions listed in the consent form and in this protocol. 10. Willing and able to wear ABPM device for at least 25 hours on two separate sessions.

Section 5.3 Exclusion Criteria

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

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

CKD-RELATED CRITERIA

1. **Dialysis modality**: Planned change from HD to peritoneal dialysis within the study time period, or on home dialysis.
2. **Renal transplant**: Planned for kidney transplant within the ~~18~~ **16** weeks following the Screening visit.
3. **High ESA dose**: An epoetin alfa dose of ≥360 IU/kg/week IV or ≥250 IU/kg/week subcutaneous (SC), or darbepoetin dose of ≥1.8 µg/kg/week IV or SC, or methoxy PEG-epoetin beta dose of ≥ 2.2 µg/kg/week within the 8 weeks prior to screening through Week -4.
4. **Mircera**: Planned or recorded administration of Mircera (methoxy PEG-epoetin beta) within the 4 weeks prior to~~start of~~ **the W**~~w~~ashout **visit** ~~at Week -4.~~

CARDIOVASCULAR DISEASE-RELATED CRITERIA

5. **Myocardial infarction or acute coronary syndrome:** Within the ~~4 weeks~~ **3 months** prior to ~~screening through Day 1~~ **Washout.**
6. **Stroke or transient ischemic attack:** Within the ~~4 weeks~~ **3 months** prior to ~~screening through Day 1~~ **Washout.**
7. **Heart failure:** Chronic Class IV heart failure (HF), as defined by the New York Heart Association (NYHA) functional classification system diagnosed prior to ~~screening through Day 1~~ **Washout.**
8. QT interval corrected for heart rate using Bazett's formula **(QTcB):** QTcB >500 msec, or QTcB >530 msec in subjects with Bundle Branch Block. There is no QTc exclusion for subjects with a predominantly paced rhythm.
9. **Current uncontrolled hypertension:** Resting systolic blood pressure >160 mmHg; or diastolic blood pressure >100 mmHg at screening.
10. **Atrial Fibrillation:** Presence of atrial fibrillation ~~or a history of atrial fibrillation~~.

OTHER DISEASE-RELATED CRITERIA

11. **Inflammatory disease:** Active chronic inflammatory disease that could impact erythropoiesis (e.g., scleroderma, systemic lupus erythematosus, rheumatoid arthritis, celiac disease) diagnosed prior to ~~screening through Day 1~~ **Washout.**
12. **Aplasias:** History of bone marrow aplasia or pure red cell aplasia.
13. **Other causes of anemia:** Pernicious anemia, thalassemia major, sickle cell disease or myelodysplastic syndrome.
14. **Liver disease (any one of the following):**
    - Alanine transaminase (ALT) >2x upper limit of normal (ULN) (screening only)
    - Bilirubin >1.5xULN (screening only)

      NOTE: Isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%.
    - Current unstable liver or biliary disease per investigator assessment, generally defined by the presence of ascites, encephalopathy, coagulopathy, hypoalbuminaemia, esophageal or gastric varices, persistent jaundice, or cirrhosis.

      NOTE: Stable chronic liver disease (including asymptomatic gallstones, chronic

hepatitis B or C, or Gilbert's syndrome) are acceptable if subject otherwise meets entry criteria.

15. **Major surgery:** Major surgery (excluding vascular access surgery) within the ~~8 weeks~~ **3 months** prior to ~~screening through Day 1~~ **Washout**, or planned during the study.
16. **Transfusion:** Blood transfusion within the 8 weeks prior to ~~screening through Day 1~~ **Washout**, or an anticipated need for blood transfusion during the study.
17. **Gastrointestinal (GI) bleeding:** Evidence of actively bleeding gastric, duodenal, or esophageal ulcer disease **OR** clinically significant GI bleeding within the ~~4~~ **8** weeks prior to ~~screening through Day 1~~ **Washout**.
18. **Acute Infection:** Clinical evidence of acute infection or history of infection requiring intravenous (IV) antibiotic therapy within the 4 **8** weeks prior to ~~Screening through Day 1~~ **Washout.**

    NOTE: IV antibiotics as prophylaxis are allowed.
19. **Malignancy:** History of malignancy within the two years prior to screening through Day 1 or currently receiving treatment for cancer, or has a known complex kidney cyst (e.g., Bosniak Category IIF, III or IV) ≥3 cm.
    NOTE: ONLY exception is localized squamous cell or basal cell carcinoma of the skin that has been definitively treated more than 4 weeks prior to screening.
20. **Blood Pressure Measurement:** Subjects with an upper arm diameter which cannot be measured by oscillometer/ sphygmomanometer cuff **OR** for whom blood pressure cannot be measured in the opposite arm of current vascular access.

CONCOMITANT MEDICATIONS
21. **Severe allergic reactions:** History of severe allergic or anaphylactic reactions or hypersensitivity to excipients in the investigational product (see Daprodustat IB for list of excipients [GlaxoSmithKline Document Number RM2008/00267/07]). 22. **Drugs and supplements:** Use of any prescription or non-prescription drugs or dietary supplements that are prohibited (See Section 6.11.3), from screening until ~~Day 1~~ **Washout**. 23. **Prior investigational product exposure**: The subject has participated in a clinical trial and has received an experimental investigational product within the 30 days prior to ~~screening through~~ Day 1 **or within 5 half lives of the investigational product prior to screening, whichever is longer.**
GENERAL HEALTH RELATED CRITERIA
24. **Other conditions:** Any other condition, clinical or laboratory abnormality, or examination finding that the investigator considers would put the subject at unacceptable risk, which may affect study compliance or prevent understanding of the aims or investigational procedures or possible consequences of the study. 25. **Females ONLY:** Subject is pregnant [as confirmed by a positive serum human chorionic gonadotrophin (hCG) test for females of reproductive potential (FRP) only], subject is breastfeeding, or subject is of reproductive potential and does not agree to follow one of the contraceptive options listed in the List of Highly Effective Methods for Avoiding Pregnancy in Section 12.4.7.
DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
26. **Vitamin B_{12}:** At or below the lower limit of the reference range (may rescreen in a minimum of 8 weeks**, following treatment**). 27. **Folate:** <2.0 ng/mL (4.5 nmol/L) (may rescreen in a minimum of 4 weeks, **following treatment**). 28. **Ferritin:** <100 ng/mL 29. **Transferrin saturation (TSAT):** <20%.

Section 5.4 Screening/Baseline/Run-in Failures

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

Screen failures are defined as subjects who consent to participate in the clinical trial but **who never enter the washout period** ~~are never subsequently randomized~~. In order to ensure transparent reporting of screen failure subjects, meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and respond to queries from Regulatory authorities, a minimal set of screen failure information is required including Demography, Screen Failure details, Eligibility Criteria, and Serious Adverse Events (see Section 7.3.1.6).

Section 5.5 Withdrawal/Stopping Criteria

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

A subject 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 or administrative reasons. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records.

If a decision is made to withdraw a subject, he/she should return to the clinic as soon as possible to complete the Early Withdrawal assessments. **The** ~~and~~ Follow-up visit **should be performed 14 ± 3 days after last dose.** (see Time and Events Table Section 7.1).

In all cases, the reason for withdrawal from the study and date of the last dose of study treatment will be recorded in the eCRF.

Section 5.5.1.1 Hemoglobin Stopping Criteria

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

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 (Table **2** ~~3~~) and recorded in the eCRF. The table below summarizes the Hgb values and corresponding action to be taken at each visit. **If the subject meets the below condition(s), then IP must be permanently stopped.**

Table 2 Hgb Stopping Criteria

~~Week 2 &~~ Day 1 (Prior to Acute Challenge 1)

Hgb 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, withdraw subject from the study.	
≥7.5-<11.5	decrease of ≥2.0 in Hgb over 2 weeks	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently withdraw subject from the study.
	increase of ≥1.~~0~~ **3** in Hgb over 2 weeks	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, withdraw subject from the study.
≥11.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed~~, permanently withdraw subject from the study~~ **avoid dosing and continue washout for one additional week. Progress with Acute Challenge 1 one week later if Hgb has decreased below 11.5; if not then withdraw subject from study.**	

Days 15, 29, & 43

Hgb 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 subject from the study.	
≥7.5-<12.0	decrease of ≥2.0 in Hgb over 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 subject from the study.
	increase of ≥1.~~0~~ **3** in Hgb over 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 subject from the study.
~~≥12.0~~	~~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 subject from the study.~~	

Day 57 (Prior to Acute Challenge 2)

Hgb 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 subject from the study.	
≥7.5-<11.~~0~~**5**	decrease of ≥2.0 in Hgb over 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 subject from the study.
	increase of ≥1.0 **3** in Hgb over 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 subject from the study.
≥11.~~0~~**5**	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, **HOLD the dose for 1 week and progress with Acute Challenge 2 one week later.**~~permanently discontinue study treatment and withdraw subject from the study.~~ **If Hgb remains above 11.5 one week later, then withdraw subject from the study.**	

Section 6.1 Investigational Product and Other Study Treatment

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

The term 'study treatment' is used throughout the protocol to describe any combination of products received by the subject as per the protocol design. Study treatment may therefore refer to the individual study treatments or the combination of those study treatments.

Product name:	**Daprodustat**
Dosage form:	Tablet
Unit dose strength(s)/Dosage level(s):	**1 mg**, 2 mg, 4 mg, 6 mg, 8 mg, 10 mg tablet strengths/**1 mg,** 2 mg, 4 mg, 6 mg, 10 mg, **12 mg** & 24 mg dosage levels
Route of Administration	Oral
Physical description:	**1 mg,** 2 mg & 4 mg tablets: 7.0 mm round, compound radius, white film coated tablets 6 mg, 8 mg & 10 mg tablets: 9.0 mm round, compound radius, white film coated tablets
Method for individualizing dosage:	See Section 6.4.1

For a description of epoetin alfa, please consult the local label.

All eligible subjects will discontinue their current ESA therapy and will undergo a 4 **2-** week ESA washout period. Following washout, subjects will be randomized to receive either daprodustat or epoetin alfa, on a dialysis day. For subjects with a three-time weekly dialysis schedule, randomization must not occur on the first dialysis session of the week (e.g., if Monday-Wednesday-Friday schedule, the study visit cannot be on Monday). For subjects with a four to five times weekly dialysis schedule, randomization can be on any hemodialysis session of the week.

Subjects can take their tablets without regard to food. For all study visits, it is recommended for subjects not to eat a heavy meal before coming to the clinic in order to avoid lipemia of the blood sample collected for the analysis of transferrin, iron and total iron binding capacity (TIBC).

Section 6.4.1 Subjects Randomized to Daprodustat
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

Subjects randomized to daprodustat will have doses adjusted, as required, to target Hgb within the range of 10.0-11.0 g/dL. Dose adjustments will be assigned **automatically** via the IVWRS based on the subject's Hgb value via onsite HemoCue assessment according to the following algorithms:

Day 1 **(HemoCue Prior to Acute Challenge 1)**

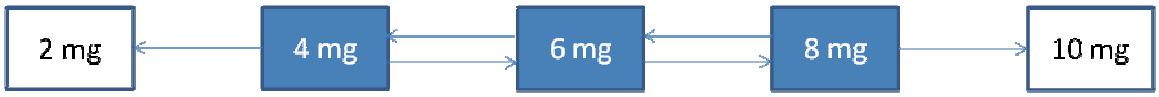
Hgb (g/dL)	Action or Dose
<7.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, withdraw subject from the study.
~~≥7.5 and <9.0~~	~~8 mg daprodustat~~
≥**7.5** ~~9~~ and <10.0	6 mg daprodustat
≥10.0 and <11.5	4 mg daprodustat
≥11.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, ~~withdraw subject from the study.~~ **avoid dosing and continue washout for one additional week. If Hgb remains above 11.5 then withdraw subject from study.**

The 8 week Hgb maintenance dosing will begin on Day 2, following Acute Challenge 1. **Acute Challenge dosing is the same for all subjects on the daprodustat arm: 24 mg.**

Days **15,** 29**, and 43**

The available dose steps for daprodustat are outlined below (highlighted boxes indicate starting doses). Dose adjustments will result in the daprodustat dose being increased or decreased by **one dose step**.

PREVIOUS IMAGE



REVISED IMAGE

Hgb (g/dL)	Hgb change since ~~Day 1~~ previous study 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 drug and withdraw subject from the study.
≥7.5 to <9.5	Decreasing or No change[1]	Increase to the next higher dose step
≥7.5 to <9.5	Increasing[2]	Maintain dose
≥9.5 to ≤11.5	Any change	Maintain dose
>11.5 to <12.0	Increasing or No change	Decrease to the next lower dose step
	Decreasing	Maintain dose
≥12.0	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.~~ **HOLD dosing for 2 weeks until next study visit.**[3]

[1]No change is defined as an increase < 0.5 g/dL
[2]Increase is defined as an increase ≥ 0.5 g/dL
[3] If Hgb remains ≥12.0 at next visit, the subject should be withdrawn from the study. If the Hgb drops below 12.0, the subject should be restarted on the next lowest dose.

Section 6.10.1 Meals and Dietary Restrictions
REVISED TEXT, where text in strikethrough has been removed.

Daprodustat can be taken without regard to meals.

All subjects will be instructed to limit caffeine- or xanthine- containing products (e.g., coffee, tea, cola drinks, chocolate) for 24h prior to both Acute Challenge 1 and Acute Challenge 2. ~~Should the Additional Acute Challenge 2 be necessary, the subject does not need to limit the products listed above.~~

Section 6.11.1 Estimated Dry Weight and Antihypertensive Medication Changes
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

It is preferred that changes to EDW and antihypertensive medication(s) are not made while the subject is part of this study, **however,** ~~Subjects~~ **subjects** should remain in the

study regardless of any changes. **All medication and dose changes should be recorded in the eCRF.**

Section 6.11.3 Prohibited Medications and Non-Drug Therapies
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

Use of any of the following prescription drugs from screening ~~(Week -8)~~ until 7 days after the last dose of randomized treatment is prohibited and will constitute a protocol violation.

- ~~The s~~**S**trong inhibitor**s** of CYP2C8 **(e.g.,** gemfibrozil**, high dose clopidogrel [300 mg])**
- **S**trong inducer**s** of CYP2C8 **(e.g.**, rifampin/rifampicin**)**

Section 7.1 Time and Events Table
Table 3 Study Procedures and Assessments

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

Procedure[1]	Screening		~~ESA~~ Washout		Treatment Period[2]					Follow-up[2]	
	~~Week -8~~	~~Week -6~~	~~Week -4~~	~~Week -2~~	Day 1	Day 15	Day 29	Day 43	Day 57	Early WD	Week 10
Informed Consent	X										
Entry Criteria	X										
Physical, Medical History, Demography	X										
HemoCue Hgb	X	~~X~~	~~X~~	~~X~~	X[4]	X	X	X	X[4]	X	**X**
Enrollment				**X**							
IVWRS					X	**X**	X	**X**			
Randomization					X						
Acute Challenge					X[4]				X[4]		
ABPM Assessment					X[4]				X[4]		
Dose Adjustment						**X**	X	**X**			
Females Only: Serum Pregnancy Test	**X**	~~X~~			X		X		X	~~X~~	X
Females Only: Estradiol & FSH (if required)[3]	**X**	~~X~~									
ECG	X				X[4]				X[4]	~~X~~	X
Vital signs & weight (Pre- & Post-dialysis)	X	~~X~~	~~X~~	**X**	X[4]		X		X[4]	~~X~~	X
Clinical Chemistry	**X**	~~X~~			X		X		X	~~X~~	X
Hematology	**X**	~~X~~			X		X		X	~~X~~	X
Folate and Vitamin B12	X										
Ferritin, transferrin, total iron,, TSAT, UIBC	X		~~X~~								
Pharmacokinetic/Biomarker Assessments					X[4]				X[4]		
Hgb Maintenance Period[5]					←==========				==========→		
Adverse Events Assessment	X[6]	~~X~~[6]	~~X~~	~~X~~	X[4]	X	X	X	X[4]	~~X~~	X
Review Concomitant Medications	X		~~X~~	~~X~~	X[4]	X	X	X	X[4]	~~X~~	X

1 All assessments should be done predialysis/predose except as noted.

2 Allowable time window ±2 days EXCEPT Follow-up Visit which is ±3 days.

3 As detailed in Inclusion Criteria.

4 Detailed timings for assessments on Acute Challenge Days are given in Table 4.

5 From the end of Acute Challenge 1 to the beginning of Acute Challenge 2.

6 Only SAEs assessed as related to study participation are collected at this visit. See Section 12.4 for additional details.

Section 7.2 Screening and Critical Baseline Assessments
REVISED TEXT, where text in strikethrough has been removed.

Medical history, including cardiovascular disease and associated risk factors, (as detailed in the eCRF) will be assessed at screening.

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

Medical/medication/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5.

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

Section 7.3.1.1 Time Period and Frequency for Collecting AE and SAE Information
REVISED TEXT, where text in strikethrough has been removed.

- Any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact.
- AEs will be collected from the start of washout ~~(Week −4)~~ until the follow-up contact (see Section 7.3.1.3), at the timepoints specified in the Time and Events Table (Section 7.1).
- Medical occurrences that begin prior to the start of washout but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF.
- All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Section 12.4.
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

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

Section 7.3.5 Electrocardiogram (ECG)
REVISED TEXT, where text in strikethrough has been removed.

ECG measurements will be taken at the time points specified in the Time and Events Table (Section 7.1). Full 12-lead ECGs will be recorded with the subject in a supine position. Heart rate (HR), PR interval, QRS duration, and QT (uncorrected) interval will be measured. QTcB will be calculated (machine-read or manually).

At the Screening visit ~~(Week -8)~~ two additional ECGs are required if the initial ECG indicates prolonged QTc (see Section 5.3) using the automated or manually calculated QTcB value. The average QTcB value of all three ECGs will be used to determine eligibility. Additional details are provided in the SRM.

ECG data will be read locally.

All ECGs will be performed before measurement of vital signs and collection of blood samples for laboratory testing.

Section 7.3.6 Clinical safety Laboratory Assessments
A spelling error was corrected in the notes of Table 5.

Section 7.4.1 Blood Sample Collection
REVISED TEXT, where bolded text has been added.

Blood samples for pharmacokinetic (PK) analysis of daprodustat and its metabolites (GSK2391220 [M2], GSK2531403 [M3], GSK2487818 [M4], GSK2506102 [M5], GSK2531398 [M6], and GSK2531401 [M13]) will be collected at the time points indicated in Section 7.1, Time and Events Table. The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

Details of PK blood sample collection (including volume to be collected), processing, storage and shipping procedures are provided in the SRM.

If the subject is unable to provide a sufficient blood quantity per time point or there is significant concern for this by the subject or investigator, these serial blood draws may be omitted.

Section 7.5.1.1 ABPM Delay Due to High Hgb
REVISED TEXT, where bolded text has been added.

Day 1: If the HemoCue Hgb is confirmed to be ≥ 11.5 g/dL then avoid dosing and continue washout for one additional week. No other assessments should be performed for that subject during the visit. When the subject returns 7 days later, re-draw the Hgb for HemoCue and assess. If confirmed to be <11.5 g/dL, then progress with the remainder of assessments and perform Acute Challenge 1. If the Hgb remains high, the subject must be withdrawn.

Day 57: If the HemoCue Hgb is confirmed to be ≥ 11.5 g/dL then hold the dose(s) of IP for 1 week. No other assessments should be performed for that subject during the visit. When the subject returns 7 days later, re-draw the Hgb for HemoCue and assess. If confirmed to be <11.5 g/dL, then progress with the remainder of

assessments and perform Acute Challenge 2. If the Hgb remains high, the subject must be withdrawn.

Section 7.5.2 Blood pressure Regulation Biomarkers
REVISED TEXT, where bolded text has been added.

Blood samples will be collected during this study to investigate the mechanism of the effect of daprodustat on blood pressure. Biomarkers selected will explore the renin-angiotensin-aldosterone axis, the nitric oxide and endothelin axes, sodium retention and HIF signalling.

Biomarkers may include erythropoietin, nitric oxide (NO), asymmetric dimethyarginine (ADMA), renin, angiotensin-II (1-8) and metabolites (1-7 and 1-5), endothelin-1 (ET-1), and noradrenalin. Samples will be collected as specified in the Time and Events Table (Section 7.1). The timing of the collections may be adjusted on the basis of emerging pharmacokinetic or pharmacodynamic (PD) data from this study or other new information in order to ensure optimal evaluation of the PD endpoints.

If the subject is unable to provide a sufficient blood quantity per time point or there is significant concern for this by the subject or investigator, these serial blood draws may be omitted.

Section 11 References
The following reference was added:

Holdstock L, Meadowcroft AM, Maier R, Johnson BM, Jones D, Rastogi A, Zeig S, Lepore JJ, Cobitz AR. Four-week studies of oral hypoxia-inducible factor-prolyl hydroxylase inhibitor GSK1278863 for treatment of anemia. *JASN* 2016; 27:1234-1244

Section 12.1 Appendix 1 – Abbreviations and Trademarks
REVISED TEXT, where text in strikethrough has been removed and bolded text has been added

ABPM	Ambulatory blood pressure monitoring
ADMA	Asymmetric dimethyarginine
AE	Adverse event
ALT	Alanine transaminase
AMD	Age-Related Macular Deg**e**neration
ANCOVA	Analysis of Covariance
AST	Aspartate transaminase
AUEC	Area under the effect curve
AUC (0-24)	Area under concentration-time curve from time zero to 24 hours
BP	Blood pressure
CHr	Reticulocyte h~~a~~emoglobin content
CI	Confidence interval
CKD	Chronic kidney disease
Cmax	Maximum observed concentration

CPK	Creatine phosphokinase
CV	Cardiovascular
DBP	Diastolic blood pressure
dL	Deciliter
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDW	Estimated Dry Weight
EPO	Erythropoietin
ESA	Erythropoiesis-stimulating agent
ET-1	Endothelin-1
FDA	Food and Drug Administration
FRP	Females of Reproductive Potential
FSH	Follicle S~~s~~timulating H~~h~~ormone
GCP	Good Clinical Practice
G	Gram
GI	Gastrointestinal
GSK	GlaxoSmithKline
hCG	Human chorionic gonadotrophin
HD	Hemodialysis dependent
HDPE	High Density Polyethylene
HF	Heart Failure
Hgb	Hemoglobin
HIF	Hypoxia-inducible factor
~~HPLC~~	~~High Performance Liquid Chromatography~~
HR	Hour/heart rate
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
INR	International N~~n~~ormalized R~~r~~atio
IRB	Institutional Review Board
ITT	Intent-to-treat
IU	International Unit
IV	Intravenous
IVWRS	Interactive Voice/Web Response System
KDIGO	Kidney Disease Improving Global Outcomes
KG	Kilogram
LDH	Lactate dehydrogenase
MAP	Mean Arterial Blood Pressure
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MG	Milligram
MI	Myocardial infarction
ML	Milliliter

mmHG	Millimeter of mercury
MSDS	Material Safety Data Sheet
ND	Non-dialysis dependent
NO	Nitric Oxide
NOAEL	No Observed Adverse Effect Level
NYHA	New York Heart Association
PD	Pharmacodynamic
PEG	Polyethylene glycol
PGI_2	Prostaglandin I_2
PHD	Prolyl hydroxylase domain
PHI	Prolyl hydroxylase inhibitor
PK	Pharmacokinetic
proADM	Proadrenomedullin
PRS	**Project Requirement Specification**
PRVP	Peak Right Ventricular Pressure
PSRAE	Possible suicidality related adverse event
QC	**Quality Control**
QTc	QT interval corrected for heart rate
QTcB	QT interval corrected for heart rate using Bazett's formula
RAP	Reporting and Analysis Plan
RBC	Red blood cell
RDW	Red blood cell distribution width
rhEPO	Recombinant human erythropoietin
RNA	Ribonucleic acid
SAE	Serious adverse event
SBP	Systolic blood pressure
SC	Subcutaneous
SD	Standard deviation
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic-pyruvic transaminase
sPAP	Systolic Pulmonary Artery Pressure
SRM	Study Reference Manual
T1/2	Terminal phase half-life
TIBC	Total iron binding capacity
Tmax	Time of occurrence of Cmax
TSAT	Transferrin saturation
UG	Microgram
UIBC	Unsaturated iron binding capacity
ULN	Upper limit of normal
URR	Urea reduction ratio
WBC	White blood cells

12.7.4. Amendment 4

Summary of Amendment Changes with Rationale

This protocol amendment applies to all centers where this study may be performed.

This protocol amendment was written to remove the interim analysis from the statistical section due to more rapid recruitment than anticipated. During the update, the objectives and endpoints were streamlined, the stratification variable of previous ESA dose was added to the models as it was inadvertently left out, the daprodustat dosing algorithm was updated, and exploratory endpoints were added to further clarify results.

During this time the safety language was updated as well as clarification of inclusion/exclusion criteria in response to an internal audit.

List of Specific Changes

Title Page

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

~~**Author (s):**~~ PPD

Sponsor Name and Legal Registered Address:

GlaxoSmithKline Research & Development Limited
980 Great West Road
Brentford
Middlesex, TW8 9GS
UK

Medical Monitor Name and Contact Information can be found in the Study Reference Manual (SRM).

Regulatory Agency Identifying Number(s): IND Number: 101,291

Medical Monitor Page

REVISED TEXT, where text in strikethrough has been removed:

~~**Medical Monitor/SAE Contact Information:**~~

~~Role~~	~~Name~~	~~Day Time Phone Number and email address~~	~~After-hours (Cell) Number~~	~~Fax Number~~

~~Primary Medical Monitor~~	PPD ~~, MD, PhD~~	~~Tel:~~ PPD	PPD	PPD
~~Secondary Medical Monitor~~	PPD ~~, MD, FACC~~	~~Tel:~~ PPD	PPD	PPD
~~Tertiary Medical Monitor~~	PPD ~~, MD~~	~~Tel:~~ PPD	PPD	PPD
~~Site and SAE Contact Address:~~	~~GlaxoSmithKline 1250 S. Collegeville Road, UP4400 Collegeville, PA 19426~~			

~~**Sponsor Legal Registered Address:**~~

~~GlaxoSmithKline Research & Development Limited~~
~~980 Great West Road~~
~~Brentford~~
~~Middlesex, TW8 9GS~~
~~UK~~

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

~~Regulatory Agency Identifying Number(s): IND Number: 101,291~~

Section 1 PROTOCOL SYNOPSIS FOR STUDY 205665 (Objective(s)/Endpoints(s))

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

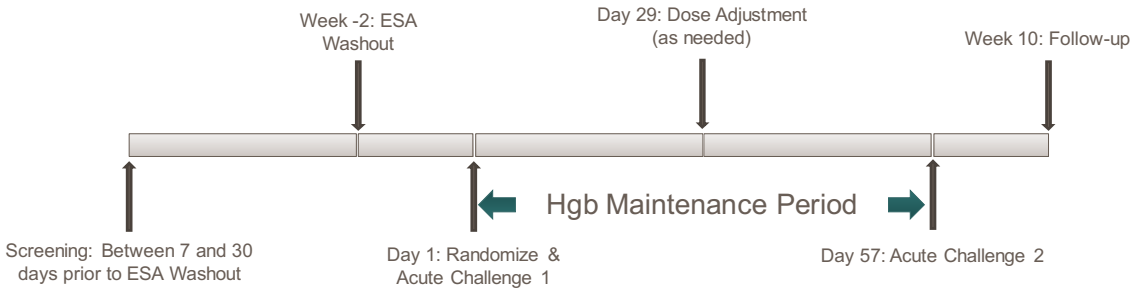
Objectives	Endpoints
Primary	
• To compare the effect of daprodustat to epoetin alfa on blood pressure (BP) after Acute Challenge 2 (8 weeks of hemoglobin [Hgb] maintenance therapy)	• Average of systolic blood pressure (SBP) as measured by ambulatory blood pressure monitoring (ABPM) over 6-hr post-dosing after 8 weeks of Hgb maintenance therapy
Secondary	
• To compare the initial effect of daprodustat to epoetin alfa on BP after Acute Challenge 1 (2 weeks of erythropoiesis-stimulating agent [ESA] washout)	• Average of SBP, diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR) as measured by ABPM over 6-hr post-dosing at Day 1

Objectives	Endpoints
	• Area under the effect curve (AUEC) of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 1
• To compare the effect of daprodustat to epoetin alfa on BP after Acute Challenge 2	• Average of DBP, MAP, and HR as measured by ABPM over 6 hr post-dosing at Day 57. • AUEC of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 57.
• To estimate the initial effect of daprodustat on SBP, DBP, HR and MAP after Acute Challenge 1	• Change from pre-dose in SBP, DBP, HR, and MAP at each timepoint at Day 1
• To characterize the pharmacokinetics of daprodustat	• Plasma concentrations of daprodustat and metabolites and derived pharmacokinetic parameters including maximum observed concentration (Cmax), time of occurrence of Cmax (tmax), terminal phase half-life (t½) and area under concentration-time curve from time zero to 24 hours (AUC[0-24]) as appropriate
Safety	
• To assess the safety and tolerability of daprodustat	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) • Reasons for discontinuation of study treatment • Absolute values and changes from baseline over time in laboratory parameters, electrocardiograms (ECGs) and vital signs
Exploratory	
• To investigate the effect of daprodustat **and epoetin alfa** on vasoactive mediators of blood pressure	• Plasma concentrations and derived parameters including Cmax, tmax, t½ and AUC(0-24) as appropriate (to include erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II and metabolites, and noradrenalin)
~~• Evaluate exposure-response relationships for daprodustat and: erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II (and metabolites), and noradrenalin~~	~~• Further models (as appropriate) to describe the daprodustat exposure-response relationship with: erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II (and metabolites), and noradrenalin.~~
• To summarize the effect of daprodustat and epoetin alfa on SBP after Acute Challenge 1 and Acute Challenge 2	• AUEC of SBP as measured by ABPM over 24-hr post-dosing

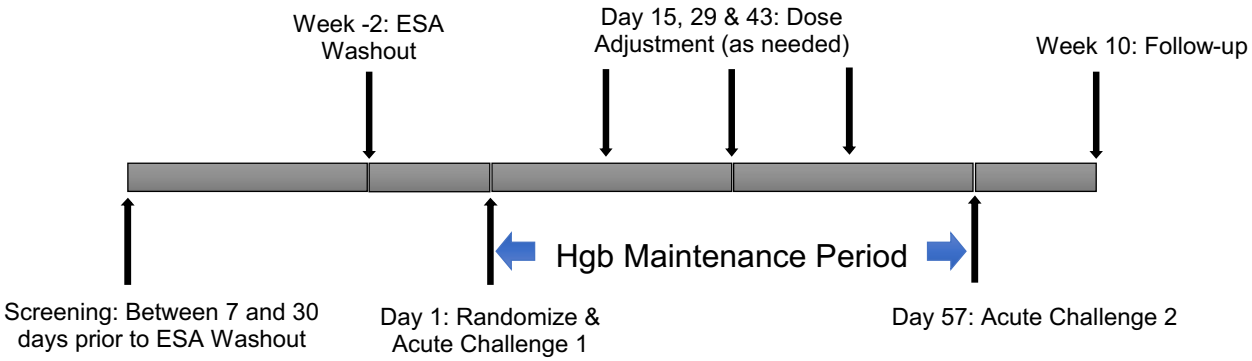
Objectives	Endpoints
• To compare the effect of daprodustat to epoetin alfa on SBP after 8 weeks of Hgb maintenance therapy	• Change from Day 1 pre-dose SBP to Day 57 pre-dose
• **To compare the effect of daprodustat to epoetin alfa on SBP at Cmax of each study treatment, as measured by ABPM**	• **Change in SBP from pre-dose on Day 1 to Cmax of the study treatment on Day 1** • **Change in SBP from pre-dose on Day 1 to Cmax of the study treatment on Day 57** • **Change in SBP from pre-dose on Day 57 to Cmax of the study treatment on Day 57**
• **To compare the effect of daprodustat to epoetin alfa on SBP at Cmax of the erythropoietin level, as measured by ABPM**	• **Change in SBP from pre-dose on Day 1 to Cmax of erythropoietin on Day 1** • **Change in SBP from pre-dose on Day 1 to Cmax of erythropoietin on Day 57** • **Change in SBP from pre-dose on Day 57 to Cmax of erythropoietin on Day 57**

Study Design

PREVIOUS IMAGE



REVISED IMAGE



REVISED TEXT, where text in strikethrough has been removed:

Following the 2-week ESA washout, subjects will be randomized 1:1 and stratified by prior ESA dose, based on the average weekly (for epoetins or darbepoetin) or monthly (for methoxy polyethylene glycol (PEG)-epoetin beta) dosing during the 12 weeks prior to ESA washout as follows:

- Low ESA dose: <100 ~~I~~U/kg/week epoetin alfa OR <0.5 µg/kg/week darbepoetin OR <0.6 µg/kg/week methoxy PEG-epoetin beta
- High ESA dose: ≥100 ~~I~~U/kg/week epoetin alfa OR ≥0.5 µg/kg/week darbepoetin OR ≥0.6 µg/kg/week methoxy PEG-epoetin beta

On Day 1, subjects will be randomized and undergo Acute Challenge 1, a single dose challenge to compare the acute effects on BP of the highest planned once-daily maintenance dose of daprodustat (24 mg) to the highest starting dose of epoetin alfa (100 ~~I~~U/kg). Subjects will have BP monitored for 24 hr using ABPM, and will have serial blood sampling to assess the pharmacokinetics of daprodustat and the time-course of various biomarkers. Acute Challenge 1 will be started promptly following completion of a mid-week dialysis session.

Analysis

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

- The primary endpoint for this study is average systolic blood pressure (SBP) over 6 hours of measurements taken at 15-minute intervals after the administration of study treatment for the Acute Challenge 2. The primary comparison of interest is daprodustat versus epoetin alfa for this challenge.
- The primary analysis of average SBP over 6 hr post-dose during Acute Challenge 2 will be an analysis of covariance (ANCOVA) with terms for treatment, **prior ESA dose (low/high)**, ~~and~~ post-HD/pre-Acute Challenge 1 SBP, difference between post-HD/pre-Acute Challenge 2 SBP and post-HD/pre-Acute Challenge 1 SBP, and treatment by (difference in post-HD SBP between Acute Challenge 1 and 2) interaction. Note that the pre-challenge SBP may change over time between Acute Challenge 1 and Acute Challenge 2 as a result of the maintenance therapy, and thus the pre-challenge SBP at Acute Challenge 2 is not a true pre-treatment covariate. As a result, the interpretation of the effect of the Acute Challenge 2 may be confounded by the impact of treatment phase on the covariate. Therefore, if the interaction term in this model is significant at the 0.10 level, then the primary model will be ANCOVA with terms for treatment, **prior ESA dose (low/high),** and post-HD/pre-Acute Challenge 1 SBP. The primary model will provide a point estimate and two-sided 95% CI for the treatment effect and a p-value for the superiority assessment. Superiority will be established if the p-value is <0.05.

- The comparison of daprodustat versus epoetin alfa on 6 hr average SBP during Acute Challenge 1 is a secondary endpoint. Average SBP over 6 hr post-dose in Acute Challenge 1 will be analyzed using ANCOVA with terms for treatment, **prior ESA dose (low/high),** and post-HD/pre-Acute Challenge 1 SBP. Similar analyses will be performed for DBP, MAP, and HR.
- ANCOVA using the primary model will be used for the analysis of average DBP, MAP, and HR over 6 hr post-dose in Acute Challenge 2 replacing the baseline SBP term with the analogous baseline measurement for DBP, MAP, and HR, respectively.
- AUECs of SBP, DBP, MAP, and HR post Acute Challenge 2 and 1 will be analyzed using ANCOVA with terms for treatment **and prior ESA dose (low/high)** with 95% CIs and p-values provided for the treatment effect.
- Null Hypothesis: The difference between 24 mg daprodustat versus 100 IU/kg epoetin alfa on 6 hr average SBP under a background of treatment (i.e., during Acute Challenge 2) is zero.
- Alternative Hypothesis: The difference between 24 mg daprodustat versus 100 IU/kg epoetin alfa on 6 hr average SBP under a background of treatment (i.e. during Acute Challenge 2) is not zero.
- ~~An interim analysis will be conducted after approximately 24 subjects have completed Acute Challenge 2.~~
- ~~If the mean change in SBP from pre-Acute Challenge 1 to the 6-hour mean following both Acute Challenge 1 and Acute Challenge 2 is less than 5 mmHg in the epoetin alfa group, then the study will be stopped. However, if a clinically meaningful difference between the two treatment groups in mean change in SBP from pre-Acute Challenge 1 to the 6-hour mean following Acute Challenge 1 (i.e., >5 mmHg) is observed, then the study will be continued in order to further characterize the effects of daprodustat on blood pressure.~~
- ~~Simulations show that under assumptions of a true mean change in SBP of 0 mmHg and SD of 18 mmHg for both treatment groups at Acute Challenge 1 and the epoetin alfa group at Acute Challenge 2, this stopping guideline will result in stopping the trial >99% of the time. Under the assumptions of a true mean change in SBP of 8 mmHg and SD of 18 mmHg for both treatment groups at Acute Challenge 1 and the epoetin alfa group at Acute Challenge 2, this rule will result in stopping the trial <1% of the time.~~

~~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.~~

Section 2 INTRODUCTION

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

Daprodustat (GSK1278863) is a small molecule, oral inhibiter of the HIF-PHD enzymes which may present several important advantages over other ESAs. It is an oral medication and does not require cold-chain storage as do some ESAs, thus increasing ease of use for patients. Moreover, data indicate that daprodustat can effectively raise

Hgb concentrations with lower EPO levels than those observed after administration of ESAs [Provenzano, 2011]. Because of the increased CV risk associated with raising Hgb concentrations through large increases in EPO levels [Pfeffer, 2009], daprodustat has the potential to raise Hgb with less CV risk than other ESAs. CCI [redacted] CCI [redacted] CCI [redacted].

Section 3 OBJECTIVES AND ENDPOINTS

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added.

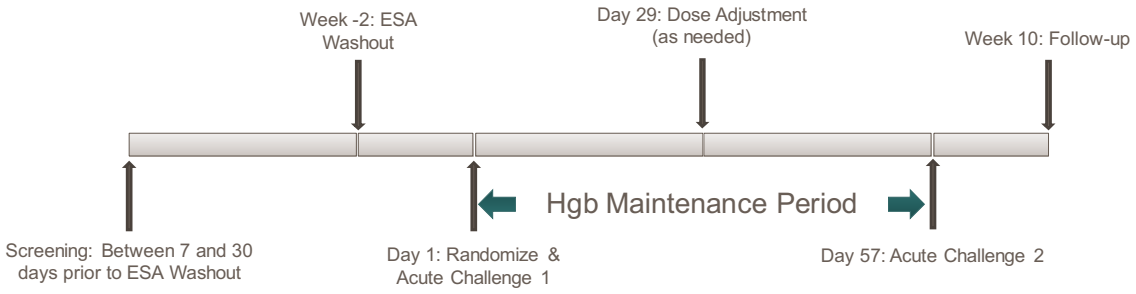
Objectives	Endpoints
Primary	
• To compare the effect of daprodustat to epoetin alfa on blood pressure (BP) after Acute Challenge 2 (8 weeks of hemoglobin [Hgb] maintenance therapy)	• Average of systolic blood pressure (SBP) as measured by ambulatory blood pressure monitoring (ABPM) over 6-hr post-dosing after 8 weeks of Hgb maintenance therapy
Secondary	
• To compare the initial effect of daprodustat to epoetin alfa on BP after Acute Challenge 1 (2 weeks of erythropoiesis-stimulating agent [ESA] washout)	• Average of SBP, diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR) as measured by ABPM over 6-hr post-dosing at Day 1 • Area under the effect curve (AUEC) of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 1
• To compare the effect of daprodustat to epoetin alfa on BP after Acute Challenge 2	• Average of DBP, MAP, and HR as measured by ABPM over 6 hr post-dosing at Day 57. • AUEC of SBP, DBP, MAP, and HR as measured by ABPM over 24-hr post-dosing at Day 57.
• To estimate the initial effect of daprodustat on SBP, DBP, HR and MAP after Acute Challenge 1	• Change from pre-dose in SBP, DBP, HR, and MAP at each timepoint at Day 1
• To characterize the pharmacokinetics of daprodustat	• Plasma concentrations of daprodustat and metabolites and derived pharmacokinetic parameters including maximum observed concentration (Cmax), time of occurrence of Cmax (tmax), terminal phase half-life (t½) and area under concentration-time curve from time zero to 24 hours (AUC[0-24]) as appropriate
Safety	
• To assess the safety and tolerability of daprodustat	• Incidence and severity of adverse events (AEs) and serious adverse events (SAEs) • Reasons for discontinuation of study treatment • Absolute values and changes from baseline over time in laboratory parameters,

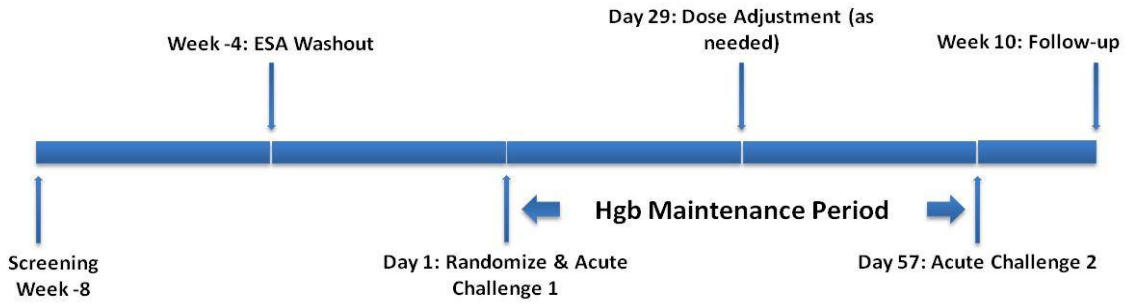
Objectives	Endpoints
	electrocardiograms (ECGs) and vital signs
Exploratory	
• To investigate the effect of daprodustat **and epoetin alfa** on vasoactive mediators of blood pressure	• Plasma concentrations and derived parameters including Cmax, tmax, t½ and AUC(0-24) as appropriate (to include erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II and metabolites, and noradrenalin)
~~• Evaluate exposure-response relationships for daprodustat and: erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II (and metabolites), and noradrenalin~~	~~• Further models (as appropriate) to describe the daprodustat exposure-response relationship with: erythropoietin, endothelin-1, nitric oxide, asymmetric dimethylarginine, renin, angiotensin-II (and metabolites), and noradrenalin.~~
• To summarize the effect of daprodustat and epoetin alfa on SBP after Acute Challenge 1 and Acute Challenge 2	• AUEC of SBP as measured by ABPM over 24-hr post-dosing
• To compare the effect of daprodustat to epoetin alfa on SBP after 8 weeks of Hgb maintenance therapy	• Change from Day 1 pre-dose SBP to Day 57 pre-dose
• **To compare the effect of daprodustat to epoetin alfa on SBP at Cmax of each study treatment, as measured by ABPM**	• **Change in SBP from pre-dose on Day 1 to Cmax of the study treatment on Day 1** • **Change in SBP from pre-dose on Day 1 to Cmax of the study treatment on Day 57** • **Change in SBP from pre-dose on Day 57 to Cmax of the study treatment on Day 57**
• **To compare the effect of daprodustat to epoetin alfa on SBP at Cmax of the erythropoietin level, as measured by ABPM**	• **Change in SBP from pre-dose on Day 1 to Cmax of erythropoietin on Day 1** • **Change in SBP from pre-dose on Day 1 to Cmax of erythropoietin on Day 57** • **Change in SBP from pre-dose on Day 57 to Cmax of erythropoietin on Day 57**

Section 4.1 Overall Design

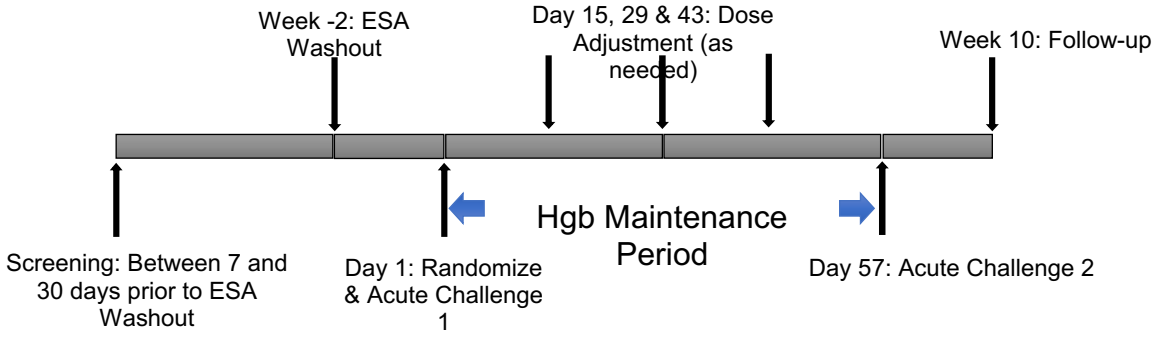
Figure 1 Study Design

PREVIOUS IMAGES





REVISED IMAGE



REVISED TEXT, where text in strikethrough has been removed:

Following the 2-week ESA washout, subjects will be randomized 1:1 and stratified by prior ESA dose, based on the average weekly (for epoetins or darbepoetin) or monthly (for methoxy polyethylene glycol (PEG)-epoetin beta) dosing during the 12 weeks prior to ESA washout as follows:

- Low ESA dose: <100 ŧU/kg/week epoetin alfa OR <0.5 µg/kg/week darbepoetin OR <0.6 µg/kg/week methoxy PEG-epoetin beta
- High ESA dose: ≥100 ŧU/kg/week epoetin alfa OR ≥0.5 µg/kg/week darbepoetin OR ≥0.6 µg/kg/week methoxy PEG-epoetin beta

On Day 1, subjects will be randomized and undergo Acute Challenge 1, a single dose challenge to compare the acute effects on BP of the highest planned once-daily maintenance dose of daprodustat (24 mg) to the highest starting dose of epoetin alfa (100 ŧU/kg). Subjects will have BP monitored for 24 hr using ABPM, and will have serial blood sampling to assess the pharmacokinetics of daprodustat and the time-course

of various biomarkers. Acute Challenge 1 will be started promptly following completion of a mid-week dialysis session.

Section 4.4 Design Justification

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

- This will be an open-label study since the primary endpoint (SBP by ABPM) is an objective measure and unlikely to be influenced by knowledge of the treatment received. ~~Additionally, an open label design will facilitate the conduct of the interim analysis. Lastly,~~ Additionally, blinding of an injectable comparator and oral study drug is impractical in this case.

Section 4.6 Benefit: Risk Assessment

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

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 Supplements, **if applicable**. ~~[GlaxoSmithKline Document Number RM2008/00267/07; GlaxoSmithKline Document Number 2015N266524_00; GlaxoSmithKline Document Number 2015N266524_01].~~

Section 5 SELECTION OF STUDY POPULATION AND WITHDRAWAL CRITERIA

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on daprodustat that may impact subject eligibility is provided in the Daprodustat Investigator's ~~Brochure [GlaxoSmithKline Document Number RM2008/00267/07]~~ and IB Supplements, **if applicable**. ~~[GlaxoSmithKline Document Number 2015N266524_00; GlaxoSmithKline Document Number 2015N266524_01]~~.

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

Section 5.2 Inclusion Criteria

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

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

AGE
1. ≥40 years of age, at the time of signing the informed consent.
TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2. **Hemoglobin:** Stable Hgb 8.5 to 11.5 g/dL inclusive (See Section 5.1). 3. **Dialysis frequency:** On hemodialysis (HD, hemofiltration or hemodiafiltration) three-to five-times weekly for at least 4 weeks prior to screening. 4. **Dialysis adequacy**: A single pool Kt/V_{urea} ≥1.2 based on a historical value obtained within the 3 months prior to screening in order to ensure the adequacy of dialysis. If Kt/V_{urea} is not available, then an average of the last 2 values of urea reduction ratio (URR) is at least 65%. **NOTE**: Only needs confirming at screening. 5. **ESA treatment:** Treated with an ESA (epoetins or their biosimilars, darbepoetin, or methoxy PEG-epoetin beta) for at least 4 weeks prior to screening. 6. **Iron replacement therapy**: Subjects may be on stable **(<50% change in overall dose and compliance of 80% of prescribed doses in the 4 weeks prior to and including the screening period)** maintenance oral or IV (≤100 mg/week) iron supplementation. If subjects are on oral or IV iron, then doses must be stable for the 4 weeks prior to Washout. 7. ~~**Estimated Dry** **Weight (EDW)**~~**:** Mid-week ~~average~~ weight ~~gain~~ **change** between dialysis treatments <5% as assessed ~~pre- and~~ post-dialysis ~~from~~ **at the** ~~s~~**S**creening ~~to~~ **and** Washout **visits**. 8. **Antihypertensive Medication:** Meets the following criteria: • On at least 1 antihypertensive medication [excluding diuretics] **AND** • On that same medication and the same dose for at least 1 week prior to Washout
INFORMED CONSENT
9. Capable of giving signed informed consent as described in Section 10.2 which includes compliance with the requirements and restrictions listed in the consent form and in this protocol. 10. Willing and able to wear ABPM device for at least 25 hours on two separate sessions.

Section 5.3 Exclusion Criteria

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

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

CKD-RELATED CRITERIA

1. **Dialysis modality**: Planned change from HD to peritoneal dialysis within the study time period, or on home dialysis.
2. **Renal transplant**: Planned kidney transplant within the 16 weeks following the Screening visit.
3. **High ESA dose**: An epoetin alfa dose of ≥360 ŧU/kg/week IV or ≥250 ŧU/kg/week subcutaneous (SC), or darbepoetin dose of ≥1.8 µg/kg/week IV or SC, or methoxy PEG-epoetin beta dose of ≥ 2.2 µg/kg/week within the 8 weeks prior to screening through Week -4.
4. **Mircera**: Planned or recorded administration of Mircera (methoxy PEG-epoetin beta) within the 4 weeks prior to the Washout visit.

CARDIOVASCULAR DISEASE-RELATED CRITERIA

5. **Myocardial infarction or acute coronary syndrome:** Within the 3 months prior to Washout.
6. **Stroke or transient ischemic attack:** Within the 3 months prior to Washout.
7. **Heart failure:** Chronic Class IV heart failure (HF), as defined by the New York Heart Association (NYHA) functional classification system diagnosed prior to Washout.
8. **QT interval** corrected for heart rate using Bazett's formula **(QTcB):** QTcB >500 msec, or QTcB >530 msec in subjects with Bundle Branch Block. There is no QTc exclusion for subjects with a predominantly paced rhythm.
9. **Current uncontrolled hypertension:** Resting **post dialysis** systolic blood pressure >160 mmHg; or diastolic blood pressure >100 mmHg at screening **or uncontrolled hypertension as determined by the investigator**.
10. **Atrial Fibrillation:** Presence of atrial fibrillation.

OTHER DISEASE-RELATED CRITERIA

11. **Inflammatory disease:** Active chronic inflammatory disease that could impact erythropoiesis (e.g., scleroderma, systemic lupus erythematosus, rheumatoid arthritis, celiac disease) diagnosed prior to Washout.
12. **Aplasias:** History of bone marrow aplasia or pure red cell aplasia.
13. **Other causes of anemia:** Pernicious anemia, thalassemia major, sickle cell disease or myelodysplastic syndrome.
14. **Liver disease (any one of the following):**
    - Alanine transaminase (ALT) >2x upper limit of normal (ULN) (screening only)
    - Bilirubin >1.5xULN (screening only)

      NOTE: Isolated bilirubin >1.5xULN is acceptable **to keep in the study** if bilirubin is fractionated and direct bilirubin <35%.
    - Current unstable liver or biliary disease per investigator assessment, generally defined by the presence of ascites, encephalopathy, coagulopathy, hypoalbuminaemia, esophageal or gastric varices, persistent jaundice, or cirrhosis.

      NOTE: Stable chronic liver disease (including asymptomatic gallstones, chronic

hepatitis B or C, or Gilbert's syndrome) are acceptable if subject otherwise meets entry criteria.

15. **Major surgery:** Major surgery (excluding vascular access surgery) within the 3 months prior to Washout, or planned during the study.
16. **Transfusion:** Blood transfusion within the 8 weeks prior to Washout, or an anticipated need for blood transfusion during the study.
17. **Gastrointestinal (GI) bleeding:** Evidence of actively bleeding gastric, duodenal, or esophageal ulcer disease **OR** clinically significant GI bleeding within the 8 weeks prior to Washout.
18. **Acute Infection:** Clinical evidence of acute infection or history of infection requiring intravenous (IV) antibiotic therapy within the 8 weeks prior to Washout.

    NOTE: IV antibiotics as prophylaxis are allowed.
19. **Malignancy:** History of malignancy within the two years prior to screening through Day 1 or currently receiving treatment for cancer, or has a known complex kidney cyst (e.g., Bosniak Category IIF, III or IV) ≥3 cm.
    NOTE: ONLY exception is localized squamous cell or basal cell carcinoma of the skin that has been definitively treated more than 4 weeks prior to screening.
20. **Blood Pressure Measurement:** Subjects with an upper arm diameter which cannot be measured by oscillometer/ sphygmomanometer cuff **OR** for whom blood pressure cannot be measured in the opposite arm of current vascular access.

CONCOMITANT MEDICATIONS
21. Severe allergic reactions: History of severe allergic or anaphylactic reactions or hypersensitivity to excipients in the investigational product ~~(see Daprodustat IB for list of excipients [GlaxoSmithKline Document Number RM2008/00267/07])~~.
22. Drugs and supplements: Use of any prescription or non-prescription drugs or dietary supplements that are prohibited (See Section 6.11.3), from screening until Washout.
23. Prior investigational product exposure: The subject has participated in a clinical trial and has received an experimental investigational product within the 30 days prior to Day 1 or within 5 half lives of the investigational product prior to screening, whichever is longer.
GENERAL HEALTH RELATED CRITERIA
24. Other conditions: Any other condition, clinical or laboratory abnormality, or examination finding that the investigator considers would put the subject at unacceptable risk, which may affect study compliance or prevent understanding of the aims or investigational procedures or possible consequences of the study.
25. Females ONLY: Subject is pregnant [as confirmed by a positive serum human chorionic gonadotrophin (hCG) test for females of reproductive potential (FRP) only], subject is breastfeeding, or subject is of reproductive potential and does not agree to follow one of the contraceptive options listed in the List of Highly Effective Methods for Avoiding Pregnancy in Section 12.4.7.
DIAGNOSTIC ASSESSMENTS AND OTHER CRITERIA
26. Vitamin B_{12}: At or below the lower limit of the reference range (may rescreen in a minimum of 8 weeks, following treatment).
27. Folate: <2.0 ng/mL (4.5 nmol/L) (may rescreen in a minimum of 4 weeks, following treatment).
28. Ferritin: <100 ng/mL
29. Transferrin saturation (TSAT): <20%.

Section 5.5.1 Criteria for Permanent Discontinuation from Study Treatment and Early Withdrawal

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

Subjects must **permanently discontinue study treatment** and be withdrawn from the study for the following reasons:

- Meets Hgb stopping criteria (See Section 5.5.1.1)
- Receives a blood transfusion
- Receives a kidney transplant
- Becomes pregnant or intends to become pregnant during the study
- Active GI bleeding

- Diagnosis of cancer, with the exception of squamous cell or basal cell carcinoma
- Liver chemistry abnormalities exceeding the threshold criteria (See Section 5.5.3)
- Misses 2 consecutive dialysis sessions
- ~~Need for chronic (more than 14 days) use~~ **Use** of prohibited medication (See Section 6.11.3)
- Myocardial infarction or acute coronary syndrome
- Stroke or transient ischemic attack
- New diagnosis of Class IV heart failure, as defined by the New York Heart Association (NYHA) functional classification system
- Active chronic inflammatory disease that could impact erythropoiesis
- Any new diagnosis of hematological disease including those affecting platelets, white or red blood cells, coagulation disorders, or any other cause of anemia of chronic disease other than renal disease

Section 5.5.1.1 Hemoglobin Stopping Criteria

Table 2 Hgb Stopping Criteria

REVISED TEXT, where text in strikethrough has been removed:

Day 1 (Prior to Acute Challenge 1)

Hgb 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, withdraw subject from the study.	
≥7.5-<11.5	decrease of ≥2.0 in Hgb over 2 weeks	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, permanently withdraw subject from the study.
	~~increase of ≥1.3 in Hgb over 2 weeks~~	~~Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, withdraw subject from the study.~~
≥11.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, avoid dosing and continue washout for one additional week. Progress with Acute Challenge 1 one week later if Hgb has decreased below 11.5; if not then withdraw subject from study.	

Days 15, 29, & 43

Hgb 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 subject from the study.	
≥7.5-<12.0	decrease of ≥2.0 in Hgb over 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 subject from the study.
	~~increase of ≥1.3 in Hgb over 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 subject from the study.~~

Day 57 (Prior to Acute Challenge 2)

Hgb 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 subject from the study.	
≥7.5-<11.5	decrease of ≥2.0 in Hgb over 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 subject from the study.
	~~increase of ≥1.3 in Hgb over 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 subject from the study.~~
≥11.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, HOLD the dose for 1 week and progress with Acute Challenge 2 one week later. If Hgb remains above 11.5 one week later, then withdraw subject from the study.	

Section 5.5.3 Liver Chemistry Stopping Criteria

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

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

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

- **a participant meets one of the conditions outlined in the algorithm below.**
- **when in the presence of abnormal liver chemistries not meeting protocol-specified stopping rules, the investigator believes study intervention discontinuation is in the best interest of the participant**.

Section 6.2 Treatment Assignment

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

Subjects will be stratified by prior ESA dose (as outlined in Section 4.1) and randomized 1:1 to receive open-label oral daprodustat or IV epoetin alfa. A central randomization approach will be used due to the small sample size and to protect against potential selection bias due to the open-label design. Once a randomization number has been assigned by ~~the~~ an **Interactive Voice/Web Response System** (IVWRS) **at time of Randomization**, it must not be re-assigned.

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

Section 6.3 Blinding

REVISED TEXT, where text in strikethrough has been removed:

This will be an open-label study. However, there are no plans to generate any aggregated unblinded summaries during the conduct of the study ~~except the summaries necessary for the planned interim analysis as defined in Section 9.3.2~~.

Section 6.4.1 Subjects Randomized to Daprodustat

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

Subjects randomized to daprodustat will have doses adjusted, as required, to target Hgb within the range of 10.0-11.0 g/dL. Dose adjustments will be assigned **automatically** via the IVWRS based on the subject's Hgb value via onsite HemoCue assessment according to the following algorithms:

Day 1 (HemoCue Prior to Acute Challenge 1)

Hgb (g/dL)	Action or Dose
<7.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, withdraw subject from the study.
≥7.5 and <10.0	6 mg daprodustat
≥10.0 and <11.5	4 mg daprodustat
≥11.5	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, avoid dosing and continue washout for one additional week. If Hgb remains above 11.5 then withdraw subject from study.

The 8 week Hgb maintenance dosing will begin on Day 2, following Acute Challenge 1. Acute Challenge dosing is the same for all subjects on the daprodustat arm: 24 mg.

Days 15, 29, ~~&~~ 43, **& 57**

The available dose steps for daprodustat are outlined below (highlighted boxes indicate starting doses). Dose adjustments will result in the daprodustat dose being increased or decreased by **one dose step**.

Hgb (g/dL)	Hgb change since previous study 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 drug and withdraw subject from the study.
≥7.5 to <9.5	Decreasing or No change[1]	Increase to the next higher dose step
≥7.5 to <9.5	Increasing[2]	Maintain dose
≥9.5 to ≤11.5	Any change	Maintain dose
>11.5 to <12.0	Increasing or No change	Decrease to the next lower dose step
	Decreasing	Maintain dose
≥12.0	Any change	Repeat HemoCue assessment on the same sample at same study visit to confirm; take average of 2 values. If confirmed, HOLD dosing for 2 weeks until next study visit.[3]
≥7.5 to <12.0	**increase of ≥1.3 in Hgb over 2 weeks[4]**	**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,**
≥7.5 to <12.0	**Increase of > 2.0 in Hgb over previous 4 weeks (Days 29, 43, 57[4] only)**	**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,**

[1]No change is defined as an increase < 0.5 g/dL
[2]Increase is defined as an increase ≥ 0.5 g/dL
[3] If Hgb remains ≥12.0 at next visit, the subject should be withdrawn from the study. If the Hgb drops below 12.0, the subject should be restarted on the next lowest dose.
[4] Only applies at Day 57 if a repeat AC2 is necessary. The dose should be decreased for the 1 week between AC2 and Additional AC2.

Section 6.11.1 ~~Estimated Dry~~ Weight and Antihypertensive Medication Changes

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added (including the section title):

It is preferred that changes to ~~EDW~~ **subject weight** and antihypertensive medication(s) are not made while the subject is part of this study, however, subjects should remain in the study regardless of any changes. All medication and dose changes should be recorded in the eCRF.

Section 6.11.3 Prohibited Medications and Non-Drug Therapies

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

Use of any of the following prescription drugs from ~~s~~**S**creening until 7 days after the last dose of randomized treatment is prohibited and will constitute a protocol violation.

- Strong inhibitors of CYP2C8 (e.g., gemfibrozil, high dose clopidogrel [300 mg])
- Strong inducers of CYP2C8 (e.g., rifampin/rifampicin)

Section 6.11.5 Iron Protocol

REVISED TEXT, where bolded text has been added:

Subjects must remain iron replete throughout the study. **If ferritin and TSAT are collected per local clinical practice while enrolled, the following is recommended.**

Iron therapy will be administered if ferritin is ≤100 ng/mL and/or TSAT is ≤20%. The investigator should choose the route of administration and dose of iron based on subject's iron status and local clinical practice.

All iron (excluding multivitamins) must be stopped and cannot be administered if:

- Ferritin >800 ng/mL **AND** TSAT >20% **OR**
- TSAT >40%

Section 7.1 Time and Events Table

Table 4 Study Procedures and Assessments on Acute Challenge Days (Treatment Period Day 1 and Day 57)

No changes made to the table itself.

Revised text in the footnotes only; text in bold has been added:

1 Procedures to be repeated if Acute Challenge 2 fails quality control criteria **(For more detail refer to Section 7.5.1)**
2 Applies to Acute Challenge 1 only
3 Timing of ABPM measurements does not correlate with specified time points in Table 4.
4 PK to be drawn from daprodustat subjects only.

Section 7.3.1.1 Time Period and Frequency for Collecting AE and SAE Information

REVISED TEXT, where bolded text has been added:

- Any SAEs assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact.

- AEs will be collected from the start of washout until the follow-up contact (see Section 7.3.1.3), at the timepoints specified in the Time and Events Table (Section 7.1).
- Medical occurrences that begin prior to the start of washout but after obtaining informed consent may be recorded on the Medical History/Current Medical Conditions section of the CRF.
- All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Section 12.4. **The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.**
- Investigators are not obligated to actively seek AEs or SAEs in former study subjects. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator must promptly notify GSK.

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

Section 7.3.1.4 Adverse Events of Special Interest

REVISED TEXT, where bolded text has been added:

The investigator or site staff will be responsible for detecting, documenting and reporting any AEs of special interest or any event that may potentially be one of the categories listed below (using preferred terms):

- Death, myocardial infarction (MI), stroke, heart failure, thromboembolic events, thrombosis of vascular access
- Thrombosis and/or tissue ischemia secondary to excessive erythropoiesis
- Cardiomyopathy
- Pulmonary artery hypertension (see also Section 7.3.1.5)
- 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**

The results of any investigation should be recorded in the relevant sections of the subject's eCRF.

Section 7.3.2 Pregnancy

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

Details of all pregnancies in female subjects **and the outcome for the neonate, if applicable**, will be collected after the start of dosing and until **14** ~~7~~ days post-last dose. If a pregnancy is reported, then the investigator should inform GSK within 2 weeks of learning of the pregnancy and should follow the procedures outlined in Section 12.4.8.

Section 7.3.6 Clinical Safety Laboratory Assessments

Table 5 Protocol Required Safety Laboratory Assessments

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

All protocol required laboratory assessments, as defined in Table 5 must be conducted in accordance with the Laboratory Manual, and Protocol Time and Events Schedule. Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, site/center number, and visit date. Details for the preparation and shipment of samples will be provided by the laboratory and are detailed in the SRM. Reference ranges for all safety parameters will be provided to the site by the laboratory responsible for the assessments.

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

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

All study-required laboratory assessments will be performed by a central laboratory, apart from HemoCue Hgb. The results of each **HemoCue** test must be entered into the eCRF.

NOTE: Local laboratory results are only required in the event that the central laboratory results are not available in time for either a treatment and/or response evaluation to be performed. If a local sample is required, it is important that the sample for central analysis is obtained at the same time. Additionally if the local laboratory results are used to make either a treatment or response evaluation, the results must be entered into the eCRF.

Hematology, clinical chemistry, and additional parameters to be tested are listed in Table 5.

Laboratory Assessments	Parameters		
Hematology	Platelet Count	*RBC Indices:*	*WBC Count with Differential:*
	RBC Count	MCV	Neutrophils
	Hemoglobin	MCH	Lymphocytes
	Hematocrit	MCHC	Monocytes
	WBC Count (absolute)	RDW	Eosinophils
	Reticulocyte Count	CHr	Basophils
Clinical Chemistry	Potassium	AST (SGOT)	Total and direct/indirect bilirubin
	Sodium	ALT (SGPT)	Total Protein
	Glucose	Calcium (Albumin adjusted)	Alkaline Phosphatase
	Albumin	Phosphate	
Other Screening Tests	Serum hCG pregnancy test	Serum ferritin	Folate
	Estradiol[1]	Serum iron	Vitamin B12
	FSH[1]	Serum transferrin	UIBC
		TSAT	

NOTE: Details of Liver Chemistry Stopping Criteria and Required Actions and Follow-Up Assessments after liver stopping or monitoring event are given in Section 12.2.

[1] As needed in women of non-child bearing potential only.

Abbreviations: ALT= Alanine transaminase; AST= Aspartate transaminase; CHr=Reticulocyte haemoglobin content; FSH=follicle stimulating hormone; MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular hemoglobin concentration; MCV= Mean corpuscular volume; RBC= Red blood cell; RDW= Red blood cell distribution width; SGOT=Serum glutamic oxaloacetic transaminase; SGPT=Serum glutamic-pyruvic transaminase; TSAT = transferrin saturation; UIBC= unsaturated iron binding capacity; WBC= White blood cells;

Section 9.2.1 Sample Size Assumptions

REVISED TEXT, where bolded text has been added:

It is expected that 31 subjects will be randomized into each treatment arm. Assuming a 20% withdrawal rate during the Hgb Maintenance Period, there will be 25 subjects per treatment arm that will undertake Acute Challenge 2. The study will continue to recruit subjects until it is projected that 25 subjects per treatment arm complete **and pass QC for** Acute Challenge 2.

Assuming a 5% significance level and a true standard deviation (SD) of 16 mmHg for average SBP over 6 hours, a sample size of 25 subjects per treatment group will provide greater than 90% power to detect a -15 mmHg difference between treatment groups. Under these assumptions, statistical significance will be obtained if there is more than a 9.1 mmHg mean difference observed in favor of daprodustat.

Section 9.3.2 Interim Analysis

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

No interim analyses are currently planned for this study.

~~An interim analysis will be conducted after approximately 24 subjects have completed Acute Challenge 2.~~

~~If the mean change in SBP from pre-Acute Challenge 1 to the 6-hour mean following both Acute Challenge 1 and Acute Challenge 2 is less than 5 mmHg in the epoetin alfa group, then the study will be stopped. However, if a clinically meaningful difference between the two treatment groups in mean change in SBP from pre-Acute Challenge 1 to the 6-hour mean following Acute Challenge 1 (i.e., >5 mmHg) is observed, then the study will be continued in order to further characterize the effects of daprodustat on blood pressure.~~

~~Simulations show that under assumptions of a true mean change in SBP of 0 mmHg and SD of 18 mmHg for both treatment groups at Acute Challenge 1 and the epoetin alfa group at Acute Challenge 2, this stopping guideline will result in stopping the trial >99% of the time. Under the assumptions of a true mean change in SBP of 8 mmHg and SD of 18 mmHg for both treatment groups at Acute Challenge 1 and the epoetin alfa group at Acute Challenge 2, this rule will result in stopping the trial <1% of the time.~~

~~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.~~

Section 9.4.1 Primary Analyses

REVISED TEXT, where bolded text has been added:

The primary analysis of average SBP over 6 hr post-dose during Acute Challenge 2 will be an analysis of covariance (ANCOVA) with terms for treatment, **prior ESA dose (low/high),** and post-HD/pre-Acute Challenge 1 SBP, difference between post-HD/pre-Acute Challenge 2 SBP and post-HD/pre-Acute Challenge 1 SBP, and treatment by (difference in post-HD SBP between Acute Challenge 1 and 2) interaction. Note that the pre-challenge SBP may change over time between Acute Challenge 1 and Acute Challenge 2 as a result of the maintenance therapy, and thus the pre-challenge SBP at Acute Challenge 2 is not a true pre-treatment covariate. As a result, the interpretation of the effect of the Acute Challenge 2 may be confounded by the impact of treatment phase on the covariate. Therefore, if the interaction term in this model is significant at the 0.10 level, then the primary model will be ANCOVA with terms for treatment, **prior ESA dose (low/high),** and post-HD/pre-Acute Challenge 1 SBP. The primary model will provide a point estimate and two-sided 95% CI for the treatment effect and a p-value for the superiority assessment. Superiority will be established if the p-value is <0.05.

Section 9.4.2 Secondary Analyses

REVISED TEXT, where bolded text has been added:

Average SBP over 6 hr post-dose in Acute Challenge 1 will be analyzed using ANCOVA with terms for treatment, **prior ESA dose (low/high),** and post-HD/pre-Acute Challenge 1 SBP. Similar analyses will be performed for DBP, MAP, and HR.

ANCOVA using the primary model will be used for the analysis of average DBP, MAP, and HR over 6 hr post-dose in Acute Challenge 2 replacing the baseline SBP term with the analogous baseline measurement for DBP, MAP, and HR respectively.

AUECs of SBP, DBP, MAP, and HR post Acute Challenge 2 and 1 will be analyzed using ANCOVA with terms for treatment **and prior ESA dose (low/high)** with 95% CIs and p-values provided for the treatment effect.

Section 11 References

REVISED TEXT, where bolded text has been added:

Campochiaro PA and the first ARVO/Pfizer Institute Working Group *et al.* Ocular versus extraocular neovascularization: Mirror images or vague resemblances. *Invest Ophthalmol & Vis Sci.* 2006; 47:462-474.

Hofherr, A., *et al*, HIF-1α drives cyst growth in advanced stages of autosomal dominant polycystic kidney disease*; Kidney International* (2018) 94, 849–851.

Kraus, A., *et al*, HIF-1α promotes cyst progression in a mouse model of autosomal dominant polycystic kidney disease; *Kidney International,* 2018.

Navaneethan SD, for CRIC investigators: Prevalence, Predictors, and Outcomes of Pulmonary Hypertension in CKD. *J Am Soc Nephrol* 2016, 27:877–886.

Section 12.1 Appendix 1 – Abbreviations and Trademarks

REVISED TEXT, where bolded text has been added:

ABPM	Ambulatory blood pressure monitoring
ADMA	Asymmetric dimethyarginine
AE	Adverse event
ALT	Alanine transaminase
AMD	Age-Related Macular Degeneration
ANCOVA	Analysis of Covariance
AST	Aspartate transaminase
AUEC	Area under the effect curve
AUC (0-24)	Area under concentration-time curve from time zero to 24 hours
BP	Blood pressure
CHr	Reticulocyte hemoglobin content
CI	Confidence interval
CKD	Chronic kidney disease
Cmax	Maximum observed concentration
CPK	Creatine phosphokinase
CV	Cardiovascular
DBP	Diastolic blood pressure
dL	Deciliter

DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
ECHO	**Echocardiogram**
eCRF	Electronic Case Report Form
EDW	Estimated Dry Weight
EPO	Erythropoietin
ESA	Erythropoiesis-stimulating agent
ET-1	Endothelin-1
FDA	Food and Drug Administration
FRP	Females of Reproductive Potential
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
G	Gram
GI	Gastrointestinal
GSK	GlaxoSmithKline
hCG	Human chorionic gonadotrophin
HD	Hemodialysis dependent
HDPE	High Density Polyethylene
HF	Heart Failure
Hgb	Hemoglobin
HIF	Hypoxia-inducible factor
HR	Hour/heart rate
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
IU	International Unit
IV	Intravenous
IVWRS	Interactive Voice/Web Response System
KDIGO	Kidney Disease Improving Global Outcomes
KG	Kilogram
LDH	Lactate dehydrogenase
MAP	Mean Arterial Blood Pressure
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MG	Milligram
MI	Myocardial infarction
ML	Milliliter
mmHG	Millimeter of mercury
MSDS	Material Safety Data Sheet
ND	Non-dialysis dependent
NO	Nitric Oxide

NOAEL	No Observed Adverse Effect Level
NYHA	New York Heart Association
PASP	**Pulmonary Artery Systolic Pressure**
PCI	**Percutaneous Coronary Intervention**
PD	Pharmacodynamic
PEG	Polyethylene glycol
PGI_2	Prostaglandin I_2
PHD	Prolyl hydroxylase domain
PHI	Prolyl hydroxylase inhibitor
PK	Pharmacokinetic
proADM	Proadrenomedullin
PRS	Project Requirement Specification
PRVP	Peak Right Ventricular Pressure
PSRAE	Possible suicidality related adverse event
QC	Quality Control
QTc	QT interval corrected for heart rate
QTcB	QT interval corrected for heart rate using Bazett's formula
RAP	Reporting and Analysis Plan
RBC	Red blood cell
RDW	Red blood cell distribution width
rhEPO	Recombinant human erythropoietin
RNA	Ribonucleic acid
SAE	Serious adverse event
SBP	Systolic blood pressure
SC	Subcutaneous
SD	Standard deviation
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic-pyruvic transaminase
sPAP	Systolic Pulmonary Artery Pressure
SRM	Study Reference Manual
T1/2	Terminal phase half-life
TIBC	Total iron binding capacity
Tmax	Time of occurrence of Cmax
TSAT	Transferrin saturation
UG	Microgram
UIBC	Unsaturated iron binding capacity
ULN	Upper limit of normal
URR	Urea reduction ratio
WBC	White blood cells

Section 12.3 Appendix 3: Daprodustat Risk Assessment

REVISED TEXT, where text in strikethrough has been removed and bolded text has been added:

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Excessive erythropoiesis ~~(polycythemia)~~ 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.** ~~Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat.~~ 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**	• Specific eligibility criteria related to requirements for entry Hgb are detailed in Section 5 • Hgb will be closely monitored throughout the dosing period as outlined in the Time and Events Table (Section 7.1) • Specific guidance for **dose adjustment, dose interruption, or** discontinuation of daprodustat based on achieved Hgb **(including rate of change)** is provided in Section 5.5.1.1 • **Instream m**~~M~~onitoring of ~~emerging~~ safety data by an internal ~~GSK~~ **s**~~S~~afety r~~R~~eview ~~T~~team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	appropriately according to target Hgb. However, experience with daprodustat is currently insufficient to fully characterize this risk.	
Worsening hypertension	**In a dog cardiovascular study, single oral doses of daprodustat (up to 90 mg/kg) did not produce effects on blood pressure.** **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)]:** ○ **The majority (>90%) of subjects had baseline history of hypertension.** ○ **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.** ○ **Although no clinically meaningful changes in blood pressure were observed, subjects in both treatment groups required increases in anti-HTN medications:**	• **Specific eligibility criteria related to blood pressure, including exclusion of subjects with uncontrolled hypertension, are detailed in Section 6.2** • **Blood pressure will be closely monitored throughout the dosing period as outlined in the Time and Events Table Section 7.1.** • **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
	○ **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.** ○ **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 (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/~~ESAs~~ **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.** **In non-clinical studies conducted to date, not observed at tolerated doses when hemoglobin/hematocrit within normal range for species.** **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**	• Specific eligibility criteria related to CV risk are outlined in Section 5.3 • Hgb will be closely monitored throughout the dosing period as outlined in the Time and Events Table Section 7.1 • **Instream m**~~M~~onitoring of ~~emerging~~ safety data by an internal ~~GSK~~ **s**~~S~~afety r~~R~~eview ~~T~~team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	makes it difficult to draw any firm conclusions. ~~Not observed at tolerated doses when hemoglobin/hematocrit within normal range for species.~~ The clinical data received to **date from completed clinical trials with daprodustat** are insufficient to ~~conclude~~ **substantiate** or refute this risk.	
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 ~~rats~~ **rodents**, stomach erosions were observed with intravenous and oral administration of daprodustat. ~~Stomach erosions/ulcers also reported in rats with marketed rhEPOs/ESAs.~~ **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 should be investigated diagnostically (i.e. endoscopic examination) as clinically warranted • **Instream m~~M~~**onitoring of ~~emerging~~ safety data by an internal ~~GSK Safety Review~~ **safety review** team
Cancer-related mortality and tumor progression and recurrence	Marketed rh~~e~~EPO **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	• Specific eligibility criteria related to personal history of malignancy or subjects with complex kidney cyst are outlined in Section 5.3.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	circulating 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 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.** ~~There were no daprodustat-related neoplastic findings in a 2-year rat oral carcinogenicity study (lifetime study).~~ ~~In clinical studies conducted to date, administration of daprodustat has been associated with:~~ ~~Once daily administration:~~ • ~~In studies up to 4 weeks duration, a dose-ordered increase in VEGF plasma concentrations at doses ranging from 10 to 150 mg.~~ • ~~In studies up to 24 weeks duration at doses up to 25 mg, changes in VEGF plasma concentration were variable but similar relative to control.~~ • ~~Systemic EPO concentrations within the physiologic range.~~	• Stopping criteria for subjects with treatment emergent malignancy are outlined in Section 5.5.1. • **Instream m**~~M~~onitoring of ~~emerging~~ safety data by an internal ~~GSK Safety Review~~ **safety review** team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	~~Three times weekly administration:~~ • ~~In studies up to 4 weeks duration at doses of 10 to 30 mg:~~ o ~~Dose dependent increases in plasma VEGF and EPO concentrations were observed.~~ o ~~Pre-dose concentrations of EPO and VEGF were near or below baseline indicating no accumulation of EPO or VEGF after three times weekly dosing.~~ ~~Following review of clinical data received to date, this has not been identified as a safety concern for daprodustat.~~	
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 (up to 13-weeks duration in ~~mice and~~ dogs, up to ~~26-weeks~~ **2 years** in rat**s 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. These hypoxia-induced PRVP changes fall within the range of PRVP differences noted among non-treated 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 ha**d**~~s~~ no clinically significant effect on **transthoracic** echocardiographically **(ECHO)** estimate**s**~~d~~ of ~~systolic~~ pulmonary artery **systolic** pressure (sPA**S**P) under either normoxic or hypoxic conditions. ECHO assessments performed in Phase 2b studies (24 weeks treatment	• **Instream m**~~M~~onitoring of **emerging** safety data by an internal ~~GSK Safety Review~~ **safety review** team

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	duration) did not identify any clinically meaningful changes in ~~sPAP~~ **PASP** in ~~subjects~~ **participants** not on dialysis **for daprodustat**. In hemodialysis ~~subjects~~ **participants**, mean absolute change from baseline in ~~sPAP~~ **PASP** was similar for both treatment groups; however, there was a numeric imbalance (Daprodustat **Total**: 8 [7%]; Control 0) in ~~subjects~~ **participants** reaching the ~~sPAP~~ **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 ~~subjects~~ **participants** with resolution of ~~sPAP~~ **PASP** on safety follow-up ECHOs had confounding conditions that could contribute to resolution other than discontinuation of study ~~treatment~~ **drug**; and there was no dose relationship for ~~subjects~~ **participants** meeting the ~~sPAP~~ **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 commonly cited in the literature [Navaneethan, 2016]. Subjects with 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**	

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	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.** ~~With lifetime exposure to daprodustat in a 2-year rat oral carcinogenicity study, an exacerbation of rat spontaneous, progressive cardiomyopathy (PCM)(focal myofiber degeneration/necrosis with inflammatory infiltrates) was observed at doses of 0.8 mg/kg/day and above, although total incidence and severity distribution within any daprodustat-group were within historical control ranges. This is consistent with an equivocal threshold for exacerbation of spontaneous, progressive cardiomyopathy at 0.8 mg/kg/day which is also the threshold dose for observing increased Hct values in individual rats.~~ 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 LVEF for	• **Instream m**~~M~~onitoring of ~~emerging~~ safety data by an internal ~~GSK~~ **s**~~S~~afety **r**~~R~~eview **t**~~T~~eam

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	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 60 mg/kg daprodustat to mice caused minimal increases in circulating VEGF while significant EPO increases were observed. ~~Aside from congestion of retinal vessels and optic disc hyperemia secondary to markedly increased red cell mass, there were no ocular abnormalities observed in non-clinical studies.~~ **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 up to 4 weeks duration, a dose-ordered increase in VEGF plasma concentrations was observed at doses ranging from 10 to 150 mg administered once daily and from 10 to 30 mg administered three times weekly. In studies up to 24 weeks duration at doses up to 25 mg, 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	• **Instream m**~~M~~onitoring of ~~emerging~~ safety data by an internal ~~GSK~~ **s**~~S~~afety **r**~~R~~eview **t**~~T~~eam

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	proliferative retinopathy, macular edema, or choroidal neovascularization with 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 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 m~~M~~**onitoring of ~~emerging~~ safety data by an internal ~~GSK~~ **s~~S~~**afety **r~~R~~**eview **t~~T~~**eam
Drug-drug interactions	**Daprodustat is a substrate of CYP2C8:** Co-administration of daprodustat with a strong CYP2C8 inhibitor 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.,**	• Co-administration of daprodustat with strong CYP2C8 inhibitors (e.g., gemfibrozil) and inducers (e.g., rifampin/rifampicin) is not

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	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 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. ~~Although 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 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**	permitted as outlined in Section 6.11.3. • 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 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 6.11. • Hgb will be closely monitored throughout the dosing period as outlined in the Time and Events Table (Section 7.1) • Specific guidance for **dose adjustment, dose interruption, or** discontinuation of daprodustat based on achieved Hgb is provided in Section 5.5.1.1. • **Instream m~~M~~**onitoring of ~~emerging~~ safety data by an internal ~~GSK~~ **s~~S~~**afety **r~~R~~**eview **t~~T~~**eam

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	100mg daprodustat with an OATP1B1/1B3 substrate (i.e., rosuvastatin) showed no PK interaction at these doses of daprodustat. ~~Population PK analysis from completed Phase 2 studies suggests that co-administration of daprodustat with a moderate CYP2C8 inhibitor, leads to a ~ 2-fold increase in AUC, with no clinically-significant increase in the measured Hgb response.~~ ~~Daprodustat is an inhibitor of CYP2C8 *in vitro*, with an IC_{50} value of 21 µM.~~ ~~Population PK analysis from completed Phase 2 studies suggests that co-administration of daprodustat with clopidogrel (a moderate CYP2C8 inhibitor) leads to a ~ 2-fold increase in AUC, with no clinically-significant increase in the measured Hgb response~~ ~~Co-administration of daprodustat with potent BCRP inhibitors has the potential to increase exposure of daprodustat. Use of BCRP inhibitors (mostly weak) was found to result in a small change in metabolite exposure (20% increase in AUC).~~ ~~Daprodustat is an inhibitor of OATP1B1/1B3 *in vitro*, with IC_{50} values of 6 µM and 11 µM, respectively. A clinical drug interaction study between 25 mg daprodustat with either a CYP2C8 substrate or an OATP1B1/1B3 substrate showed that there is no PK interaction at this dose of daprodustat.~~	
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. However, the dose of FG-2216 (a PHI) used resulted in a significant erythropoietic response as reflected by ≥10% relative increases in hematocrit over the course of the study (Kraus, 2018; Hofherr, 2018). (Kraus, 2018; Hofherr,**	• **Kidney function will be monitored throughout the dosing period as outlined in the Time and Events Table (Section7.1).** • **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
	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.**	
Other		
rhEPO risks (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 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, thromboembolic events, thrombosis of vascular access; and for **increased** cancer-related mortality and tumor progression. • Specific eligibility criteria related to blood pressure management are outlined in Section 5. • Specific eligibility criteria related to personal

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		history of pure red cell aplasia are outlined in Section 5.3