Statistical Analysis Plan

Study ID: 201771

Official Title of Study: A repeat dose, open label, two period, randomized, cross over study to compare the effect of daprodustat to recombinant, human erythropoietin (rhEPO) on oral iron absorption in adult participants with anemia associated with chronic kidney disease who are not on dialysis

NCT ID: NCT03457701

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TITLE PAGE

Protocol Title:

A repeat dose, open label, two period, randomized, cross over study to compare the effect of daprodustat to recombinant, human erythropoietin (rhEPO) on oral iron absorption in adult participants with anemia associated with chronic kidney disease who are not on dialysis

Study Number: 201771

Compound Number: GSK1278863

Abbreviated Title: **A**nemia **S**tudies in **C**KD: **E**rythropoiesis via a **N**ovel PHI **D**aprodustat − **I**ron (**ASCEND-Fe**)

Sponsor Name: GlaxoSmithKline Research & Development Limited

Regulatory Agency Identifier Number(s)

Registry	**ID**
Clintrials.gov	**NCT03457701**
IND Number	**101,291**

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1. INTRODUCTION

The purpose of this SAP is to describe the planned analysis to be included in the Clinical Study Report for Protocol 201771. Details of the planned final analysis provided.

Additional detail with regards to data handling conventions and the specification of data displays will be provided in the Output and Programming Specification (OPS) document. Listings not required by ICH guidelines may be produced via the RAPIDO data viewer tool.

1.1. Objectives, Estimands and Endpoints

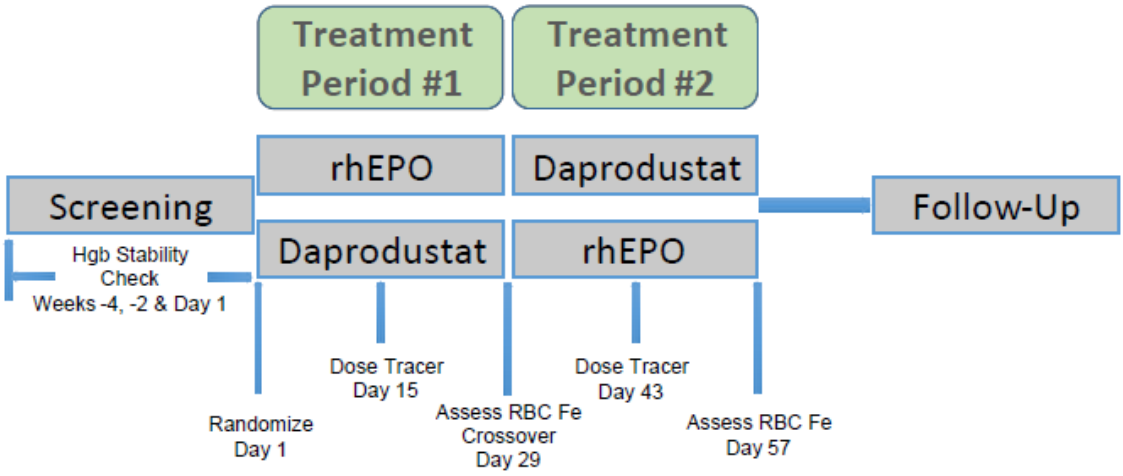
Since the primary endpoint analyses are based on the Evaluable Population, subjects with intercurrent events who discontinue treatment in either period will be excluded from the primary analysis. Missing data post withdrawal will not be imputed.

Objectives	Endpoints
Primary	
• Compare the efficacy of daprodustat to rhEPO (i.e., epoetin alfa or darbepoetin alfa) on iron absorption	• Difference in fractional oral iron absorption (%) between daprodustat and rhEPO
Secondary	
• Characterize the effect of daprodustat or rhEPO on markers of iron status • Characterize the effect of daprodustat or rhEPO on indices of erythropoiesis	• Change from baseline (Day 1) at 14 and 28 days following treatment with daprodustat or rhEPO in serum iron, transferrin, transferrin saturation, soluble transferrin receptor, ferritin, hepcidin and erythroferrone • Change from baseline (Day 1) at 14 and 28 days following treatment with daprodustat or rhEPO in Hgb, hematocrit, red blood cell number, mean corpuscular volume, reticulocyte Hgb and reticulocyte number

Objectives	Endpoints
Safety	
• Assess safety and tolerability	• Incidence and severity of adverse events and serious adverse events • Reasons for discontinuation of study medication Vital signs assessments

1.2. Study Design

Overview of Study Design and Key Features



Design Features	This study will be a randomized, repeat dose, open label, two period cross-over study in adult, male and female participants with anemia associated with chronic kidney disease who are not on dialysis currently treated with stable doses (≤ 50% change in 4-weekly dose) for at least 8 weeks prior to and including the screening period, of rhEPO (i.e.,epoetin alfa or darbepoetin alfa). • There will be a 4-week screening period in order to confirm that participants have stable Hgb levels and to assess entry criteria • A 28-day Treatment Period 1 for incorporation of iron into erythrocytes while on randomized study treatment • A 28-day Treatment Period 2 for a second incorporation of iron into erythrocytes while on randomized study treatment • A follow-up visit 14 ± 3 days after completing treatment.
Study intervention	• Daprodustat dosing information: **Darbepoetin alfa (µg/4 wk SC/IV)** | **Epoetin alfa (convert SC to IV U/week)[1]** | **Daprodustat dose (mg, once daily)** 20 to 30 | 1500 to 2000 | 1 >30 to 300 | >2000 to <20000 | 2 >300 | ≥20000 | 4 1 Standardized rhEPO IV dose (U/week) = 161/113 x (epoetin SC dose (units)/frequency) • rhEPO Dosing Information ➢ Participants are treated with stable doses (≤ 50% change in 4-weekly dose) of rhEPO for at least 8 weeks prior to and including the screening period.

<table>
<tr><th colspan="2">Overview of Study Design and Key Features</th></tr>
<tr><td></td><td>

57Fe Dosing Information

A 57Fe ferrous sulfate oral solution (10 mL) will be administered containing 10 mg of 57Fe (iron concentration at 1 mg/mL with 1:1 molar ratio ascorbic acid in the solution).

58Fe Dosing Information

A 58Fe ferrous sulfate oral solution (3 mL) will be administered containing 3 mg of 58Fe followed by a 56Fe (natural abundance) ferrous sulfate oral solution (7 mL) containing 7 mg of 56Fe. Both solution contains iron at 1 mg/mL with ascorbic acid at 1:1 molar ratio.
</td></tr>
<tr><td>Study intervention Assignment</td><td>
Participants will be randomly assigned either to remain on their current therapy (either epoetin alfa or darbepoetin alfa) or be switched to daprodustat for Treatment Period 1.
For assessment of incorporation of iron into erythrocytes, enrolled participants will be administered ferrous sulfate containing a stable isotope of iron (57Fe or 58Fe) orally in a randomized fashion following 2 weeks of administration of randomized study treatment. Two weeks following oral iron administration a blood sample will be taken in order to assess the incorporation of the iron isotope into red blood cells by thermal ionization mass spectrometry(TIMS).

At Day 29 participants will be crossed over to the study treatment they did not receive in Treatment Period 1. At 2 weeks following initiation of dosing, participants will again be administered ferrous sulfate containing the stable iron isotope not administered following the assessment in Treatment Period 1 orally. Two weeks following oral iron administration a blood sample will again be taken in order to assess the incorporation of the iron isotope into red blood cells by TIMS.
Participants will attend a follow-up visit 14 ± 3 days following the final blood sample for assessment of red blood cell iron.

There are 4 possible treatment sequences:

Possible Treatment Sequences:
<table>
<tr><th></th><th>Sequence A
N=3</th><th>Sequence B
N=3</th><th>Sequence C
N=3</th><th>Sequence D
N=3</th></tr>
<tr><td>Treatment Period 1</td><td>rhEPO
^{57}Fe</td><td>rhEPO
^{58}Fe</td><td>Daprodustat
^{57}Fe</td><td>Daprodustat
^{58}Fe</td></tr>
<tr><td>Treatment Period 2</td><td>Daprodustat
^{58}Fe</td><td>Daprodustat
^{57}Fe</td><td>rhEPO
^{58}Fe</td><td>rhEPO
^{57}Fe</td></tr>
</table>
</td></tr>
<tr><td>Interim Analysis</td><td>

No interim analysis will be performed in this study.

</td></tr>
</table>

2. STATISTICAL HYPOTHESES

The primary analysis will test whether daprodustat is superior to rhEPO (i.e., epoetin alfa or darbepoetin alfa) according to the following statistical hypotheses:

Null: The difference in fractional iron absorption between treatment arms (daprodustat-rhEPO (i.e., epoetin alfa or darbepoetin alfa)) is equal to zero.

Alternative: The difference in fractional iron absorption between treatment arms (daprodustat-rhEPO (i.e., epoetin alfa or darbepoetin alfa)) is not equal to zero.

2.1. Multiplicity Adjustment

No multiplicity adjustments will be performed.

3. ANALYSIS SETS

Analysis Set	Definition / Criteria	Analyses Evaluated
Screened Population	• This population consists of all participants who signed an ICF to participate in the clinical trial. • This population will be used for summarizing screening failure rates and reasons for screening failure.	• Study Population
Enrolled population	• This population consists of all randomized subjects.	• Study Population
Safety Population	• This population consists of all randomized participants who received at least one dose of study treatment.	• Study Population • Safety
Evaluable Population	• This population consists of all randomized participants who received study medication and completed the iron absorption assessment for both treatment periods.	• Primary Endpoint • Secondary Endpoints
Modified Intent to Treat (mITT) Population	• This population consists of all randomized participants who received study medication and completed the iron absorption assessment at the end of the first treatment period. • This population will be used for the sensitivity analysis of the primary endpoint.	Sensitivity analyses of Primary Endpoint

4. STATISTICAL ANALYSES

4.1. General Considerations

4.1.1. General Methodology

Unless otherwise specified, continuous data will be summarized using descriptive statistics: n, mean, standard deviation (SD), median, minimum, and maximum. Categorical data will be summarized as the number and percentage of participants in each category.

It is anticipated that patient accrual will be spread thinly across centers and summaries of data by center would unlikely be informative and may not, therefore, be provided.

4.1.2. Baseline Definition

For all endpoints the baseline value will be the latest pre-dose assessment with a non-missing value, including those from
unscheduled visits in the associated treatment period. If time is not collected, Day 1 and Day 29
assessments are assumed to be taken prior to first dose and used as baseline.

Parameter	Study Assessments Considered as Baseline				Baseline Used in Data Display
	Screening (Week -4)	Screening (Week -2)	Day 1 (Pre-Dose)	Day 29 (Pre-Dose)	
Vital Signs	X		X		Day 1
Iron Status Markers	X	X	X	X	Day 1 for Period 1, Day 29 for Period 2
Indices of erythropoiesis			X	X	Day 1 for Period 1, Day 29 for Period 2

Unless otherwise stated, if baseline data is missing no derivation will be performed and baseline will be set to missing.

4.2. Primary Endpoint(s) Analyses

The primary objective of this study is to compare daprodustat to rhEPO (i.e., epoetin alfa or darbepoetin alfa) on fractional iron absorption. Evaluable population will be used for this analysis.

4.2.1. Definition of endpoint(s)

Objectives	Endpoints
Primary Objectives	**Primary Endpoints**
• Compare the efficacy of daprodustat to rhEPO (i.e., epoetin alfa or darbepoetin alfa) on iron absorption	• Difference in fractional oral iron absorption (%) between daprodustat and rhEPO

The process for calculating the factional iron absorption will depend on the sequence and is defined separately for each iron isotope. Formulas will be based on IAEA guidance for assessment of iron bioavailability in humans (IAEA 2012).

Sequences A and C:

S. No.	Parameters	Period 1 -57Fe Fractional Oral Iron Absorption	Period 2 -58Fe Fractional Oral Iron Absorption	Details
Parameters provided in data				
1	Hgb in g/dL	$Hgb\ Day\ 15$	$Hgb\ Day\ 43$	We will use the Hgb (g/dL) measurement from the day of isotope dosing (Day 15 for Period 1, Day 43 for Period 2)
2	BV in mL	$70 * Day\ 15\ Weight$	$70 * Day\ 43\ Weight$	We will assume a blood volume (BV) of 70 mL/kg and a fixed volume per kg. Weight represents the

S. No.	Parameters	Period 1 -57Fe Fractional Oral Iron Absorption	Period 2 -58Fe Fractional Oral Iron Absorption	Details
				weight measurement (kg) taken on the day of dosing (Day 15 for Period 1, Day 43 for Period 2).
3a	Baseline 57Fe/56Fe	0.02317	N/A	Natural abundance measure for iron isotope, which is an assumed constant for this analysis.
3b	Baseline 58Fe/56Fe	N/A	Value3b	Baseline measurement for 58Fe in

S. No.	Parameters	Period 1 -57Fe Fractional Oral Iron Absorption	Period 2 -58Fe Fractional Oral Iron Absorption	Details
				Period 2 will utilize the Day 43 blood sampling for isotopic iron, as the 57Fe solution in Period 1 may contain small quantities of 58Fe. This measurement will be provided by Cornell University.
4a	Enriched 57Fe/ 56Fe	Value4a	N/A	Enriched iron value is calculated internally on the

S. No.	Parameters	Period 1 -57Fe Fractional Oral Iron Absorption	Period 2 -58Fe Fractional Oral Iron Absorption	Details
				spectroscopy instrument and provided by Cornell University.
4b	Enriched 58Fe/56Fe	N/A	Value4b	Enriched iron value is calculated internally on the spectroscopy instrument and provided by Cornell University.
Parameters derived by S&P				
5	% excess	$\frac{Value4a - Value3a}{Value3a} * 100$	$\frac{Value4b - Value3b}{Value3b} * 100$	S&P will derive this value. Δ% excess is used

S. No.	Parameters	Period 1 -57Fe Fractional Oral Iron Absorption	Period 2 -58Fe Fractional Oral Iron Absorption	Details
				to express measurements of enrichment relative to baseline. We will use the following formula suggested by the IAEA for the assessment of iron bioavailability (IAEA pg.20).
6	Total circulating Fe (mg)	$Hgb * BV * 3.47 * 0.01$	$Hgb * BV * 3.47 * 0.01$	S&P will derive this value, using the formula

S. No.	Parameters	Period 1 -57Fe Fractional Oral Iron Absorption	Period 2 -58Fe Fractional Oral Iron Absorption	Details
				suggested by the IAEA for the assessment of iron bioavailability (IAEA pg.33).
7	Total incorporated iron isotope (mg)	$Total\ incorporated\ 57Fe = Parameter5 * Parameter6 * 0.00287 *$	$Total\ incorporated\ 58Fe = Parameter4 * Parameter6 * 0.00287 *$	S&P will derive this value. We assume the fractional abundance of 57Fe in mg is (0.0214 g * 0.01), and the fractional abundance of 58Fe in mg is (0.0028 7 g *

S. No.	Parameters	Period 1 -57Fe Fractional Oral Iron Absorption	Period 2 -58Fe Fractional Oral Iron Absorption	Details
				0.01).
8	% iron isotope incorporated	$\%\ 57Fe\ Incorporated = \frac{Parameter7}{57Fe\ Dose}$	$\%\ 58Fe\ Incorporated = \frac{Parameter7}{58Fe\ Dose}$	S&P will derive percent of iron isotope incorporated. 57Fe and 58Fe doses (mg) will be determined using the density of ferrous sulfate (FeSO4) oral solution (1g/mL), the dose of oral solution (in mL),

S. No.	Parameters	Period 1 -57Fe Fractional Oral Iron Absorption	Period 2 -58Fe Fractional Oral Iron Absorption	Details
				and concentration of elemental iron (1mg/mL).
9	**Assumed % iron isotope absorbed if 80%**	$\%\ 57Fe\ absorbed = Parameter8 * \frac{100}{80}$	$\%\ 58Fe\ absorbed = Parameter8 * \frac{100}{80}$	S&P will derive this value, using the formula suggested by the IAEA for the assessment of iron bioavailability (IAEA pg.38). Parameter 9 defines the fractional oral iron absorption

S. No.	Parameters	Period 1 -57Fe Fractional Oral Iron Absorption	Period 2 -58Fe Fractional Oral Iron Absorption	Details
				endpoint, assuming 80% of the absorbed Fe is incorporated into RBCs.

Sequences B and D:

S. No.	Parameters	Period 1 -58Fe Fractional Oral Iron Absorption	Period 2 -57Fe Fractional Oral Iron Absorption	Details
Parameters provided in data				
1	Hgb in g/dL	*Hgb Day 15*	*Hgb Day 43*	We will use the Hgb (g/dL) measurement from the day of isotope dosing (Day 15 for Period 1, Day 43 for

S. No.	Parameters	Period 1 -58Fe Fractional Oral Iron Absorption	Period 2 -57Fe Fractional Oral Iron Absorption	Details
				Period 2)
2	BV in mL	$70 * Day\ 15\ Weight$	$70 * Day\ 43\ Weight$	We will assume a blood volume (BV) of 70 mL/kg and a fixed volume per kg. Weight represents the weight measurement (kg) taken on the day of dosing (Day 15 for Period 1, Day 43 for Period 2).
3a	Baseline 57Fe/	N/A	Value3a	Baseline measurement

S. No.	Parameters	Period 1 -58Fe Fractional Oral Iron Absorption	Period 2 -57Fe Fractional Oral Iron Absorption	Details
	56Fe			for 57Fe in Period 2 will utilize the Day 43 blood sampling for isotopic iron, as the 58Fe solution in Period 1 may contain small quantities of 57Fe. This measurement will be provided by Cornell University.
3b	Baseline 58Fe/56Fe	0.00307	N/A	Natural abundance measure for iron

S. No.	Parameters	Period 1 -58Fe Fractional Oral Iron Absorption	Period 2 -57Fe Fractional Oral Iron Absorption	Details
				isotope, which is an assumed constant for this analysis.
4a	Enriched 57Fe/56Fe	N/A	Value4a	Enriched iron value is calculated internally on the spectroscopy instrument and provided by Cornell University.
4b	Enriched 58Fe/56Fe	Value4b	N/A	Enriched iron value is calculated internally on the

S. No.	Parameters	Period 1 -58Fe Fractional Oral Iron Absorption	Period 2 -57Fe Fractional Oral Iron Absorption	Details
				spectroscopy instrument and provided by Cornell University.
Parameters derived by S&P				
5	% excess	$\frac{Value4b - Value3b}{Value3b} * 100$	$\frac{Value4a - Value3a}{Value3a} * 100$	S&P will derive this value. Δ% excess is used to express measurements of enrichment relative to baseline. We will use the following formula suggested by

S. No.	Parameters	Period 1 -58Fe Fractional Oral Iron Absorption	Period 2 -57Fe Fractional Oral Iron Absorption	Details
				the IAEA for the assessment of iron bioavailability (IAEA pg.20).
6	Total circulating Fe (mg)	$Hgb * BV * 3.47 * 0.01$	$Hgb * BV * 3.47 * 0.01$	S&P will derive this value, using the formula sugges ted by the IAEA for the assessment of iron bioavailability (IAEA pg.33).
7	Total incorporated iron isotope (mg)	$Total\ incorporated\ 58Fe = Parameter5 * Parameter6 * 0.0214 *$	$Total\ incorporated\ 57Fe = Parameter5 * Parameter6 * 0.0214 *$	S&P will derive this value. We assume

S. No.	Parameters	Period 1 -58Fe Fractional Oral Iron Absorption	Period 2 -57Fe Fractional Oral Iron Absorption	Details
				the fractional abundance of 57Fe in mg is (0.0214 g * 0.01), and the fractional abundance of 58Fe in mg is (0.00287 g * 0.01).
8	% iron isotope incorporated	$\%\ 58Fe\ Incorporated = \frac{Parameter7}{58Fe\ Dose} * 100$	$\%\ 57Fe\ Incorporated = \frac{Parameter7}{57Fe\ Dose} * 100$	S&P will derive percent of iron isotope incorporated. 57Fe and 58Fe doses (mg) will be determined using the

S. No.	Parameters	Period 1 -58Fe Fractional Oral Iron Absorption	Period 2 -57Fe Fractional Oral Iron Absorption	Details
				density of ferrous sulfate (FeSO4) oral solution (1g/mL), the dose of oral solution (in mL), and concentration of elemental iron (1mg/mL).
9	**Assumed % iron isotope absorbed if 80%**	$\%\ 58Fe\ absorbed = Parameter8 * \frac{100}{80}$	$\%\ 57Fe\ absorbed = Parameter8 * \frac{100}{80}$	S&P will derive this value, using the formula suggested by the IAEA for the assessment

S. No.	Parameters	Period 1 -58Fe Fractional Oral Iron Absorption	Period 2 -57Fe Fractional Oral Iron Absorption	Details
				of iron bioavailability (IAEA pg.38). Parameter 9 defines the fractional oral iron absorption endpoint, assuming 80% of the absorbed Fe is incorporated into RBCs.

4.2.2. Main analytical approach

Endpoint / Variables
• Fractional oral iron absorption (%)
Model Specification
• To compare daprodustat to rhEPO on fractional iron absorption, a mixed effects model will be fitted • Terms fitted in the mixed effect model will include:

Endpoint / Variables
• Fixed effect: treatment, period, and iron isotope • Random effect: participant • The Kenward and Roger method for approximating the denominator degrees of freedom and correcting for bias in the estimated variance-covariance of the fixed effects will be used. • Using PROC MIXED in SAS, the covariance for the random participant effect (G matrix) will be fit using an unstructured covariance structure by specifying 'type=UN' on the RANDOM line. • In the event that this model fails to converge, alternative covariance structures may be considered such as Variance Components or Compound Symmetry. • Akaike's Information Criteria (AIC) will be used to assist with the selection of covariance structure.
Model Checking & Diagnostics
• For the Mixed Model, model assumptions will be checked, and appropriate adjustments may be applied based on the data. • Distributional assumptions underlying the model used for analysis will be examined by obtaining a normal probability plot of the residuals and a plot of the residuals versus the fitted values (i.e. checking the normality assumption and constant variance assumption of the model respectively) to gain confidence that the model assumptions are reasonable. • If there are any departures from the distributional assumptions, alternative transformations, such as data squared or square root of data, will be explored. • Non-parametric analyses will be conducted if the normality assumption does not hold.
Model Results Presentation
Fractional oral iron absorption will be summarized using n, mean, SD, median, minimum, and maximum. Point estimates, corresponding 95% confidence intervals, and two-sided p-values will be constructed for the comparison of efficacy of daprodustat to rhEPO.

4.2.3. Sensitivity analyses

A sensitivity analysis for the primary analysis will be performed using the same mixed modelling approach, except with the mITT population.

4.3. Secondary Endpoint(s) Analyses

Markers of iron status endpoints will include serum iron, transferrin, transferrin saturation, soluble transferrin receptor, ferritin, hepcidin, and erythroferrone.

Indices of erythropoiesis endpoints will include Hgb, haematocrit, red blood cell number, mean corpuscular volume, reticulocyte Hgb, and reticulocyte number.

Change from baseline (n, mean, standard deviation, 95% CI of mean, median, minimum, and maximum) at Day 14, Day 29, Day 43, and Day 57 will be calculated for iron markers and indices of erythropoiesis by treatment group. Day 1 (Pre-dose) and Day 29 (Pre-dose) will be the baseline for both iron markers as well as indices of erythropoiesis for their respective periods.

Descriptive statistics (n, arithmetic mean, standard deviation, 95% CI, minimum, median, and maximum) will be calculated for iron markers and indices of erythropoiesis at each scheduled visit by treatment group. No formal statistical testing will be performed. Hepcidin and ferritin values will be log-transformed prior to summary.

All analyses on secondary endpoints will be based on the "Evaluable" population, unless otherwise specified.

4.4. Safety Analyses

The safety analyses will be based on the "Safety" population, unless otherwise specified.

4.4.1. Extent of Exposure

- Number of days of exposure to study drug will be calculated based on the formula: **Duration of Exposure in Days = Treatment Stop Date – (Treatment Start Date) + 1**
- Duration will be summarized by treatment group. Each subject will contribute duration of exposure to the treatment taken.
- Participants who were randomized but did not report a treatment start date will be categorised as having zero days of exposure.

A listing and summary table of exposure will be created.

4.4.2. Adverse Events

Adverse events analyses including the analysis of adverse events (AEs), Serious AEs (SAEs) and other significant AEs will be based on GSK Core Data Standards. An overview summary of AEs, including counts and percentages of participants with any AE, AEs related to study intervention, SAEs related to study intervention, fatal SAEs, and fatal SAEs related to study intervention will be produced. Adverse events will be coded using the standard Medical Dictionary for Regulatory Affairs (MedDRA dictionary). A summary of number and percentage of participants with any adverse events by maximum severity will be produced. A separate summary will be provided for

study intervention-related AEs. A study intervention-related AE is defined as an AE for which the investigator classifies the possible relationship to study intervention as "Yes". A worst-case scenario approach will be taken to handle missing relatedness data, i.e. the summary table will include events with the relationship to study intervention as 'Yes' or missing. The summary table will be displayed by PT only. All SAEs will be tabulated based on the number and percentage of participants who experienced the event. Separate summaries will also be provided for study intervention-related SAEs. The summary tables will be displayed by PT and SOC. A study intervention-related SAE is defined as an SAE for which the investigator classifies the relationship to study intervention as "Yes". A worst-case scenario approach will be taken to handle missing data, i.e. the summary table will include events with the relationship to study intervention as 'Yes' or missing**.** Summaries and listings of adverse events will include treatment sequence. A listing of AEs that occur during Follow-up will be provided. AEs leading to permanent discontinuation of study intervention and study intervention related AEs leading to permanent discontinuation of study intervention will be listed.

4.4.2.1. Adverse Events of Special Interest

Adverse Events of Special Interest (AESI) have been identified based on non-clinical studies with daprodustat, clinical experience with rhEPOs, and current information regarding HIF-regulated pathways in mediating hypoxia-associated pathophysiology. The following will be considered adverse events of special interest for the purpose of analyses:

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

The summary of event characteristics will be provided for each AESI respectively, including number of participants with any event, number of events, number of participants with any event that is serious. The percentage will be calculated in two ways, one with number of participants with event as the denominator and the other with total number of participants as the denominator. The worst-case approach will be applied at participant level for the maximum grade, i.e. a participant will only be counted once as the worst case from all the events experienced by the participant. For action taken to an event, a participant will be counted once under each action, e.g. if a participant has an event leading to both study intervention discontinuation and dose reduction, the participants will be counted once under both actions.

4.4.2.2. COVID-19 Assessment and COVID-19 AEs

A standardized MedDRA Query (SMQ) will be used to identify all COVID-19 AEs. COVID-19 assessments for participants with COVID-19 AEs will be summarized.

4.4.3. Additional Safety Assessments

4.4.3.1. Possible Suicidality Related Adverse Events

A listing of possible suicidality related- adverse events (PSRAE) will be produced based on GSK Core Data Standards. The listing will be sorted by site id, unique subject id, then chronologically within subject.

4.4.3.2. Laboratory Data

Laboratory evaluations including the analyses of Chemistry laboratory tests, Hematology laboratory tests, Urinalysis, and liver function tests will be based on GSK Core Data Standards. When collected throughout the treatment period, laboratory data will be summarized based on observed values by visit and treatment sequence, as well as change from baseline by visit and treatment sequence. For laboratory data collected only at screening and follow-up visits, no summary will be produced. A listing of all laboratory data and a listing of PCI laboratory data will be produced.

Laboratory data includes the following variables:

Assessments	Parameters		
Haematology	• Platelet Count • Hematocrit • Hgb	RBC Indices: • MCV • MCH • %Reticulocytes	WBC count with Differential: • Neutrophils • Lymphocytes • Monocytes • Eosinophils • Basophils
Clinical Chemistry	• BUN • Creatinine • Glucose • Potassium • Sodium • Calcium	• Aspartate Aminotransferase (AST)/Serum Glutamic-Oxaloacetic Transaminase (SGOT)	• Total and direct bilirubin
	• Alanine Aminotransferase (ALT)/ Serum Glutamic-Pyruvic Transaminase (SGPT)	• Alkaline phosphatase	• Total Protein

Assessments	Parameters
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick • Microscopic examination (if blood or protein is abnormal)
Other Laboratory Analyses	• Follicle-stimulating hormone and estradiol (as needed in women of non-childbearing potential only) • Urine human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential) • HIV antibody • Folate, high sensitivity C-reactive protein, Vitamin B12

Separate summary tables for haematology, and chemistry laboratory tests will be produced. Liver function laboratory tests will be included with chemistry lab tests. Summaries of other laboratory analyses collected only at screening will not be produced.

Summaries of hepatobiliary laboratory events including possible Hy's law cases will be provided in addition to what has been described above. Possible Hy's law cases are defined as any elevated alanine aminotransferase (ALT)>3×upper limit of normal (ULN), total bilirubin≥2×ULN and alkaline phosphatase (ALP)<3×ULN/missing. Total bilirubin≥2×ULN can be within 28 days following the ALT elevation and if direct bilirubin is available on the same day, it must be ≥ 35% of total bilirubin. ALP<3×ULN/missing means it is satisfied unless the ALP is ≥3xULN at the time of bilirubin elevation. The summary will be produced for worst case post baseline only.

4.4.3.3. Vital Signs

Vital signs data will be summarized based on observed values by treatment sequence and visit, as well as change from baseline by treatment sequence and visit. Change from baseline will be calculated using one baseline for both periods (Day 1). A listing of all Vital signs data and a listing of PCI Vital signs data will be produced.
Vital signs data includes the following variables:

- Heart rate
- Blood pressure (SBP, DBP)

4.4.3.4. ECG

A listing of all ECG data and a listing of PCI ECG data will be produced.

4.5. Changes to Protocol Defined Analyses

The fixed sequence effect has been removed from the mixed model specified for the primary analysis in protocol amendment 3 (Dated: 14-JUL-2021) to better align with recommendations in the literature (Kenward and Roger, 2009).

5. SAMPLE SIZE DETERMINATION

Sufficient participants will be enrolled such that at least 12 participants comprise the Evaluable Population. A sample size of 12 participants provides 90% power to detect a paired mean difference in fractional iron absorption of 11% between the two treatment groups under an assumed between-participant standard deviation (SD) of 10.7%. Note this is a conservative estimate of the SD of the paired differences as it is based on a parallel design study (Young, 2009). No sample size re-estimation will be performed.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 Study Population Analyses

Unless otherwise specified, the study population analyses will be based on the "Safety" population, unless otherwise specified. Screen failures will be summarized or listed based on the "Screened" population. A summary of the number of participants in each of the participant level analysis set will be provided.

6.1.1. Participant Disposition

A summary of the number and percentage of participants who completed the study as well as those who prematurely withdrew from the study will be provided overall and by study period. Reasons for study withdrawal will be summarized overall and by study period. For those who have neither completed nor withdrawn, they will be categorized as on study intervention or in follow up.

A summary of study intervention status will be provided. This display will show the number and percentage of participants who have completed the scheduled study intervention, are ongoing with study intervention, or have discontinued study intervention prematurely, as well as primary reasons for discontinuation of study intervention.

A summary of the number of subjects in the "Enrolled" population by country and site ID will be provided

6.1.2. Demographic and Baseline Characteristics

The demographic characteristics including age, sex, ethnicity, height at screening and race will be summarized with descriptive statistics. In addition, the following age categories will be summarized: 18-64, 65-84 and >=85 based on the Enrolled Analysis Set.

Listings of demographic characteristics and race will also be produced.

6.1.3. Protocol Deviations

Important protocol deviations will be summarized.

Protocol deviations will be tracked by the study team throughout the conduct of the study. These protocol deviations will be reviewed to identify those considered as important as follows:

- Data will be reviewed prior to freezing the database to ensure all important deviations (where possible without knowing the study intervention details) are captured and categorised in the protocol deviations dataset.

- This dataset will be the basis for the summaries of important protocol deviations.

In addition to the overall summary of important protocol deviations, separate summaries will be produced for important protocol deviations related to COVID-19, and important protocol deviations not related to COVID-19 respectively.

6.1.4. Prior and Concomitant Medications

Prior and concomitant medications will be coded using both the GSK Drug and WHO Drug dictionaries. However, the summary will be based on GSK Drug dictionary only. However, they will only be presented in the form of ICH Listing.

For classifying study phase for concomitant medication, use the following definition.

Study Phase	Definition
Prior	If medication end date is not missing and is before 28 days prior to screening visit
Concomitant	Any medication that is not a prior

Please refer to 6.2.6: for handling of missing and partial dates for concomitant medication. Use the rules in this table if concomitant medication date is completely missing.

6.1.5. Study Intervention Compliance

A summary of overall compliance for daprodustat based on the exposure data will be produced. Overall compliance will be summarized using descriptive statistics as well as the categories <80%, 80%-105%, and >105%.

Study intervention Compliance (%) = [Total cumulative actual dose / Total cumulative scheduled dose] *100.

6.1.6. Additional Analyses Due to the COVID-19 Pandemic

A participant is defined as having a suspected, probable, or confirmed COVID-19 infection during the study if the answer is “Confirmed”, “Probable” or “Suspected” to the case diagnosis question from the COVID-19 coronavirus infection assessment eCRF. Numbers of participants with a suspected, probable, or confirmed COVID-19 infection, and of COVID-19 test results will be summarized.

6.2. Appendix 2 Data Derivations Rule

6.2.1. Criteria for Potential Clinical Importance

A laboratory value that is outside the reference range is considered either high abnormal (value above the upper limit of the reference range) or low abnormal (value below the lower limit of the reference range). Note: a high abnormal or low abnormal laboratory value is not necessarily of clinical concern.

In addition, the following criteria will be used to flag potential clinical importance (PCI) in the Safety population:

• **Haematology**				
Laboratory Parameter	**Units**	**Category**	**Clinical Concern Range**	
			Low Flag (< x)	**High Flag (>x)**
Hematocrit	Ratio of 1	Male	0.225	0.54
		Female	0.225	0.46
Hemoglobin	g/L	Male	75	175
		Female	75	153
Platelet Count	$x10^9$/ L		<80	>500
RBC Count	$x10^{12}$/ L		<4	>5
WBC Count with Differential:			< LLRR	> 5x ULRR
Neutrophils	mm3		< 0. 5	N/A
Lymphocytes	mm3		<0.5	N/A
Monocytes	mm3		<0. 2	>1.0
Eosinophils	mm3		N/A	>0. 5
Basophils	mm3		N/A	>0. 1
RBC Indices:				
MCV	Femtolitres/cell		<80	>96
MCH	picograms/cell		<27	>33
%Reticulocytes	%		<0.5	>1.5

Clinical Chemistry				
Laboratory Parameter	**Units**	**Category**	**Clinical Concern Range**	
			Low Flag (< x)	**High Flag (>x)**
Calcium	mmol/L		<1.75	>2.74
Creatinine	umol/L		<44.21	>884
Glucose	mmol/L		<3.9	>18
Total Protein	g/L		<60	>90
Potassium	mmol/L		<3.5	> 6.0

Clinical Chemistry				
Laboratory Parameter	**Units**	**Category**	**Clinical Concern Range**	
			Low Flag (< x)	**High Flag (>x)**
Sodium	mmol/L		< 130	> 150

Liver Function			
Test Analyte	**Units**	**Category**	**Clinical Concern Range**
ALT/SGPT	U/L	High	>= 3x ULRR
AST/SGOT	U/L	High	>= 3x ULRR
AlkPhos	U/L	High	>150
T Bilirubin	µmol/L	High	>= 2x ULRR
Direct Bilirubin	µmol/L	High	>8.0

Other Laboratory analyses				
Laboratory Parameter	**Units**	**Category**	**Clinical Concern Range**	
			Low Flag (< x)	**High Flag (>x)**
Ferritin	ng/ml		<100	>800
TSAT	%		<15	>40
Folate	ng/ml		<2.0	N/A
high sensitivity C-reactive protein	micrograms /ml		N/A	>/=20
Vitamin B12			</= LLRR at screening	N/A

ECG Parameter	**Units**	**Clinical Concern Range**	
		Lower	**Upper**
Absolute			
Heart rate	bpm	40	125
PR Interval	msec	80	230
QRS Duration	msec	50	130
RR	breaths/mit	8	25
Uncorrected QT Interval	msec	300	500
QTcF	msec	300	500
QTcB	msec	300	500
QTc (Unspecified)	msec	300	500
RR Interval	msec	545	1500

Vital Sign Parameter (Absolute)	Units	Clinical Concern Range	
		Lower	Upper
Systolic Blood Pressure	mmHg	85	170
Diastolic Blood Pressure	mmHg	50	110
Heart Rate	bpm	40	125
Change from baseline			
Systolic Blood Pressure	mmHg		>30
Diastolic Blood Pressure	mmHg		>50
Heart Rate	bpm		>30

6.2.2. Study Period

Assessments and events (e.g., study withdrawal) will be classified according to the time of occurrence relative to the study intervention period.

Study Phase	Definition
Pre-Intervention	Reference Day <= Previous Day of Dosing Start Date in Period 1
Period 1	Dosing Start Date in Period 1 < Reference Day <= Previous Day of Dosing Start Date in Period 2
Period 2	Dosing Start Date in Period 2 <= Reference Day

6.2.3. Study Day and Reference Dates

The safety reference date is the study intervention start date and will be used to calculate study day for safety measures.

The efficacy reference date is the study intervention start date and will be used to calculate study day for efficacy measures and baseline characteristics, as well as efficacy durations.

The study day is calculated as below:

- Assessment Date = Missing → Study Day = Missing
- Assessment Date < Reference Date → Study Day = Assessment Date – Ref Date
- Assessment Data ≥ Reference Date → Study Day = Assessment Date – Ref Date + 1

6.2.4. Assessment Window

For data summaries by visit, scheduled visits with nominal visit description will be displayed. Unscheduled visits will not be displayed or slotted into a visit window but will be included in the derivation of worst-case post baseline assessment. All un-scheduled visits will be displayed in listings, as appropriate.

6.2.5. Multiple measurements at One Analysis Time Point

When triplicate ECG assessments are taken, mean of the measurement will be calculated first and summary statistics will be based on the calculated mean. This will apply to both baseline and post baseline assessments.

For lab tests on a study day, if more than one assessment is taken on the same day, the test from a central lab will be taken over the test from a local lab. If multiple assessments are taken from the same type of lab, the worst case will be used.

6.2.6. Handling of Partial Dates

<table>
<tr><th>Element</th><th>Reporting Detail</th></tr>
<tr><td>General</td><td>

Partial dates will be displayed as captured in participant listing displays.
However, where necessary, display macros may impute dates as temporary variables for sorting data in listings only. In addition, partial dates may be imputed for ‘slotting’ data to study phases or for specific analysis purposes as outlined below.
Imputed partial dates will not be used to derive study day, time to onset or duration (e.g., time to onset or duration of adverse events), or elapsed time variables (e.g., time since diagnosis).

</td></tr>
<tr><td>Adverse Events</td><td>

Partial dates for AE recorded in the CRF will be imputed using the following conventions:

<table>
<tr><td>Missing start day</td><td>If study intervention start date is missing (i.e. participant did not start study intervention), then set start date = 1st of month.

Else if study intervention start date is not missing:

o If month and year of start date = month and year of study intervention start date, then

▪ If stop date contains a full date and stop date is earlier than study intervention start date, then set start date= 1st of month.

▪ Else set start date = study intervention start date.

Else set start date = 1st of month.</td></tr>
<tr><td>Missing start day and month</td><td>If study intervention start date is missing (i.e. participant did not start study intervention), then set start date = January 1.</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Element</th><th colspan="2">Reporting Detail</th></tr>
<tr><td></td><td></td><td>Else if study intervention start date is not missing:

If year of start date = year of study intervention start date, then

If stop date contains a full date and stop date is earlier than study intervention start date, then set start date = January 1.

Else set start date = study intervention start date.

Else set start date = January 1.</td></tr>
<tr><td></td><td>Missing end day</td><td>A '28/29/30/31' will be used for the day (dependent on the month and year).</td></tr>
<tr><td></td><td>Missing end day and month</td><td>No Imputation</td></tr>
<tr><td></td><td>Completely missing start/end date</td><td>No imputation</td></tr>
<tr><td>Concomitant Medications/ Medical History</td><td colspan="2">• Partial dates for any concomitant medications recorded in the CRF will be imputed using the following convention:</td></tr>
<tr><td></td><td>Missing start day</td><td>If study intervention start date is missing (i.e. participant did not start study intervention), then set start date = 1st of month.

Else if study intervention start date is not missing:

o If month and year of start date = month and year of study intervention start date, then

▪ If stop date contains a full date and stop date is earlier than study intervention start date, then set start date= 1st of month.
▪ Else set start date = study intervention start date.
Else set start date = 1st of month.</td></tr>
<tr><td></td><td>Missing start day and month</td><td>If study intervention start date is missing (i.e. participant did not start study intervention), then</td></tr>
</table>

Element	Reporting Detail		
		set start date = January 1. *Else if study intervention start date is not missing:* o *If year of start date = year of study intervention start date, then* ▪ *If stop date contains a full date and stop date is earlier than study intervention start date, then set start date = January 1.* ▪ *Else set start date = study. intervention start date.* *Else set start date = January 1.*	
	Missing end day	A '28/29/30/31' will be used for the day (dependent on the month and year).	
	Missing end day and month	A '31' will be used for the day and 'Dec' will be used for the month.	
	Completely missing start/end date	No imputation	

6.2.7. Early PK Access Key Activities

NA

Trademarks

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HARP

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SAS

7. REFERENCES

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Young MF, Glahn RP, Ariza-Nieto M, Inglis J, Olbina G, Westerman M, O'Brien KO. Serum hepcidin is significantly associated with iron absorption from food and supplemental sources in healthy young women. Am J Clin Nutr. 2009; 89:533-538.