

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

216951Orig1s000

INTEGRATED REVIEW

%Integrated Review

Table 1. Application Information

Application type	NDA
Application number(s)	216951
Priority or standard	STANDARD
Submit date(s)	2/1/2022
Received date(s)	2/1/2022
PDUFA goal date	2/1/2023
Division/office	Division of Nonmalignant Hematology (DNH)
Review completion date	1/31/2023
Established/proper name	daprodustat
(Proposed) proprietary name	Jesduvroq
Pharmacologic class	Hypoxia-inducible factor prolyl-hydroxylase (HIF-PHI) inhibitor
Other product name(s)	Jesduvroq (daprodustat)daprodustat, GSK1278863
Applicant	GLAXOSMITHKLINE INTELLECTUAL PROPERTY NO 2 LTD ENGLAND
Dosage form(s)/formulation(s)	Daprodustat tablets 1 mg, 2 mg, 4 mg, 6 mg, 8 mg
Dosing regimen	One tablet daily with or without food.
Applicant-proposed indication(s)/ population(s)	Treatment of anemia due to chronic kidney disease in adult patients on dialysis and not on dialysis
SNOMED CT code for proposed indication disease term(s)¹	234348004 Anemia of renal disease (disorder)
Regulatory action	Approval
Approved dosage (if applicable)	One tablet daily with maximum recommended once daily dose of 24 mg.
Approved indication(s)/ population(s) (if applicable)	Treatment of anemia due to chronic kidney disease in adults who have been receiving dialysis for at least four months.
SNOMED CT code for approved indication disease term(s)¹	234348004 Anemia of renal disease (disorder)

¹ For internal tracking purposes only.

Abbreviations: PDUFA, Prescription Drug User Fee Act; SNOMED CT, Systematized Nomenclature of Medicine Clinical Terms

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Glossary

ACM	all-cause mortality
ADME	absorption, distribution, metabolism, and excretion
ADPKD	autosomal dominant polycystic kidney disease
AE	adverse event
AESI	adverse event of special interest
AKI	acute kidney injury
ANCOVA	analysis of covariance
AUC	area under the concentration-time curve
BCRP	breast cancer resistance protein
BID	twice daily
CFR	Code of Federal Regulations
CI	confidence interval
CKD	chronic kidney disease
CL	clearance
C _{max}	maximum plasma concentration
CMH	Cochran-Mantel-Haenszel
CSR	clinical study report
CV	cardiovascular
CVOT	cardiovascular outcome trials
DCOA	Division of Clinical Outcome Assessment
DD	dialysis dependent
DILI	Drug Induced Liver Injury
DNH	Division of Non-Malignant Hematology
DPMH	Division of Pediatric and Maternal Health
EP	Evaluation Period
EPO	erythropoietin
E-R	exposure-response
ESA	erythropoietin stimulating agent
ESRD	End-Stage Renal Disease
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FMQ	Food and Drug Administration Medical Dictionary for Regulatory Activities query
GCP	good clinical practice
GI	gastrointestinal
GLP	good laboratory practice
Hb	hemoglobin
HD	high dose
HHF	hospitalization for heart failure
HIF	hypoxia-inducible factor
HIF-PIH	hypoxia-inducible factor prolyl-hydroxylase inhibitors
HR	hazard ratio
IC ₅₀	half maximal inhibitory concentration

NDA 216951
Jesduvrog (daprodustat)

ICH	International Council for Harmonisation
ID	incident dialysis
IDMC	Independent Data Monitoring Committee
IND	investigational new drug
IRD	incidence rate difference
ITT	intent-to-treat
MACE	major adverse cardiovascular events
MAR	missing at random
MedDRA	Medical Dictionary for Regulatory Activities
MI	myocardial infarction
MMRM	mixed model repeated measures
MNAR	missing not at random
MP	maintenance phase
ND	non-dialysis
NDA	new drug application
NDD	non-dialysis dependent
NI	non-inferiority
NOAEL	no observed adverse effect level
OPQ	Office of Pharmaceutical Quality
PD	pharmacodynamic
PGI-C	Patient Global Impression of Severity
PGI-S	Patient Global Impression of Change
PI	Prescribing Information
PK	pharmacokinetic
PMR	postmarketing requirement
PP	per protocol
PREA	Pediatric Research Equity Act
PRO	patient-reported outcome
PT	preferred term
PY	person-years
QD	once daily
RBC	red blood cell
SAE	serious adverse event
SOC	system organ class
TEAE	treatment-emergent adverse event
TEE	thromboembolic event
TK	toxicokinetic
T _{max}	time to maximum concentration
TSAT	transferrin saturation
VAT	vascular access thrombosis
VPC	visual predictive check

I. Executive Summary

1. Summary of Regulatory Action

On February 1, 2022, GlaxoSmithKline, Inc. submitted NDA 216951 to the FDA for approval of daprodustat for the treatment of anemia associated with chronic kidney disease (CKD) in adults on dialysis and not on dialysis.

If approved, daprodustat would be the first-in-class inhibitor of hypoxia inducible factor-prolyl hydroxylase (HIF-PH), which improves anemia of CKD by inducing endogenous production of erythropoietin, rather than supplementing it exogenously. Daprodustat's oral administration route differs from the parenteral administration route of erythropoietin stimulating agents (ESAs), the current standard of care (together with iron supplementation) for treating anemia associated with CKD.

Hematologic response has been used to establish efficacy for traditional approval for drugs intended to treat anemia of CKD. In head-to-head, randomized, controlled trials, daprodustat increased hemoglobin (Hb) to a similar extent as approved ESAs in the non-dialysis dependent (NDD) population and in the dialysis dependent (DD) population with similar continued need for red blood cell (RBC) transfusions or rescue therapy. Daprodustat was also efficacious in raising Hb in the NDD population in a randomized, placebo-controlled trial. These multiple adequate and well-controlled trials establish substantial evidence of effectiveness of daprodustat for the treatment of anemia in patients with CKD, for both the NDD and DD populations. The trials did not establish any other benefits on how patients feel, function, or survive.

Safety findings for the two populations diverged. For the DD population, for the co-primary safety analysis, daprodustat demonstrated non-inferiority to the ESAs for adjudicated major adverse cardiovascular events (MACE, defined as a composite of all-cause mortality, nonfatal myocardial infarction, and nonfatal stroke). In addition, daprodustat did not have unfavorable effects on other cardiovascular events (e.g., myocardial infarction (MI), stroke, thromboembolism, vascular access thrombosis) compared to darbepoetin alfa or epoetin alfa in the DD population, with the exception of hospitalization for heart failure (HHF). However, this risk was particularly confined to those patients with a history of heart failure. Daprodustat also appears to increase the risk of bleeding gastric and esophageal erosions. The clinical review team, statistical team, and division director are aligned with the position that the benefits outweigh the risks in the DD population as described in the Benefit-Risk Framework below, and the office director, who is the signatory authority, concurs. While the advantage of an oral route is less clear for the vast majority of patients undergoing hemodialysis in the U.S. who typically receive an ESA during the dialysis session, it will provide another treatment option and will be preferred by some patients over parenteral administration of ESAs.

For detailed information supporting the basis for this action, refer to the detailed sections included in this Interdisciplinary Assessment document.

In the NDD population, for the co-primary safety endpoint of MACE, daprodustat demonstrated non-inferiority to ESAs. However, in contrast to the DD population, daprodustat showed several other important risks in addition to the risks of heart failure and bleeding gastric erosions. Our

findings, based on adjudicated cardiovascular endpoints, show elevated HRs for stroke, thromboembolic disease, vascular access thrombosis, and to a lesser extent MI, relative to the ESA comparator, which itself already carries these risks. The estimated harms were further elevated in the U.S. subgroup of patients, which is noteworthy because daprodustat will be used in the U.S. if approved. Lastly, we also identified a possible increased risk of acute kidney injury with daprodustat in the NDD population. The oral route also has potential downsides in this population, which is not as closely followed as the DD population, such as risks of inadequate Hb monitoring, which might lead to worse outcomes (e.g., if Hb is increased too much or too quickly).

We could not identify a risk mitigation strategy or postmarketing approach that will allow for the safe administration of daprodustat at the proposed dosing regimen in the NDD population to ensure the benefits of daprodustat outweigh its risks. The clinical team, statistical team, and division director conclude that the overall benefit/risk is unfavorable in the non-dialysis population as described in the Benefit-Risk Framework below, and the office director, who is the signatory authority, concurs. For detailed information supporting the basis for this action, refer to the detailed sections included in this Interdisciplinary Assessment document.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of condition	• CKD is a progressive loss of kidney function over time due to irreversible kidney damage due to a variety of underlying causes. • Approximately 15% of the U.S. adult population (about 1 in 7) has CKD, with 17.2 million having advanced CKD. Certain populations, such as older adults, African Americans, and people with hypertension or diabetes, are at a higher risk for developing CKD. • Patients with CKD may be on dialysis or not on dialysis and patients with advanced CKD are commonly awaiting kidney transplant as a definitive therapy for their disease. Patients who are on dialysis have frequent but vital vascular access procedures and healthcare interactions. • Anemia is a common early and progressive complication of CKD and it is estimated that about two-thirds of patients who develop CKD will also develop anemia. Anemia of CKD is caused by decreased production of erythropoietin by renal interstitial cells, iron deficiency, and chronic inflammation. • CKD is associated with increased risk of cardiovascular disease, morbidity due to symptomatic anemia and early mortality due to cardiac disease, stroke and renal-associated complications.	CKD is a prevalent and serious disease with significant morbidity and early mortality. It is associated with an increased risk of cardiovascular disease, anemia and anemia-related signs and symptoms. In patients with CKD, anemia is highly prevalent. Untreated anemia of CKD may lead to a variety of signs and symptoms, including fatigue, dyspnea, tachycardia, myocardial ischemia and decreases in cognitive function and mental acuity, resulting in significant morbidity and mortality.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current treatment options	• Options for the treatment of anemia of CKD include iron (oral or intravenously), ESAs, and RBC transfusions. The treatment depends on the severity of anemia and iron deficiency. • RBC transfusions are used primarily for acute or life threatening anemia, in part due to attendant risks (including iron overload, fluid overload, alloimmunization, hyperkalemia, alloimmunization and hypersensitivity reactions). • <u>ESAs:</u> • Epogen/Procrit (epoetin alfa), Aranesp (darbepoetin alfa), Mircera (methoxy polyethylene glycol-epoetin beta), and Retacrit (epoetin alfa-epbx) • Parenteral therapies; standard-of-care for the treatment of anemia in patients with CKD on dialysis and not on dialysis. Current products include formulations/routes/devices that allow self-administration of SC injection. • All ESA labels contain a boxed warning for increased risk of CV mortality and morbidity associated with targeting a higher Hb level, compared to a lower Hb level. • There is no identified Hb target value, ESA dose or dosing strategy that does not increase these risks. • The general dosing recommendation for ESAs is to use the lowest dose sufficient to reduce the need for RBC transfusions, while targeting a Hb value less than 11 g/dL. • There are no approved oral treatments for patients with anemia of CKD.	ESAs are the current standard therapy for the treatment of anemia in patients with CKD, while RBC transfusions are mainly considered in acute scenarios. ESAs are associated with cardiovascular mortality and morbidity when higher Hb values (>11 g/dL) are targeted, compared to lower Hb values. A Hb target has not been identified below which ESA cardiovascular risks no longer occur. Importantly, RBC transfusions are associated with foreign antigen exposure that may result in alloimmunization, thus, RBC transfusions may adversely affect a patient’s eligibility for kidney allograft. There is a need for alternative treatments including an oral anemia treatment, but more importantly, a treatment which increases hemoglobin (thus avoiding transfusions) but does not have increased cardiovascular risk.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit	- Hematological response and reduction in RBC transfusions have been used to establish efficacy (for traditional approval) for drugs indicated to treat anemia of CKD. - The ASCEND program included 4 active controlled studies in DD-CKD, NDD-CKD, an incident dialysis (ID) population, and a three times a week (TIW) dosing regimen in the DD population. In addition, there was one placebo-controlled study in the NDD-CKD population. The clinical program represented reasonable clinical practice by implementing consistent Hb target windows and prespecified treatment algorithms (including titration of daprodustat and comparator, iron management, and rescue). - In the 4 active-controlled studies, the primary efficacy analysis of change from baseline in hemoglobin to the average hemoglobin from weeks 28 to 52 showed that daprodustat was non-inferior to an ESA (ASCEND-ND: SC darbepoetin, ASCEND-D: IV epoetin alfa for hemodialysis subjects or SC darbepoetin alfa for peritoneal dialysis subjects, TIW trial: IV epoetin alfa, ID: SC or IV darbepoetin alfa). The non-inferiority conclusion was robust in all pertinent patient subgroups (ages, gender, race, prior ESA use, dialysis type, pertinent medical history) as well as sensitivity analyses (based on estimand strategy, missing data handling, definition of treatment window). - Supplementary analyses of hemoglobin (e.g., visit-to-visit variability, time in range, incidence of excessive erythropoiesis) as well as transfusions and anemia rescue were balanced between arms across all four studies. - A placebo-controlled study in NDD patients (ASCEND-NHQ) targeted a higher Hb range (11 to 12 mg/dL). The primary endpoint of mean change from baseline Hb was superior to placebo from weeks 24 to 28. Although a statistically significant treatment difference favoring daprodustat was demonstrated on the Standard Form 36 Vitality domain (a commonly used Patient Reported Outcome), the review team concluded the modest to small treatment differences and minimal magnitude of changes were not clinically meaningful to patients.	The studies comprising the ASCEND program were adequate and well controlled. Daprodustat demonstrated non-inferiority to ESA therapy in subjects with DD-CKD and NDD-CKD in raising and maintaining Hb up to a treatment period of at least 52 weeks. There were no clinically meaningful differences in RBC transfusion or rescue incidence. The findings from multiple analysis approaches and endpoints support a noninferiority conclusion of efficacy. We expect the similarity of Hb response between daprodustat and the ESA control arm to translate into similar reductions in the need for RBC transfusions, an accepted clinical benefit for treatment of anemia of CKD, because of avoiding the procedure and its attendant risks. The Applicant did not demonstrate any other benefits of daprodustat on how patients feel, function or survive. As an oral therapy, daprodustat would offer convenience compared to the parenteral route of ESAs, especially in the NDD-CKD population. The convenience of an oral drug in the DD-CKD population is less clear, particularly for the majority of DD patients in the U.S. who receive in-center hemodialysis and who are administered ESA at the time of hemodialysis; however, daprodustat will provide an oral option that some may prefer. The higher hemoglobin target of 11 to 12 g/dL studied in the ASCEND-NHQ does not reflect current clinical practice of available treatments (i.e. ESAs) nor does it reflect the clinical scenario in ASCEND-D and ASCEND-ND studies.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and risk management	The primary safety database (ASCEND-D and ASCEND-ND) included approximately 7000 patient-years of follow-up for the NDD and DD populations combined, with most of the follow-up time spent on treatment (77 to 85%). The median duration of follow up was approximately 30 months for the DD population, and 22 months for the NDD population. The pre-specified primary safety endpoint in both ASCEND-ND and ASCEND-D was the time to first occurrence of adjudicated MACE (composite of all-cause mortality, nonfatal MI, and nonfatal stroke). Adjudicated secondary safety endpoints included all-cause mortality, CV mortality, fatal or nonfatal MI, stroke, HHF, and thromboembolic events. In the ASCEND-ND study: (NDD-CKD population) - The primary analysis (on-study analysis) of time to first MACE was similar between arms in ASCEND-ND: HR: 1.03 [95% CI 0.89 - 1.19]. The upper bound of the 95% CI was lower than the prespecified risk margin of 1.25, demonstrating non-inferiority. All-cause mortality, a prespecified secondary endpoint, was similar between arms (HR: 1.03 [0.87 - 1.20]). - There were elevated hazard ratios for stroke, thromboembolic disease, and vascular access thrombosis, and to a lesser extent MI, with daprodustat compared to darbepoietin alfa in the ASCEND-ND study (HRs ranging from 1.06 to 1.49). The treatment difference was more pronounced for these endpoints (except for stroke) in the U.S. subgroups (HRs ranging from 1.21 to 2.03). - There was a higher incidence of hospitalization for heart failure with daprodustat compared to ESA in ASCEND-ND (HR: 1.22 [95% CI 0.95 - 1.56]). This risk was particularly seen in patients with pre-existing heart failure. - There was a higher incidence of serious AKI in ASCEND-ND based on serious adverse event analyses of AKI showing a relative risk of 1.47 (95% CI 1.07, 2.00) and risk difference of 1.54% [95% CI 0.30, 2.79]).	The safety database for both NDD and DD populations was adequate to evaluate the safety profile for the once daily regimen. The breadth, ascertainment, and adjudication of the prespecified CV safety endpoints were reasonable and agreed upon by the FDA at the development stage, based on the known safety profile of ESAs (which share overlapping mechanisms). <u>In ASCEND-ND (NDD population):</u> Daprodustat demonstrated non-inferiority for time to first adjudicated MACE compared to darbepoietin alfa on the primary analysis. There were elevated estimated HRs for CV mortality, stroke, thromboembolic disease, vascular access thrombosis, and to a lesser extent MI, with daprodustat compared to darbepoietin alfa. The consistently increased risk estimates across different CV endpoints measuring related aspects of CV risk in a CV outcomes trial raises concerns as to whether daprodustat is safe relative to darbepoietin alfa, which itself carries an increased CV risk. Since ESAs already carry some of these risks, a further increase in these risks beyond that seen with the ESAs is concerning and not supportive of approval. With an oral agent, there is also the risk in the real-world of less optimal monitoring of Hb in the NDD population (which is not followed as closely as the DD population) that could lead to instances of inappropriately high Hb, potentially leading to an even more adverse profile than that seen in the trials where there was close Hb monitoring. CV risks (with the exception of stroke) also appear higher in the U.S. subgroup of patients compared to the non-U.S. subgroup. The elevated risks in the U.S. subgroup across multiple CV endpoints is noteworthy because daprodustat would be used in the U.S. if FDA approved.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	In the ASCEND-D study: (DD-CKD population) - The primary safety analysis of first occurrence of MACE was similar between arms in ASCEND-D: hazard ratio (HR) 0.93 [95% CI 0.81 - 1.07]. The upper bound of the 95% CI was lower than the prespecified risk margin of 1.25, demonstrating non-inferiority. - All-cause mortality, a pre-specified secondary endpoint, was similar between treatment arms: HR 0.96 [95% CI 0.82 - 1.13]. - There was a higher incidence of hospitalization for heart failure with daprodustat compared to ESA: HR: 1.10 [95% CI 0.84 - 1.45]). This risk was particularly seen in patients with pre-existing heart failure. In both studies (ASCEND-D and ASCEND-ND), there was a higher incidence of serious gastric/esophageal erosions (including gastrointestinal bleeding and need for RBC transfusion) reported in the daprodustat arm compared to ESA with a more notable difference in the ASCEND-ND population (HR [95% CI]: 1.63 [1.17,2.27]) than in ASCEND-D (HR [95% CI]: 1.16 [0.78, 1.73]). There were no treatment differences disfavoring daprodustat compared to ESA for other known risks of ESAs, including malignancy, seizure, hypertension, infection, or hypersensitivity reactions.	Other notable risks included HHF (particularly in patients with a history of heart failure), serious AKI, and bleeding gastric/esophageal erosions. <u>In the DD population: ASCEND-D:</u> Daprodustat demonstrated non-inferiority for time to first adjudicated MACE comparing daprodustat to epoetin alfa (hemodialysis) and darbeopoetin alfa (peritoneal dialysis). Unlike the results for the NDD population, there was not a consistent pattern of increased risk estimates in the CV endpoints (CV mortality, MI, stroke, thromboembolic disease, and vascular access thrombosis) for daprodustat vs. ESA. However, similar to the results for the NDD population, there were other notable risks including HHF (particularly in patients with a history of heart failure) and bleeding gastric/esophageal erosions. Daprodustat and the ESA comparators had comparable risks of worsening hypertension. Whether daprodustat may increase the risk of malignancy like ESAs is uncertain. While such a risk was not seen in the completed trials, their duration and the number of malignancy events are insufficient to definitively exclude a risk. Given the more reassuring findings when daprodustat is compared to ESA in the DD population, there is a favorable benefit/risk assessment in this population, with the oral route of administration offering another treatment option that some may prefer over the parenteral ESAs. Consistent with the approach used with other products for treatment of anemia in CKD (ESAs), the risks of daprodustat can be sufficiently mitigated in the DD population with labeling: Because the trials demonstrated non-inferiority on MACE with respect to the ESA comparators, a similar boxed warning will be added for the increased risk of death,

Dimension	Evidence and Uncertainties	Conclusions and Reasons
		myocardial infarction, stroke, venous thromboembolism and thrombosis of vascular access. • Labeling will include a contraindication for patients with uncontrolled hypertension and warnings and precautions for other significant risks including the risk of hypertension, hospitalization for heart failure (particularly in those with a history of heart failure), and gastric/esophageal erosions and malignancy. • A Medication Guide that must be dispensed to patients with each prescription alerting them to the serious risks. To further evaluate MACE, thrombotic vascular events to including vascular access thrombosis, hospitalization for heart failure, serious gastrointestinal bleeds, and malignancy, we are issuing Post Marketing Requirements for the Applicant to conduct observational studies in patients with anemia with DD-CKD treated with daprodustat. (b) (4)

NDA 216951
Jesduvroq (daprodustat)

Dimension	Evidence and Uncertainties	Conclusions and Reasons
		(b) (4)

Abbreviations: AESI, adverse event of special interest; AKI, acute kidney injury; CI, confidence interval; CKD, chronic kidney disease; CV, cardiovascular; DD, dialysis dependent; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; HR, hazard ratio; ID, incident dialysis; IV, intravenous; MACE, major adverse cardiovascular events; ND, non-dialysis; NDD, non-dialysis dependent; RBC, red blood cell; SC, subcutaneous; TESAE, treatment emergent serious adverse event

2.2. Conclusions Regarding Benefit-Risk

Unmet Need

CKD is a highly prevalent condition in the United States. Anemia associated with CKD is common but can be serious if severe and left untreated. Treatment options for the anemia of CKD include iron (oral or injection), ESAs, and RBC transfusions. Risks with RBC transfusions include alloreactivity (a risk factor for renal allograft rejection in CKD patients who subsequently undergo renal transplantation), volume overload, iron overload, hyperkalemia, infection, and hypersensitivity reactions. ESAs, as current standard of care, are effective at increasing and maintaining hemoglobin levels but are associated with significant cardiovascular and thrombotic adverse reactions, especially when higher hemoglobin values are targeted. There is an unmet need for safer products and orally available therapies that allow patients to avoid the need for ESA injections and RBC transfusions.

Benefits

Daprodustat's evidence of effectiveness for the treatment of anemia due to CKD in adults is based primarily on ASCEND-ND and ASCEND-D, which are event-driven, multicenter, multinational, open-label, active-controlled, randomized, parallel-group trials. ASCEND-ND compared daprodustat to darbepoetin alfa (SC) in the NDD population. ASCEND-D compared daprodustat to darbepoetin alfa (SC) for those on peritoneal dialysis and to epoetin alfa (IV) for those on hemodialysis.

Daprodustat demonstrated non-inferiority to the approved ESA comparators in raising and maintaining hemoglobin levels up to a treatment period of 52 weeks in subjects with CKD and anemia in both the NDD and DD populations. Rates of RBC transfusions were similar in the treatment arms. We expect the similarity in Hb response and similarity in the need for rescue therapy between daprodustat and the ESA control arms to translate into similar reductions in the need for RBC transfusions, an accepted clinical benefit for treatments for anemia of CKD, because of avoiding the procedure and its attendant risks.

Daprodustat is orally administered compared to the parenteral administration route of ESAs. The benefit of an oral agent is most apparent for the NDD population, which does not frequent health care facilities as often as the DD population. The benefit of the oral route is less clear for most DD patients receiving in-center dialysis where ESAs are typically given as part of the dialysis session. The oral route may reduce the need to travel for parenteral ESA (e.g., for patients on home dialysis), although this benefit is also less clear when compared to self-administration of subcutaneous ESAs. Regardless, the oral route will provide another treatment option and will be preferred by some patients.

The trials established no other benefits of daprodustat in the NDD-CKD or DD-CKD populations. Evidence of improved quality of life was not demonstrated convincingly.

Risks

In both ASCEND-D and ASCEND-ND trials, daprodustat demonstrated non-inferiority with respect to the ESAs on the time to first occurrence of MACE (defined as a composite of all-cause mortality, nonfatal myocardial infarction, and nonfatal stroke).

In the NDD population, there were treatment differences not favoring daprodustat for adjudicated cardiovascular endpoints, including stroke, thromboembolism, including vascular access thrombosis, and to a lesser extent, MI. These CV risks (with the exception of stroke), appear higher in the U.S. subgroup of patients compared to the non-U.S. subgroup. There remains uncertainty as to whether there is a true increase in harm associated with daprodustat use relative to darbepoetin on these CV outcomes. However, the consistent pattern of unfavorable treatment differences among different risks raise the level of concern. Because the ESAs already carry these risks, an apparent further increase in these risks beyond that seen with the ESAs is concerning and not supportive of approval.

In ASCEND-ND, we also noted a treatment difference not favoring daprodustat in adjudicated HHF (particularly in those with a history of heart failure), investigator-reported serious gastrointestinal erosions (mostly related to gastrointestinal bleeding, and over half required transfusion), and a risk for investigator-reported serious acute kidney injury.

In the DD population, the safety signals seen with the NDD population were not observed in the DD population except for the risks of HHF (again, particularly in those with a history of HF) and serious gastric erosions (which appeared less notable than that seen in the NDD population).

(b) (4)

Benefit/Risk in the DD Population

The increased risk for HHF was also observed in the DD population, especially in the subgroup with a history of heart failure. Acknowledging the precision and post-hoc nature of this subgroup

analysis, there is a plausible mechanism for the subgroup interaction and an appreciable effect modification observed in both the ASCEND-ND and ASCEND-D studies. For these reasons, we concluded that the HHF risk could be mitigated in the DD population by including a warning for patients with a history of heart failure. In the DD population in contrast to the NDD population, there was not a consistent pattern of increased risk estimates in the other CV endpoints (CV mortality, MI, stroke, thromboembolic disease, and vascular access thrombosis). The risk of serious erosions will be included as a warning so that healthcare providers consider it when deciding whether to prescribe daprodustat, particularly in patients already at increased risk of erosions and gastrointestinal bleeding.

In summary, in contrast to the NDD population, we could identify a risk mitigation strategy (labeling, including a Boxed Warning, Warnings and Precautions, and a Medication Guide) and post-marketing approach that will allow for the safe administration of daprodustat at the proposed daily dosing regimen to ensure that the benefits of daprodustat outweigh its risks. Therefore, we are approving daprodustat for the DD population.

(b) (4)

Additional Considerations

The Applicant should consider exploratory analyses, if they haven't already, to try to identify patient factors that predicted thrombosis and thromboembolic events to inform the design and analysis of future trial(s).

The Applicant should be encouraged to study daprodustat in patients who are considered to have a poor response to an ESA, since such patients may have a different benefit/risk, given lack of alternative therapies.

II. Interdisciplinary Assessment

3. Introduction

The Applicant, GlaxoSmithKline, Inc., is seeking approval of daprodustat under the 505(b)(1) regulatory pathway, for the treatment of anemia associated with chronic kidney disease in adults on dialysis and not on dialysis. Daprodustat is a new molecular entity, and if approved would be the first marketed drug in the class of hypoxia-inducible factor prolyl-hydroxylase inhibitors (HIF-PHI). Two other members of this class, ruxodustat and vadadustat, received Complete Response letters because of serious risks. Daprodustat is administered orally as opposed to the

¹ U.S. Renal Data System, USRDS 2016 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2016.

erythropoietin stimulating agents (ESAs), which are administered intravenously or subcutaneously and are the current standard-of-care treatments for anemia of chronic kidney disease (CKD).

Daprodustat stimulates endogenous erythropoietin production by stabilizing the hypoxia-inducible factor (HIF)- α subunit, allowing it to dimerize with the HIF- β subunit and to stimulate genes involved in protection against hypoxia, including the erythropoietin gene. HIF-PHI also influence iron homeostasis through effects on transferrin, transferrin receptor expression, hepcidin, and other iron-related proteins (Singh et al. 2021). The starting dose of daprodustat is based on the patient's dialysis status and hemoglobin level and for those switching from an erythropoiesis stimulating agent (ESA) to daprodustat, the dose is based on their existing ESA dose. The dose ranges from 1 to 24 mg orally daily with or without food.

CKD affects 8% to 16% of the population worldwide and is a leading cause of death (Chen et al. 2019). The estimated prevalence of CKD in the U.S. adult population is 15%, with an estimated 17 million people having CKD stages 3 (mild to moderate renal impairment, estimated glomerular filtration rate (eGFR) 30 to 59 mL/min/1.73 m²) to stage 5 (severe renal impairment, kidney failure; eGFR <15 mL/min/1.73 m²). The prevalence of anemia increases as the GFR declines (Hsu 2002; Meier-Kriesche et al. 2002). An estimated 90% of patients requiring dialysis are anemic.

Anemia is a common complication in CKD and is associated with a reduced quality of life, and increased morbidity and mortality. The mechanisms involved in anemia associated with CKD are diverse and complex. They include a decrease in endogenous erythropoietin (EPO) production, absolute and/or functional iron deficiency, and inflammation with increased hepcidin levels, among others. Patients are most commonly managed with red blood cell (RBC) transfusions, oral or intravenous iron supplementation and with ESAs.

ESAs are a class of recombinant glycoproteins that have an identical or nearly identical primary amino acid sequence to endogenous human erythropoietin and have the same biological effects as erythropoietin. ESAs bind to and activate the human erythropoietin receptor and stimulate RBC production in bone marrow. ESA use for the treatment of anemia due to CKD in patients on dialysis and not on dialysis has spanned over 30 years.

Approved ESAs include Epogen/Procrit (epoetin alfa), Aranesp (darbepoetin alfa), Mircera (methoxy polyethylene glycol-epoetin beta) and Retacrit (epoetin alfa-epbx); all of which are approved for the treatment of anemia in patients who are non-dialysis dependent (NDD) and those who are dialysis dependent (DD) (see [Table 3](#)).

Table 3. US-Licensed ESAs for the Treatment of Anemia Due to Chronic Kidney Disease

Product Name	Established Name (Trade Name)	Year of Approval
Epoetin alfa	(Epogen/Procrit)	1989
Darbepoetin alfa	(Aranesp)	2001
Methoxy polyethylene glycol-epoetin beta	(Mircera)	2007
Epoetin alfa-epbx	(Retacrit)	2018

Source: Clinical Reviewer's table

Abbreviations: ESA, erythropoiesis-stimulating agent; US, United States

After the first ESA approval for patients with CKD in 1989, the ESA labeling has undergone significant revisions because of risks that emerged in the postmarketing setting and in subsequent clinical trials (summarized below). These labeling revisions have included the following additions:

- A Boxed Warning for increased mortality and serious cardiovascular (myocardial infarction [MI] and stroke) and thromboembolic events. Of note, there is no identified Hb target value, ESA dose or dosing strategy that does not increase these risks.
- Warnings for hypertension, seizures, and thrombotic events including vascular access thromboses.
- In the dosing and administration section, a reduction in the recommended “target Hb,” and a recommendation to discontinue the ESA in patients in whom Hb does not respond adequately over a 12-week dose-escalation period.
- Inclusion of other major adverse reactions of ESAs including hypertension, seizures, and pure red cell aplasia.

ESA use in patients with CKD increases the risk of major adverse cardiovascular events (MACE). Clinical trials have established that targeting higher rather than lower Hb levels further increases the risk of MACE, although the ideal Hb target that best balances benefits and risks has not been identified for any of the ESAs.

- The US Normal Hematocrit trial (4) was the first in a series of randomized, controlled trials designed to test the hypothesis that a higher target hematocrit in subjects receiving hemodialysis would result in improved outcomes. In this trial, 1233 patients with End-Stage Renal Disease (ESRD) on hemodialysis with symptomatic heart failure or ischemic heart disease were randomized to either partial treatment of anemia (hematocrit of $30\pm 3\%$) or full correction (hematocrit of $42\pm 3\%$). The primary endpoint was time to death or first nonfatal MI. The trial was terminated at the third interim analysis for futility and potential harm in the full anemia correction group. There were 202 primary endpoint events in the full correction group compared to 164 events in the partial correction group: risk ratio 1.3 (95% confidence interval [CI] 0.9, 1.9). Also, 39% of subjects in the full anemia correction group had vascular access thrombosis compared to 29% of subjects in the partial treatment arm.
- The CHOIR study (5) was a randomized, active-controlled clinical trial in patients with NDD-CKD that aimed to show superiority of full anemia correction by ESA administration in terms of cardiovascular (CV) events and death. In this trial, 1432 patients with CKD and anemia (Hb <11 g/dL) received epoetin alfa and were randomly assigned to a target Hb of either 13.5 g/dL or 11.3 g/dL. The primary endpoint was a composite of death, MI, hospitalization for heart failure, or stroke. The study was prematurely stopped for futility after an interim analysis at a median study duration of 16 months because it was considered unlikely that benefit would be demonstrated for the primary composite CV endpoint. There were 125 events among 715 subjects in the high-Hb group versus 97 events among 717 subjects in the low-Hb group (hazard ratio [HR], 1.34; 95% CI 1.03, 1.74; [P=0.03]), with death and hospitalization for heart failure accounting for 75% of the events.

- The CREATE study (6) in 603 patients with CKD stages 3 to 5 (26% with diabetes) failed to demonstrate the superiority of full anemia correction (Hb target 13.0 to 15.0 g/dL) with respect to CV events, as compared to partial correction of anemia (Hb target 11.0 to 12.5 g/dL), when starting ESA therapy at an earlier stage than ESRD.
- Subsequently, TREAT, by far the largest trial, examined CV and renal outcomes in 4038 patients with stages 3 and 4 CKD (7). TREAT was the only large placebo-controlled study to assess CV outcomes. Patients received either darbepoetin alfa to achieve a Hb target of 13.0 g/dL or matching placebo with rescue darbepoetin alfa when the Hb concentration was <9.0 g/dL. The HR for the first coprimary endpoint, a composite of death or a CV event, was 1.05 (95% CI 0.94, 1.17). The HR for the second coprimary endpoint, death or ESRD, was 1.06 (P=NS). There was, however, a nearly two-fold increased risk of stroke (HR 1.92; 95% CI 1.38, 2.68) with darbepoetin alfa compared to placebo. In addition, venous thromboembolic events occurred at a significantly higher incidence in the darbepoetin alfa arm (2.0%) compared to the placebo arm (1.1%, P=0.02).

As a result of these concerns, FDA convened the Cardiovascular and Renal Drugs Advisory Committee in 2010 to discuss whether the ESA indication for the treatment of anemia due to chronic kidney disease should be withdrawn in the NDD patient population. Votes were strongly in favor of continued marketing: 1 “yes”, 15 “no”, and 1 abstention.

Current Clinical Practice

The international 2012 Kidney Disease Improving Global Outcomes Clinical Practice Guideline for the Evaluation and Management of Anemia and Chronic Kidney Disease (8) recommends addressing all correctable causes of anemia (including iron deficiency and inflammatory states) prior to initiation of ESA therapy. The guideline recommends balancing the potential benefits of reducing blood transfusions and improving anemia-related symptoms against the risks of harm in individual patients (e.g., stroke, vascular access loss, hypertension) (recommendation 1B). For adults with NDD-CKD and Hb concentrations <10.0 g/dL, the decision whether to initiate ESA therapy should be individualized based on the rate of fall of Hb concentration, prior response to iron therapy, the risk of needing a transfusion, the risks related to ESA therapy, and the presence of symptoms attributable to anemia (recommendation 2C). For adults with NDD-CKD and Hb concentrations $\geq$ 10.0 g/dL, ESA therapy is not recommended (recommendation 2D). For adults with stage 5 CKD on dialysis, ESA therapy is recommended when Hb is between 9.0 and 10.0 g/dL (recommendation 2B), and the Kidney Disease Improving Global Outcomes Guideline advises against use to maintain Hb above 11.5 g/dL (recommendation 2C).

3.1. Review Issue List

3.1.1. Key Efficacy Review Issues

3.1.1.1. Use of Rescue Therapy in the ASCEND Program

3.1.1.2. Lack of Clinical Meaningfulness on Key Secondary Endpoint of SF-26 V2.0 Vitality Domain Score in ASCEND-NHQ

3.1.2. Key Safety Review Issues

3.1.2.1. Difference Between On-Study Versus On-Treatment Analyses Results - ASCEND-ND

3.1.2.2. Higher Incidence of Cardiovascular Endpoints - ASCEND-ND

3.1.2.3. Higher Incidence of Cardiovascular Endpoints in the USA Subgroup – ASCEND-ND

3.1.2.4. Higher Incidence of Hospitalization for Heart Failure - ASCEND-ND, ASCEND-D

3.1.2.5. Three Times a Week (TIW) Dosing in ASCEND-TD

3.1.2.6. CV Safety in Incident Dialysis

3.1.2.7. Gastric Erosions and Gastrointestinal Bleeding

3.1.2.8. Acute Kidney Injury

3.1.2.9. Malignancy

3.1.2.10. Drug Induced Liver Injury

3.2. Approach to Clinical Review

The early phase studies, summarized in Section [12](#), allowed the determination of bioavailability, bioequivalence, food-effect, pharmacokinetic (PK), and pharmacodynamic (PD) characteristics of several drug formulations and dosing approaches, in addition to providing preliminary safety and tolerability data. Review of this portion of the drug development plan was addressed by the clinical pharmacology team.

[Table 4](#) provides an overview of the late phase clinical trials conducted to support the benefit-risk assessment of daprodustat (the ASCEND program). Overall, these trials were similar in study design and statistical methods (particularly the cardiovascular outcome trials (CVOTs), ASCEND-D and ASCEND-ND) but had different eligibility criteria. The primary statistical team reviewed data and analyses for efficacy outcomes of the individual phase 3 trials, the safety statistical team reviewed safety data and analyses for adjudicated safety events, and the clinical team reviewed the study design and provided interpretation of the statistical findings. The clinical team, with assistance from the clinical data scientist (who furnished standardized safety reports) reviewed other safety data.

Table 4. Clinical Studies/Trials Submitted in Support of Efficacy and/or Safety Determinations¹ for [Daprodustat]

Study/ Trial Identifier (NCT#)	Study/Trial Population	Study/Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized²	Number of Centers and Countries
200807 ASCEND- D	Subjects with anemia associated with CKD who are on dialysis who are switched from rhEPO or its analogs	<u>Control type:</u> AC <u>Randomization:</u> 1:1 <u>Blinding:</u> OL <u>Biomarkers:</u> N/A <u>Innovative design features:</u> Oral Dapro OD or rhEPO (hemodialysis-1500 to 60000 U total weekly dose IV epoetin alfa; peritoneal dialysis-20 to 400 µg total 4-weekly dose SC darbepoetin alfa); doses adjusted based on Hb during treatment period (1 y)	<u>Drug:</u> oral Dapro OD <u>Dosage:</u> 1, 2, 4, 6, 8, 10, 12, 16, or 24 mg <u>Number treated:</u> Dapro: 1487 R/1370 C rhEPO: 1477 R/1366 C <u>Duration (quantity and units):</u> ³ 2.48 y	<u>Primary:</u> Mean change in Hb between BL and EP (mean over Wks 28 to 52); <u>Co-primary:</u> Time to first occurrence of MACE, a composite of: All-cause mortality Nonfatal MI Nonfatal stroke <u>Secondary:</u> • Hb change from BL to Wk 52; • (%) responders, defined as mean Hb within the Hb analysis range 10 to 11.5 g/dL during EP	2964 randomized/ 2736 completed	431 centers 35 countries
200808 ASCEND- ND	Subjects with anemia associated with CKD not on dialysis and who are non-users or users of rhEPO or its analogs	<u>Control type:</u> AC <u>Randomization:</u> 1:1 <u>Blinding:</u> OL <u>Biomarkers:</u> N/A <u>Innovative design features:</u> Oral Dapro OD or rhEPO (hemodialysis-1500 to 60000 U total weekly dose IV epoetin alfa; peritoneal dialysis-20 to 400 µg total 4-weekly dose SC darbepoetin alfa); doses adjusted based on Hb during treatment period (1 y)	<u>Drug:</u> oral Dapro OD <u>Dosage:</u> 1, 2, 4, 6, 8, 10, 12, 16, or 24 mg <u>Number treated:</u> Dapro: 1937 R/1873 C Darbe: 1935 R/1870 C <u>Duration (quantity and units):</u> ³ 1.86 y	<u>Primary:</u> Mean change in Hb between BL and EP (mean over Wks 28 to 52); <u>Co-Primary:</u> Time to first occurrence of MACE, a composite of: All-cause mortality Nonfatal MI Nonfatal stroke <u>Secondary:</u> • Hb change from BL to Wk 52; • (%) responders, defined as mean Hb within the Hb analysis range 10 to 11.5 g/dL during EP	3872 randomized/ 3743 completed	506 centers 39 countries

Study/ Trial Identifier (NCT#)	Study/Trial Population	Study/Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized²	Number of Centers and Countries
205270 ASCEND- NHQ	Non-dialysis subjects with anemia associated with CKD who are naïve to rhEPO or its analogs and had limited IV iron exposure at BL	<u>Control type:</u> PC <u>Randomization:</u> 1:1 <u>Blinding:</u> DB <u>Biomarkers:</u> N/A <u>Innovative design features:</u> 1, 2, 4, 6, 8, 10, 12, or 16 mg oral Dapro OD or matching placebo; doses adjusted based on Hb during treatment period of 28 wks	<u>Drug:</u> oral Dapro OD <u>Dosage:</u> 1, 2, 4, 6, 8, 10, 12, or 16 mg <u>Number treated:</u> Placebo: 307 R/290 C Dapro: 307 R/300 C <u>Duration (quantity and units):</u> 0.5 y	<u>Primary:</u> Mean change in Hb between BL and EP (mean over Wk 24 to Wk 28 inclusive). <u>Secondary:</u> • % of participants having a Hb increase of ≥1.0 g/dL from BL to EP • Mean change in SF-36 v2.0 Vitality domain between BL and Wk 28	614 randomized/ 590 completed	142 centers 14 countries
201410 ASCEND- ID	Subjects with anemia associated with CKD who are not regularly using routine rhEPO or its analogs and who are initiating dialysis	<u>Control type:</u> AC <u>Randomization:</u> 1:1 <u>Blinding:</u> OL <u>Biomarkers:</u> N/A <u>Innovative design features:</u> 1, 2, 4, 6, 8, 10, 12, 16, or 24 mg oral Dapro OD or 20 to 400 µg total 4-weekly dose IV or subcutaneous darbepoetin alfa; doses adjusted based on Hb during the treatment period of 52 wks	<u>Drug:</u> oral Dapro OD <u>Dosage:</u> 1, 2, 4, 6, 8, 10, 12, 16, or 24 mg <u>Number treated:</u> Placebo: 307 R/290 C Dapro: 307 R/300 C <u>Duration (quantity and units):</u> 1 y	<u>Primary:</u> Mean change in Hb between BL and EP (mean over Wks 28 to 52); <u>Secondary:</u> Average monthly IV dose (mg)/participant from BL to Wk 52	312 randomized/ 306 completed	90 centers 14 countries

Study/ Trial Identifier (NCT#)	Study/Trial Population	Study/Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized²	Number of Centers and Countries
204837 ASCEND- TD	Subjects with anemia of CKD who are on hemodialysis who are switched from rhEPO or its analog	Control type: AC Randomization: 1:1 Blinding: DB Biomarkers: N/A Innovative design features: 0, 2, 4, 8, 12, 16, 20, 24, 32 or 48 mg oral or matching placebo three times weekly and 0, 1500 to 60000 U total weekly dose IV epoetin alfa or saline; doses adjusted based on Hb during Wk 52 treatment period	<u>Drug:</u> oral Dapro <u>Dosage:</u> 0, 2, 4, 8, 12, 16, 20, 24, 32 or 48 mg <u>Number treated:</u> Dapro: 270 R/269 C rhEPO: 137 R/135 C <u>Duration (quantity and units):</u> 1 y	<u>Primary:</u> Mean change in Hb between BL and EP (mean over Wks 28 to 52 <u>Secondary:</u> Average monthly IV dose (mg)/participant from BL to Wk 52	407 randomized/ 404 completed	90 centers 13 countries

Source: Reviewer.

¹ Includes all submitted clinical trials, even if not reviewed in-depth, except for phase 1 and pharmacokinetic studies.

² If no randomization, then replace with "Actual Enrolled."

³ Median years of follow-up for cardiovascular endpoints.

Abbreviations: AC, active-controlled; BID, twice daily; BL, baseline; C, completed; CKD, chronic kidney disease; Dapro, daprodustat; Darbe, Darbepoetin alfa; DB, double-blind; EP: evaluation period; Hb: hemoglobin; IV: intravenous; LTE, long-term extension; MACE, major adverse cardiovascular events; MC, multicenter; MI, myocardial infarction; N, number of subjects; NCT, national clinical trial; OD: once daily; OL, open-label; PC, placebo-controlled; PG, parallel group; R, randomized; rhEPO, recombinant human erythropoietin; h, hour; d, day; wk, week(s); mo, month(s); y, year(s)

4. Patient Experience Data

Table 5. Patient Experience Data Submitted or Considered

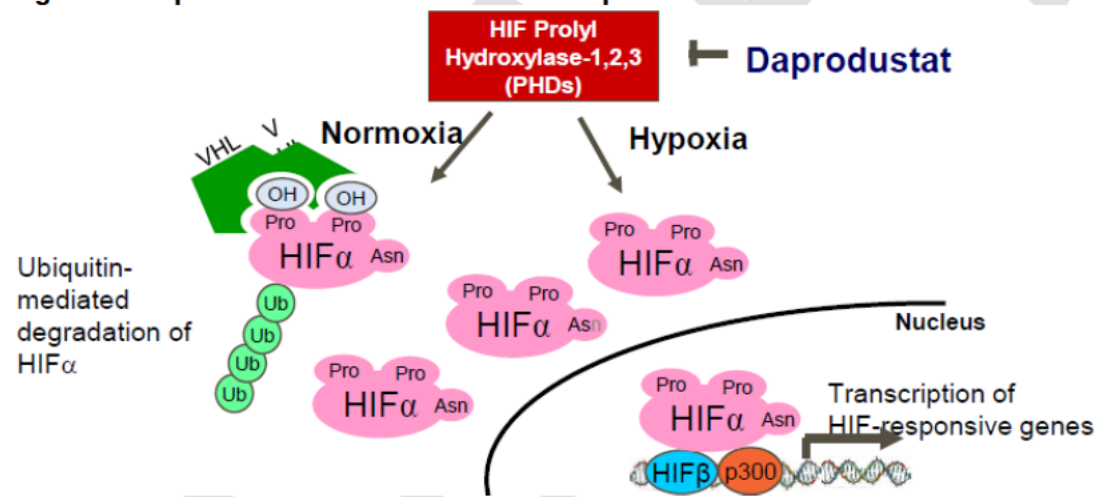
Data Submitted in the Application		
Check if Submitted	Type of Data	Section Where Discussed, if Applicable
Clinical Outcome Assessment Data Submitted in the Application		
☒	Patient-reported outcome	Section 6.3.2 and Section 16.1
☐	Observer-reported outcome	
☐	Clinician-reported outcome	
☐	Performance outcome	
Other Patient Experience Data Submitted in the Application		
☐	Patient-focused drug development meeting summary	
☒	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	See the full Division of Clinical Outcome Assessment Review
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
☒	Other: (please specify)	
☐	If no patient experience data were submitted by Applicant, indicate here.	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	Type of Data	Section Where Discussed, if Applicable
☐	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

5.1. Nonclinical Assessment of Potential Effectiveness

Daprodustat is a small molecule inhibitor of HIF prolyl-4-hydroxylases (PH1, 2, and 3). Hypoxic conditions or inhibition of PHs leads to increased accumulation of unhydroxylated HIF α subunits. These unhydroxylated HIF α subunits travel into the nucleus and form dimers with HIF β subunits. These dimers promote transcription of HIF-responsive genes that stimulate erythropoietic processes for the treatment of anemia in a similar manner to those resulting from hypoxia.

Figure 1. Proposed Mechanism of Action of Daprodustat



Source: Applicant's submission

Abbreviations: Asn, asparagine; HIF, hypoxia-inducible factor; OH, hydroxylated prolyl residual; P300, transcriptional coactivator p300; PHD, prolyl hydroxylase; Pro, proline residue; Ub, ubiquitin

Daprodustat reversibly binds HIF-PH1, 2, and 3 (K_i of 1.7-4.4nM) and selectively inhibits all three PH isoforms (half maximal inhibitory concentration [IC_{50}] of 3.3-8.9nM), in contrast to IC_{50} s with close homologues collagen prolyl hydroxylase (CP4H) and factor inhibiting HIF (FIH), which were in the μ M range.

Daprodustat was evaluated in cell-based assays, which confirmed that inhibition of PH isoforms by daprodustat stabilizes dimers of unhydroxylated HIF α with HIF β subunits and induces erythropoiesis by stimulating the downstream cascade normally induced by hypoxia.

Specifically, PH inhibition resulted in decreased hepcidin mRNA expression, leading to increased utilization of iron (increased hemoglobin production), and increased transcription of HIF-responsive genes, including EPO, heme oxygenase-1, phosphoglycerate kinase-1 (PGK1), and VEGF, leading to stimulation of erythropoietic processes. Likewise, in vivo, in wild-type (non-anemic) female B6D2F1 (C57BL/6 and male DBA/2 hybrid) mice given daprodustat orally

once at 60 mg/kg, liver EPO mRNA expression was increased up to 449-fold. Kidney EPO mRNA expression was increased up to 56-fold. Female B6D2F1 mice given daprodustat orally at doses up to 30 mg/kg/day for 8 days, showed increases in RBC count, hemoglobin, hematocrit, and reticulocytes (up to 17%, 17%, 25% and 673%, respectively vs. control) and decreased platelet/white cell counts with a clear dose-response. Male Sprague-Dawley rats were given daprodustat orally at doses up to 10 mg/kg/day for 21 days, which resulted in dose-related increases in RBC parameters (up to 18%, 22%, and 26% for RBC, hemoglobin, and hematocrit, respectively, compared to control) without significant drug-related effects on white cell counts. Metabolism studies indicate that the major human metabolites of daprodustat [M2; M3, M4, M5, M6 (both stereoisomers); M13] have comparable activity to the parent drug.

While the sponsor did not conduct studies in animal models of anemia, overall, these pharmacology data support the Applicant's therapeutic rationale for the use of daprodustat as a treatment for anemia, especially with regards to the drug's ability to potently induce EPO expression and increase production of red blood cells. However, given that pharmacology models described above consisted of healthy animals lacking either anemia or features of chronic kidney disease, these data do not directly address the impact of underlying disease on the efficacy of daprodustat.

The established pharmacologic class term for daprodustat is *hypoxia inducible factor prolyl hydroxylase inhibitor*, which is based on extensive prior internal discussions for previous HIF-PHD drug candidates.

5.2. Clinical Pharmacology/Pharmacokinetics

Table 6. Summary of Clinical Pharmacology and Pharmacokinetics

Characteristic	Drug Information																							
	Pharmacologic Activity																							
Established pharmacologic class (EPC)	Hypoxia inducible factor prolyl hydroxylase inhibitor																							
Mechanism of action	Daprodustat is a reversible inhibitor of HIF-prolyl-4-hydroxylases (PH)1, PH2 and PH3 (IC ₅₀ in the nM range). This activity results in the stabilization and nuclear accumulation of HIF-1 α and HIF-2 α transcription factors, leading to increased transcription of the HIF-responsive genes, including erythropoietin.																							
Active moieties	Daprodustat																							
QT prolongation	At a dose 10 times the maximum recommended dose (48 mg in dialysis dependent CKD), daprodustat does not prolong the QTc interval to any clinically relevant extent.																							
	General Information																							
Bioanalysis	Validated LC-MS/MS methods were used to determine the concentrations of daprodustat and its metabolites in human plasma																							
Healthy subjects versus patients	PK of daprodustat in healthy subjects (normal renal function) and patients with stage 3/4/5 CKD are similar.																							
Drug exposure at steady state following the therapeutic dosing regimen (or single dose, if more relevant for the drug)	<table border="1"> <thead> <tr> <th>Dose</th> <th>Parameter</th> <th>Mean (%CV)</th> </tr> </thead> <tbody> <tr> <td rowspan="2">2 mg</td> <td>AUC</td> <td>74.3 (11.6)</td> </tr> <tr> <td>C_{max}</td> <td>29.4 (15.3)</td> </tr> <tr> <td rowspan="2">5 mg</td> <td>AUC</td> <td>198 (32.0)</td> </tr> <tr> <td>C_{max}</td> <td>93.8 (31.5)</td> </tr> <tr> <td rowspan="2">15 mg</td> <td>AUC</td> <td>647 (18.4)</td> </tr> <tr> <td>C_{max}</td> <td>286 (30.1)</td> </tr> <tr> <td rowspan="2">50 mg</td> <td>AUC</td> <td>1685 (30.7)</td> </tr> <tr> <td>C_{max}</td> <td>521 (42.4)</td> </tr> </tbody> </table> Source: CSR Study PHX111427, data from healthy adults, following single dose Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CSR, clinical study report; CV, coefficient of variation	Dose	Parameter	Mean (%CV)	2 mg	AUC	74.3 (11.6)	C _{max}	29.4 (15.3)	5 mg	AUC	198 (32.0)	C _{max}	93.8 (31.5)	15 mg	AUC	647 (18.4)	C _{max}	286 (30.1)	50 mg	AUC	1685 (30.7)	C _{max}	521 (42.4)
Dose	Parameter	Mean (%CV)																						
2 mg	AUC	74.3 (11.6)																						
	C _{max}	29.4 (15.3)																						
5 mg	AUC	198 (32.0)																						
	C _{max}	93.8 (31.5)																						
15 mg	AUC	647 (18.4)																						
	C _{max}	286 (30.1)																						
50 mg	AUC	1685 (30.7)																						
	C _{max}	521 (42.4)																						
Maximally tolerated dose or exposure	Maximum tolerated dose not identified. 500 mg single oral dose is the highest single oral dose evaluated in healthy adults (Study PHI113635 for QT analysis). The highest multiple daily dose evaluated is 100 mg once daily for 14 days in healthy adults (Study PHI112842).																							
Dose proportionality	Daprodustat exhibited linear PK with a generally dose-proportional increase in plasma exposure (C _{max} , AUC) from 2 mg to 300 mg.																							
Accumulation	Following repeat-dose administration, no accumulation of daprodustat is observed.																							

Characteristic	Drug Information
Time to achieve steady-state	Steady-state PK is achieved by 2 days.
Bridge between to-be-marketed and clinical trial/study formulations	The tablet formulation used in the pivotal Phase 3 trials is different from the final to-be-marketed tablet formulation. The Applicant conducted a relative bioavailability study (Study 213022 Part B) in healthy adults to establish a PK bridge between the clinical trial formulation and the to-be-marketed formulation. The 90% confidence interval for the least square means for the PK parameters (C_{max} and AUC) are within the 80-125% bioequivalence range for the 1 mg, 2 mg, 4 mg, 6 mg, and 8 mg tablet strengths.
Absorption	
Bioavailability	The oral bioavailability of the daprodustat tablet is 65%.
T_{max}	1-4 hours
Food effect (fed/fasted)	Food (high-fat, high-calorie meal, or CKD meal [500 to 700 kcal, comprised of 12 to 16 g protein, 1 to 2 g salt, and up to 500 mg potassium]) did not have a clinically significant effect on the extent of absorption (AUC) and C_{max} of daprodustat in healthy subjects.
Geometric least square mean and 90% CI	Daprodustat may be taken with or without food. $AUC_{0-\infty}$ fed/fasted ratio 0.92 [90% CI 0.84, 1.01] C_{max} fed/fasted ratio 0.71 [90% CI 0.61, 0.82] T_{max} median (range): fed state: 2.00 (1.00-6.00); fasted state: 1.00 (1.00-4.00) A standard CKD meal (500 to 700 kcal, comprised of 12 to 16 g protein, 1 to 2 g salt, and up to 500 mg potassium) did not have a clinically significant effect on the extent of absorption (AUC) and C_{max} of daprodustat (4 mg) in healthy Japanese subjects. $AUC_{0-\infty}$ fed/fasted ratio 0.91 [90% CI 0.82, 1.01] C_{max} fed/fasted ratio 0.89 [90% CI 0.73, 1.08] T_{max} median (range): fed state: 2.75 (1.00, 3.00); fasted state: 1.75 (1.00, 4.00)
Distribution	
Volume of distribution	14.3 L
Plasma protein binding	The binding of daprodustat to plasma protein is high (99.4%). Binding was also high to human serum albumin (>99.3%), while low to alpha-1-acid glycoprotein (<20%). In general, there was no evidence of concentration-dependent protein binding of daprodustat over the concentration range evaluated (0.2, 1, 10 and 50 µg/mL).
Drug as substrate of transporters	- Daprodustat is a substrate of human breast cancer resistance protein (BCRP) in vitro but not a substrate of human drug transporters P-glycoprotein (P-gp), OATP1B1, OATP1B3, OATP2B1, OCT1, OAT1, OAT3, OCT2, MATE1, or MATE2-K. Concomitant administration with BCRP inhibitors is not expected to have a clinically significant effect on daprodustat PK in vivo. - All 6 predominant circulating metabolites showed some degree of active uptake activity in transporter-overexpressed cells (OATP1B1, OATP1B3), but did not exhibit active uptake in human hepatocytes. - The 6 metabolites are not in vitro substrates of the renal transporters OAT1, OAT3, OCT2, MATE1, or MATE2-K, with the exceptions of M2, M3, and M13, which are weak substrates for OAT3, and M2, which is a weak substrate for OAT1.
Elimination	
Mass balance results	In a mass-balance study in 6 healthy male subjects, a single IV micro tracer dose of 50 µg [^{14}C]-daprodustat concomitant with an oral, 6 mg tablet dose of non-radiolabeled daprodustat was administered in treatment period 1. Following a 14-day washout period, treatment period 2 consisted of a single, oral 25 mg solution dose of [^{14}C]-daprodustat. Following administration of 25 mg [^{14}C]-daprodustat as an

Characteristic	Drug Information
	oral solution, 94.6% of the original dose was recovered by 168 hours post-dose (recovery percentage includes both parent and metabolites), with 21.4% in urine and 73.6% in feces. Unchanged daprodustat was the principal circulating drug related material representing 40% of the pooled plasma radioactivity. Predominant metabolites M2, M3, M4, and M13 were observed, individually representing 5.7 to 8.3% of the plasma radioactivity. Unchanged daprodustat accounted for <0.5% in feces and <0.05% in urine. Following oral solution administration, approximately 80% of daprodustat is absorbed across the GI tract. The oral absolute bioavailability of the daprodustat tablet based on AUC(0-inf) is 65%.
Clearance	Average renal clearance (CL) of daprodustat is 0.05 mL/min, with a urinary recovery of 0.01% of unchanged daprodustat.
Half-life	Half-life of daprodustat ranges from 1-4 h.
Metabolic pathway(s)	Daprodustat is metabolized by hydroxylation primarily by CYP2C8, with a minor contribution of CYP3A4, producing 6 predominant circulating metabolites, of which, 3 (M2, M3, M13) are major ($\geq 10\%$ drug related material) metabolites.
<i>Intrinsic Factors and Specific Populations</i>	
Body weight	Using population PK modeling, the geometric mean ratio (GMR) of C_{max} for the 5 ^h and 95 th percentile body weights relative to the median was 0.73 and 1.28, and the GMR for AUC was 0.76 and 1.24, respectively. Body weight does not have a clinically significant effect on daprodustat PK and dose adjustment based on body weight is not needed.
Age	The PK of daprodustat has not been evaluated in children and adolescents less than 18 years of age. The PopPK analyses using data from Phase 2/3 trials in adults with anemia associated with CKD (ranging in age in from 22 to 93 years (median 59 years) did not show age as significant covariate.
Renal impairment	Renal impairment does not have a clinically significant effect on daprodustat PK. Daprodustat is not significantly cleared by hemodialysis.
Hepatic impairment	Daprodustat C_{max} and AUC geometric least square mean ratios [90% CI] were 1.9-fold [1.06, 3.71] and 2.0-fold [1.10, 3.61] higher, respectively, in subjects with moderate hepatic impairment compared with their matched healthy control subjects. C_{max} for daprodustat was similar between subjects with mild hepatic impairment (Cohort 3) and their matched healthy controls (Cohort 4). Daprodustat AUC geometric least square mean ratio [90% CI] was 1.46-fold [1.03, 2.05] higher in subjects with mild hepatic impairment compared with their matched healthy control subjects. Reducing the starting dose of daprodustat by approximately half is recommended when initiating treatment in patients with moderate hepatic impairment (Child-Pugh Class B) with the exception for those patients who have a recommended starting dose of 1 mg due to the unavailability of a lower tablet strength. No adjustment of the starting dose is required in patients with mild hepatic impairment. The effect of severe hepatic impairment (Child-Pugh Class C) on daprodustat PK has not been evaluated. Efficacy and safety of daprodustat in patients with severe hepatic impairment is not known. Use of daprodustat in patients with severe hepatic impairment is not recommended because of the expectation for much higher daprodustat exposures and lack of clinical experience.
<i>Drug Interaction Liability (Drug as Perpetrator)</i>	
Inhibition/induction of metabolism	In vitro, daprodustat and the 6 predominant circulating metabolites are not competitive or time-dependent inhibitors of CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4. Daprodustat is not an in vitro inducer of CYP1A2, CYP2B6, or CYP3A4.
Inhibition/induction of transporter systems	Daprodustat is not an inhibitor of transporters P-gp or BCRP in vitro. Daprodustat is an in vitro inhibitor of human OATP1B1 and OATP1B3. The 6 daprodustat metabolites did not inhibit OATP1B1 at concentrations up to 50 μ M. Daprodustat and the 6 metabolites are not inhibitors of the human renal transporters OAT1, OAT3, OCT2, MATE1 and MATE2-K.

Abbreviations: AUC, area under the concentration-time curve; AUC(0-inf), area under the concentration-time curve from time zero to infinity; C_{max} , maximum plasma concentration; CI, confidence interval; CKD, chronic kidney disease; GI, gastrointestinal; HIF, hypoxia-inducible factor; IC_{50} , half maximal inhibitory concentration; IV, intravenous; LC-MS/MS, liquid chromatography with tandem mass spectrometry; PK, pharmacokinetic; PopPK, population pharmacokinetics

6. Efficacy (Evaluation of Benefit)

6.1. Assessment of Dose and Potential Effectiveness

Applicant's Proposed Dosing Regimen

(b) (4)

Table 7. Dose Levels of JESDUVROQ

Daily dose of JESDUVROQ	1 mg	2 mg	4 mg	6 mg	8 mg	12 mg	16 mg	24 mg ^a

^a 24 mg is the maximum recommended once daily dose.

Selection of Dosing Regimen for the Phase 3 Trials

Based on an integrated analysis of Phase 1 and 2 longitudinal Hb data, a modeling and simulation approach was taken to select the starting doses and dose levels for once-daily administration of daprodustat in the global Phase 3 trials. The Applicant developed a mixed effect population model, which included dose, PK exposure, and time as key independent variables, and Hb as a repeatedly measured response variable. The dynamics (relationship of hemoglobin with respect to time) of red blood cell production and lifespan was modeled using 12 transit compartments (see Section [14.5.2](#)), where production rates were influenced by baseline Hb and daprodustat. After model validation, clinical trial simulations were used to assess the average Hb and the % of time within the target range of 10 to 11 g/dL over an evaluation period from Week 28 to 52, as planned in the Phase 3 studies.

Starting doses of 1, 2, 4, 6, 8 and 12 mg were selected. These doses were estimated to maintain equivalent Hb concentrations to that achieved in the participants on prior rhEPO therapy and its analogue or for participants not receiving prior rhEPO to increase the Hb concentration from Baseline (Day 1) to the target Hb range of 10 to 11 g/dL (11 to 12 g/dL in 205270/ASCEND-NHQ). As CKD patients receiving hemodialysis are typically treated three times a week (TIW), rhEPO treatment regimens are often TIW, coincident with the hemodialysis schedule. Therefore, a TIW regimen was assessed for daprodustat in the hemodialysis population. For TIW dosing in 204837/ASCEND-TD the once daily doses used in Study 200807/ASCEND-D were converted to corresponding TIW doses using the estimated dose conversion ratio of approximately 2.0 that was determined in the Phase 2A TIW daprodustat dose ranging study. For TIW dosing, starting doses of 8, 12, 16 and 24 mg were selected. These doses were estimated to maintain equivalent Hb concentrations to that achieved in the participants on prior rhEPO therapy and its analogues.

6.2. Clinical Studies/Trials Intended to Demonstrate Efficacy

The Applicant submitted clinical study reports (CSRs) from five Phase 3 studies to evaluate the efficacy and safety of daprodustat (GSK1278863) in the treatment of anemia associated with chronic kidney disease. An overview of the studies is provided in [Table 8](#).

Table 8. Clinical Studies in the ASCEND Program

Parameter	Non-Dialysis Studies		Dialysis Studies		
	ASCEND-ND	ASCEND-NHQ	ASCEND-D	ASCEND-TD	ASCEND-ID
Population	NDD ESA user or non-user	NDD	HD or PD ESA user	HD ESA user	Incident dialysis (ID) ESA non-user
Daprodustat dosing	Once daily	Once daily	Once daily	Three times a week	Once daily
Control	SC darbepoetin alfa	Oral placebo	IV epoetin alfa SC darbepoetin alfa	IV epoetin alfa	SC or IV darbepoetin alfa
Number of participants	4500	600	3000	402	300
Blinding	Open-label (sponsor blind)	Double-blind	Open-label (sponsor blind)	Double-blind, double dummy	Open-label (sponsor blind)
Randomization	1:1	1:1	1:1	2:1	1:1
Stratification	Region Current ESA use Participation in ABPM substudy	Region	Dialysis type (HD or PD) Region Participation in ABPM substudy	Region	Dialysis type (HD or PD) Dialysis start planned or unplanned
Evaluation period	Weeks 28-52	Weeks 24-28	Weeks 28-52	Weeks 28-52	Weeks 28-52
Hb target range	10-11 g/dL	11-12 g/dL	10-11 g/dL	10-11 g/dL	10-11 g/dL

Source: FDA

Abbreviations: ABPM, ambulatory blood pressure monitoring; Hb, hemoglobin; HD, hemodialysis; ID, incident dialysis; IV, intravenous; ND, non-dialysis; PD, peritoneal dialysis; ESA, erythropoiesis-stimulating agent; SC, subcutaneous

Daprodustat's evidence of safety and effectiveness for the treatment of anemia due to CKD in adults is based primarily on ASCEND-ND and ASCEND-D, which are event-driven, multicenter, multinational, open-label, active-controlled, randomized, parallel-group trials.

ASCEND-ND assessed the efficacy and safety of daprodustat once daily compared to darbepoetin alfa in the NDD population. Subjects not currently receiving ESAs and subjects currently receiving ESAs were included. Those on an ESA continued their ESA therapy during the screening and run-in periods.

ASCEND-D assessed the efficacy and safety of daprodustat once daily compared to intravenous (IV) epoetin alfa in subjects on hemodialysis and compared to SC darbepoetin alfa in subjects on peritoneal dialysis. Subjects switched their ESA to these study drugs on the day of randomization.

The following three additional smaller, fixed duration studies are considered supportive for efficacy and safety:

- ASCEND-NHQ was a double-blind, placebo-controlled study in a non-dialysis population with anemia with objectives of providing evidence of efficacy, safety, and improved quality of life versus placebo.
- ASCEND ID was a 1-year, open-label, active-controlled study that evaluated subjects with anemia associated with CKD who were not regularly using an ESA and who were initiating dialysis (unplanned or planned) to provide evidence for the efficacy and safety of daprodustat in the incident dialysis population.
- ASCEND-TD was a 1-year, double-blind, active-controlled study that enrolled subjects who were hemodialysis dependent (hemodiafiltration and hemofiltration with hemodialysis) who had anemia of CKD and were being treated with ESAs or its analogues to provide support for a three-times a week dosing regimen.

6.2.1. Study 200807/ASCEND-D

6.2.1.1. Design

Overview

ASCEND-D (NCT02879305): The Applicant conducted a randomized, Phase 3 Study, 200807/ASCEND-D, titled "A phase 3 randomized, open-label (sponsor-blind), active controlled, parallel-group, multi-center, event driven study in dialysis subjects with anemia associated with chronic kidney disease to evaluate the safety and efficacy of daprodustat compared to recombinant human erythropoietin, following a switch from erythropoietin-stimulating agents."

Coprietary Safety and Efficacy Endpoint

Safety: Time to first occurrence of adjudicated MACE (composite of all-cause mortality, nonfatal MI, and nonfatal stroke)

Efficacy: Mean change in Hb between Baseline and Evaluation Period (EP) (Weeks 28 to 52)

Key Secondary Efficacy Endpoint

Average monthly IV iron dose (mg)/participant to Week 52

Secondary Efficacy Endpoints

- Hb change from Baseline to Week 52
- N (%) responders, defined as mean Hb within the Hb analysis range 10 to 11.5 g/dL during EP
- % time Hb in analysis range during the Evaluation Period
- Time to stopping randomized treatment due to meeting rescue criteria

Exploratory Endpoints

- Number (%) of participants who receive at least one RBC or whole blood transfusions by Week 52 and by end of treatment
- Number of RBC and whole blood transfusions per 100 person years

The key secondary safety endpoints tested for superiority and adjusted for multiplicity included:

- Time to first MACE²
- Time to first occurrence of MACE or thromboembolic event (TEE) (vascular access thrombosis, deep vein thrombosis, pulmonary embolism)
- Time to first occurrence of MACE or hospitalization for heart failure (HHF)

Adjudicated secondary safety endpoints are listed below. These endpoints are time-to-event endpoints.

- All-cause mortality
- CV mortality
- Fatal or nonfatal MI
- Fatal or nonfatal stroke
- Fatal or nonfatal HHF
- Fatal or nonfatal TEE
- Composite of MACE and HHF³
- Composite of CV mortality and nonfatal MI
- Composite of MACE, HHF, and TEE

Study Design

Study 200807/ASCEND-D was a randomized, open-label (sponsor blind), active controlled, parallel-group, multi-center, event-driven study in dialysis participants with anemia associated with CKD currently treated with ESAs.

² Tested for noninferiority as a coprimary endpoint and tested for superiority as a key principal secondary endpoint.

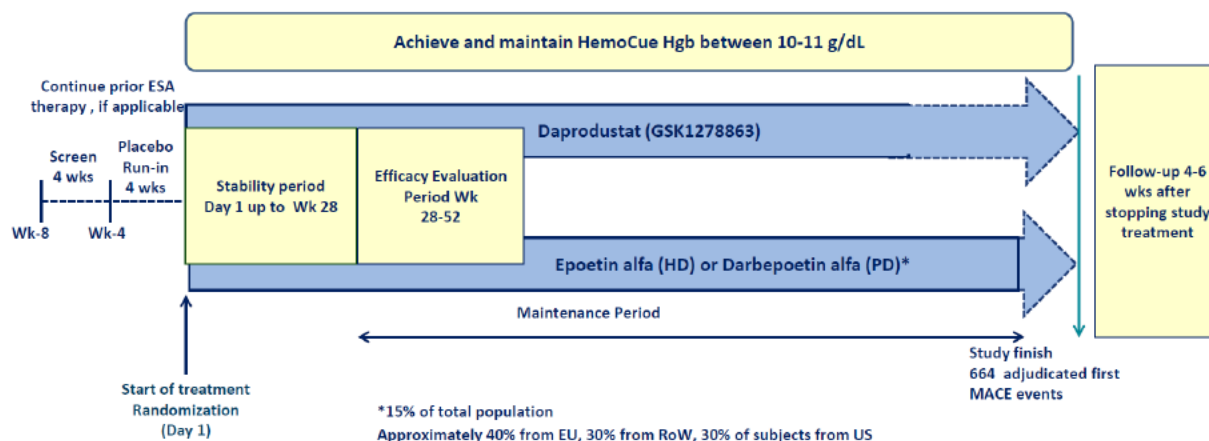
³ A recurrent event with data on multiple events for analysis

This study comprised four study periods: a 4-week screening period, a 4-week placebo run-in period, a treatment period, and a follow-up period (Figure 2). Prior ESA therapy continued during the screening and run-in periods.

The Treatment Period consists of:

- Stabilization Period (SP), defined as the period from Day 1 to Week 28 (up to but excluding the Week 28 visit), during which study treatment was dose-titrated to achieve and maintain Hb in the target range.
- Maintenance Period (MP), defined as the period from the end of the SP to the end of treatment (variable per participant), to assess long term safety and efficacy
- EP, defined as the period from Week 28 to Week 52, during which Hb efficacy was primarily assessed.
- Alternative EP: Because of the importance of patients not spending undue time with suboptimal Hb levels, a shorter EP, defined as the period from Week 28 to Week 36, was also assessed for Hb efficacy.

Figure 2. Study Schematic for Study 200807/ASCEND-D



Source: Sponsor CSR

Abbreviations: CSR, clinical study report; ESA, erythropoiesis-stimulating agents; EU, European Union; HD, hemodialysis; MACE, major adverse cardiovascular events; PD, peritoneal dialysis; RoW, rest of the world; US, United States

In ASCEND-D, subjects were randomized in a 1:1 ratio to oral daprodustat or to IV epoetin alfa (hemodialysis) or SC darbepoetin alfa (peritoneal dialysis). In the ASCEND-D study, daprodustat was also dosed daily while the control was dosed weekly, every 2 weeks, or every 4 weeks (darbepoetin alfa) or every 2 days or weekly (epoetin alfa), depending on the subject's prior ESA use.

An anemia rescue algorithm, which included a provision for the use of intravenous iron, an RBC transfusion, or both, was provided in the protocol. Additionally, the protocol required subjects to be iron replete throughout the study – and initiate iron therapy (investigator's choice) if ferritin was less than or equal to 100 ng/mL, transferrin saturation was less than or equal to 20%, or both.

Study Population Inclusion/Exclusion Criteria

Inclusion Criteria

The study recruited male and female (non-pregnant, non-lactating) participants, aged ≥ 18 years to ≤ 99 years of age with anemia of CKD who were being treated with an ESA for at least the 6-weeks prior to screening and up to randomization. Participants were required to have been on dialysis for greater than 90 days prior to screening and were either receiving hemodialysis ≥ 2 times per week or peritoneal dialysis ≥ 5 times per week. Hb concentration (measured by HemoCue) for inclusion are defined as follows:

- Week -8: Hb 8 to 12 g/dL (5 to 7.4 mmol/L); If Hb is 12.1 to 12.4 g/dL (7.5-7.7 mmol/L), up to two retests are allowed; the retest value must be between 8 to 12 g/dL (5 to 7.4 mmol/L).
- Day 1: Hb 8 to 11 g/dL (5 to 6.8 mmol/L) and receiving at least the minimum ESA dose; Hb > 11 to 11.5 g/dL (6.8 to 7.1 mmol/L) and receiving greater than the minimum ESA dose.

Exclusion Criteria

- Planned kidney transplant within 52 weeks
- Ferritin ≤ 100 ng/mL (≤ 100 $\mu\text{g/L}$) at screening
- Transferrin saturation (TSAT) $\leq 20\%$ at screening (If TSAT was 18-20%, a retest using a new blood sample could be obtained within 7 days of the final laboratory report; the final retest value must be $> 20\%$ to confirm eligibility)
- History of bone marrow aplasia or pure red cell aplasia
- Anemia other than anemia of CKD.
- MI, acute coronary syndrome, stroke, or transient ischemic attack within 4 weeks prior to screening through to randomization (Day 1)
- QTcB > 500 msec, or QTcB > 530 msec in participants with bundle branch block at randomization (Day 1)
- Chronic Class IV heart failure
- Uncontrolled hypertension
- Prior (within 2 years) or current history of cancer (with the exception of localized squamous cell or basal cell carcinoma of the skin), or complex kidney cyst (e.g., Bosniak Category II F, III or IV) > 3 cm.; current unstable liver or biliary disease or known hepatic or biliary abnormalities; or gastrointestinal bleeding.
- Participants requiring treatment with strong inhibitor of CYP2C8 or a strong inducer of CYP2C8 were excluded.

6.2.1.2. Statistical Analysis Plan

Sample Size Determination

The size of this event driven trial was based on the co-primary CV safety objective and determined by a fixed event target of 664 adjudicated first MACE. This provided approximately 90% power to establish non-inferiority assuming a true underlying 3% lower relative risk of MACE in favor of daprodustat compared to rhEPO (i.e., hazard ratio=0.97) and 82% power for

pre-specified non-inferiority of 1.25 under the assumption that the true underlying risk of MACE was the same in both arms (i.e., hazard ratio=1.00).

For 90% power to test the co-primary non-inferiority Hb hypothesis, with a two-sided 95% CI, pre-specified margin of -0.75 g/dL and a two-sample t-test, a total of 86 evaluable participants per treatment group was required. The planned study size far exceeded this requirement and provided more than 99% power for non-inferiority and a high level of precision to estimate the treatment effect (two-sided 95% CI half width of 0.128 g/dL, assuming 30% of participants would be non-evaluable for efficacy). The largest (most negative) difference between arms that would meet the statistical criterion for non-inferiority would be -0.622 g/dL. If the two-sided 95% CI for the treatment difference is completely negative (i.e., lies fully within the range -0.75 to < 0 g/dL) non-inferiority would still be concluded.

For all four of the active-controlled trials, the pre-specified margin for efficacy of -0.75 g/dL was determined based on Hb changes that would result in a clinically meaningful difference to a patient, the percentage of the rhEPO efficacy preserved by the margin and consideration of changes that could be due to variability and precedent for margins used in past trials for comparative Hb efficacy assessments (Aranesp, Omontys and Mircera).

Interim Analysis

There were no prospectively defined interim analyses planned to stop the study early for benefit. While the planned futility analysis described below had a small impact on reducing study-wise Type I error rate, there were no plans to adjust the alpha level used for the final analysis.

The Independent Data Monitoring Committee (IDMC) evaluated the co-primary MACE endpoint to assess for futility of achieving non-inferiority at study completion. Pre-specified guidelines governing the decision to continue or stop the study considered signals for harm, the predictive probability of achieving at least non-inferiority at trial end and the risk of incorrectly stopping for futility.

Missing Data Handling for Efficacy Endpoint

For the analysis of the co-primary efficacy endpoint, a multiple imputation approach was used to handle missing Hb values. Both intermittent missing and monotone missing scheduled Hb data were imputed based on the missing at random (MAR) assumption. However, it is important to examine the sensitivity of inferences to departures from the MAR assumption for the monotone missing data. A tipping point analysis was performed as a sensitivity analysis for the assumptions of missing data, that is, to evaluate missing not at random (MNAR) in which the missing observations depend on unobserved values.

Analysis for Primary Efficacy Endpoint

The primary population for efficacy analyses was the intent-to-treat (ITT) population. The primary efficacy evaluation was the effect of daprodustat relative to rhEPO on the change in Hb from Baseline to the average of all Hb values in the EP, regardless of adherence to treatment including interruptions and discontinuation, the use of non-randomized ESA medication for any reason including rescue therapy, or the use of blood transfusions, in participants on dialysis currently treated with an ESA with anemia secondary to CKD and assuming participants did not die before the end of the EP.

For each participant, Baseline Hb was the value obtained prior to taking randomized treatment (or if this was missing, Baseline Hb was the value obtained at the latest non-missing pre-dose assessment on or before the randomization date), and Hb during the EP was determined by calculating the mean of all available and imputed Hb values between Weeks 28 to 52 inclusive regardless of adherence to randomized treatment. Multiple imputation was used to impute missing Hb data, and an ANCOVA model, adjusting for dialysis type, region, Baseline Hb and treatment, was used to analyze the data and calculate a point estimate, two-sided 95% CI for the treatment effect and an associated one-sided non-inferiority test p-value. Analysis results from the multiple imputation were combined using Rubin's rules. Non-inferiority was established if the lower limit of the two-sided 95% CI was greater than the margin of -0.75 g/dL.

Sensitivity Analyses

- Post-randomization Hb (Observed Values Only Analysis): An analysis using available observed Hb values (on and off-treatment) only for post-randomization hemoglobin. Missing Hb data were not imputed.
- Evaluable Hb: An alternative “while on-treatment” estimand using only evaluable Hb data. Missing Hb data were not imputed.
- Per Protocol (PP) Hb: A PP population analysis based on the evaluable Hb analysis.
- Alternative EP (Week 28-36) Analyses:
 - An alternative EP analysis from Week 28-36 using all available Hb values (on and off treatment). Missing post-Baseline Hb values were imputed.
 - An alternative EP analysis from Week 28-36 using only evaluable Hb values. Missing Hb data were not imputed.
- A tipping point analysis using all available Hb values (on and off-treatment) as a sensitivity for the co-primary estimand and using evaluable Hb values as supportive analysis
- Excluding sites with suspected fraud: The co-primary analysis, using on and off treatment Hb values with imputation for missing values, was repeated excluding data from sites with suspected fraud.

In the 4 active-controlled studies, a target level Hb during the Evaluation Period was set as 10.0 to 11.0 g/dL. In the protocols, to account for within participant variability between methods used for measuring Hb values (HemoCue at study visits for inclusion/dose titration, compared with central laboratory tests), 0.5 g/dL was added to the upper end of the target range to create a defined analysis range of 10.0 to 11.5 g/dL.

Analyses for Secondary Efficacy Endpoints

IV iron use for all participants was recorded in the eCRF and the average on-treatment monthly IV iron dose from Day 1 up to Week 52, prior to receipt of a red blood cell or whole blood transfusion, was calculated. An analysis of covariance (ANCOVA) model was used to compare the difference in on-treatment average monthly IV iron dose per participant between treatment arms, including factors for Baseline dose, treatment, and the prognostic randomization stratification factors (dialysis type and region).

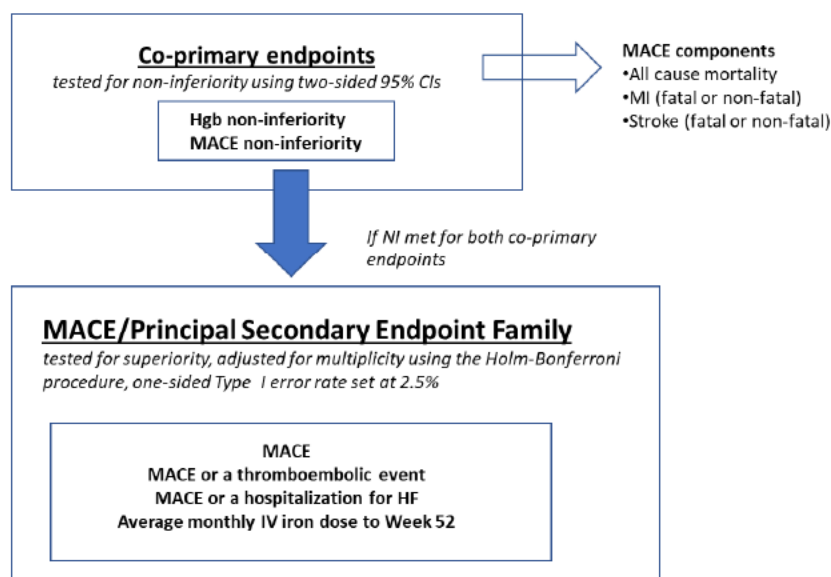
All analyses of additional secondary endpoints were unadjusted for multiplicity; summary statistics, treatment effects and nominal p-values were used for any treatment comparisons.

Multiple Comparison

The multiplicity strategy for this trial used a combination of a gatekeeper approach on the co-primary endpoints, followed by a closed-test multiplicity procedure wrapped around the family of superiority hypotheses consisting of MACE and the key secondary endpoints.

[Figure 3](#) illustrates the structure of the statistical testing plan. First, the co-primary endpoints were evaluated for non-inferiority by comparing each two-sided 95% CI to the appropriate non-inferiority margin. Conditional on both co-primary endpoints achieving non-inferiority (i.e., passing the gatekeeper), the family of MACE and the key secondary endpoints were formally tested for superiority using the widely known Holm-Bonferroni procedure. The procedure was conducted based on a familywise Type I error rate set at the one-sided 2.5% level.

Figure 3. Multiplicity Controlled Statistical Testing Plan for Study 200807/ASCEND-D



Source: Sponsor CSR
Abbreviations: CI, confidence interval; CSR, clinical study report; HF, heart failure; IV, intravenous; MACE, major adverse cardiovascular events; MI, myocardial infarction

Subgroup Analysis

A subgroup analysis for age, sex, region, and race were performed for the primary ITT analysis of post-randomization mean change in Hb between Baseline and over the EP (Weeks 28 to 52).

6.2.1.3. Results of Analyses

Patient Disposition

Participants were considered to have completed the study if they completed all visits through the End of Study visit irrespective of whether they discontinued randomized treatment. Participants were also considered to have completed the study if they died while on study. Of 2964 participants in the ITT population, 2736 (92%) participants completed the study through the End of Study visit or died while on study. A total of 117 (8%) participants in the daprodustat group

and 111 (8%) participants in the rhEPO group discontinued randomized treatment and withdrew from the study (see [Table 9](#)). Reasons for discontinuing randomized treatment were balanced between treatment groups.

Table 9. Summary of Subject Status and Reasons for Withdrawal for Study 200807/ASCEND-D

	Daprodustat N=1487	rhEPO N=1477	Total N=2964
Overall subject status			
Completed	1370 (92%)	1366 (92%)	2736 (92%)
Withdrawn	117 (8%)	111 (8%)	228 (8%)
Primary reason/sub-reason for study withdrawal			
Study closed/terminated	0	0	0
Lost to follow-up	17 (1%)	12 (<1%)	29 (<1%)
Withdrew consent	44 (3%)	42 (3%)	86 (3%)
Logistical circumstances	1 (<1%)	1 (<1%)	2 (<1%)
Privacy preferences	17 (1%)	15 (1%)	32 (1%)
Adverse experience	1 (<1%)	0	1 (<1%)
Other subject decision	25 (2%)	26 (2%)	51 (2%)
Investigator site closed	55 (4%)	57 (4%)	112 (4%)
Missing	1 (<1%)	0	1 (<1%)
Outcome of adverse events which led to study withdrawal			
Nonfatal	1 (<1%)	0	1 (<1%)
Fatal	0	0	0

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; rhEPO, recombinant human erythropoietin

Of the 2964 participants in the ITT population, 1390 (47%) completed randomized treatment. The proportion of participants who discontinued randomized treatment was high and similar in the daprodustat group [785 (53%) participants] compared to rhEPO [781 (53%) participants]. Of the participants who discontinued randomized treatment 114 (8%) in the daprodustat group and 119 (8%) in the rhEPO group died while on randomized treatment (see [Table 10](#)). The primary reasons for discontinuation were similar in both treatment groups and were mostly due to adverse events or reaching protocol-defined stopping criteria.

Table 10. Summary of Randomized Treatment Status and Reasons for Discontinuation of Randomized Treatment for Study 200807/ASCEND-D

	Daprodustat (N=1487)	rhEPO (N=1477)	Total (N=2964)
Overall study treatment status			
Never received study treatment	5 (<1%)	3 (<1%)	8 (<1%)
Prematurely discontinued study treatment	785 (53%)	781 (53%)	1566 (53%)
Did not die while taking study treatment	671 (45%)	662 (45%)	1333 (45%)
Died while taking study treatment	114 (8%)	119 (8%)	233 (8%)
Did not prematurely discontinue study treatment	697 (47%)	693 (47%)	1390 (47%)

	Daprodustat (N=1487)	rhEPO (N=1477)	Total (N=2964)
Overall primary reason/subreason for all study treatment discontinuation:			
Adverse event	233 (16%)	236 (16%)	469 (16%)
Protocol deviation	9 (<1%)	5 (<1%)	14 (<1%)
Subject reached protocol-defined stopping criteria	237 (16%)	222 (15%)	459 (15%)
Kidney transplant	136 (9%)	131 (9%)	267 (9%)
Rescue	53 (4%)	53 (4%)	106 (4%)
Pregnancy	3 (<1%)	0	3 (<1%)
Cancer	36 (2%)	29 (2%)	65 (2%)
Subject met liver chemistry stopping criteria	6 (<1%)	5 (<1%)	11 (<1%)
Prohibited medication use	2 (<1%)	3 (<1%)	5 (<1%)
Transition to peritoneal dialysis	0	0	0
Missing	1 (<1%)	1 (<1%)	2 (<1%)

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; rhEPO, recombinant human erythropoietin

Demographic Characteristics and Baseline Characteristics

Baseline demographic characteristics, and Hb levels were generally similar between treatment groups. Baseline characteristics are summarized in [Table 11](#).

Table 11. Summary of Demographic Characteristics for Study 200807/ASCEND-D

	Daprodustat (N=1487)	rhEPO (N=1477)
Age (years), n	1487	1477
Mean (SD)	57.2 (14.3)	57.3 (14.7)
Median	58.0	59.0
Min, max	18, 95	20, 94
Age category, n (%)	1487	1477
<65 years	1007 (68)	978 (66)
65-74 years	321 (22)	325 (22)
≥75 years	159 (11)	174 (12)
Missing	0	0
Gender, n (%)	1487	1477
Female	636 (43)	630 (43)
Male	851 (57)	847 (57)
Ethnicity, n (%)	1487	1477
Hispanic or Latino	367 (25)	371 (25)
Not Hispanic or Latino	1120 (75)	1106 (75)
Missing	0	0
Race, n (%)	1487	1477
American Indian or Alaska native	19 (1)	32 (2)
Asian	176 (12)	181 (12)
Black or African American	228 (15)	233 (16)
Native Hawaiian or other Pacific Islander	26 (2)	25 (2)
White	995 (67)	982 (66)
Mixed race	43 (3)	24 (2)
Missing	0	0
Baseline weight (kg)	1474	1461
Mean (SD)	77.8 (21.5)	77.5 (20.3)
Median	74.6	74.9
Min, max	36.0, 193.8	29.0, 176.5

	Daprodustat (N=1487)	rhEPO (N=1477)
Baseline weight group, n (%)	1487	1477
<75 kg	747 (50)	733 (50)
≥75 kg	727 (49)	728 (49)
Missing	13 (<1)	16 (1)
Baseline hsCRP	1474	1462
Mean (SD)	10.43 (21.5)	9.85 (20.1)
Median	4.0	4.0
Min, max	0.1, 328.5	0.1, 288.9
Dialysis vintage at screening, n (%)	1487	1477
<2 years	453 (30)	451 (31)
≥2 and <5 years	535 (36)	529 (36)
≥5 years	499 (34)	497 (34)
Dialysis type at screening, n (%)	1487	1477
Hemodialysis conventional	1265 (85)	1252 (85)
Hemofiltration/hemodiafiltration	51 (3)	56 (4)
Peritoneal dialysis	171 (11)	169 (11)
Standardized prior ESA dose (U/week)	1486	1475
Mean (SD)	8017 (7336)	7656.3 (6430.55)
Median	5850	5699
Min, max	270, 69958	147, 69958
rhEPO hypo-responder, n (%) ^a	1487	1477
No	1284 (86)	1279 (87)
Yes	183 (12)	180 (12)
Missing	20 (1)	18 (1)
Baseline Hb (g/dL)	1487	1477
Mean (SD)	10.4 (1.0)	10.4 (1.0)
Median	10.4	10.5
Min, max	6.8, 13.3	5.3, 17.8
Baseline Hb group, n (%)	1487	1477
<9 g/dL	135 (9)	122 (8)
≥9 and <10 g/dL	356 (24)	324 (22)
≥10 and ≤11 g/dL	614 (41)	644 (44)
>11 g/dL	382 (26)	387 (26)

Source: Sponsor CSR

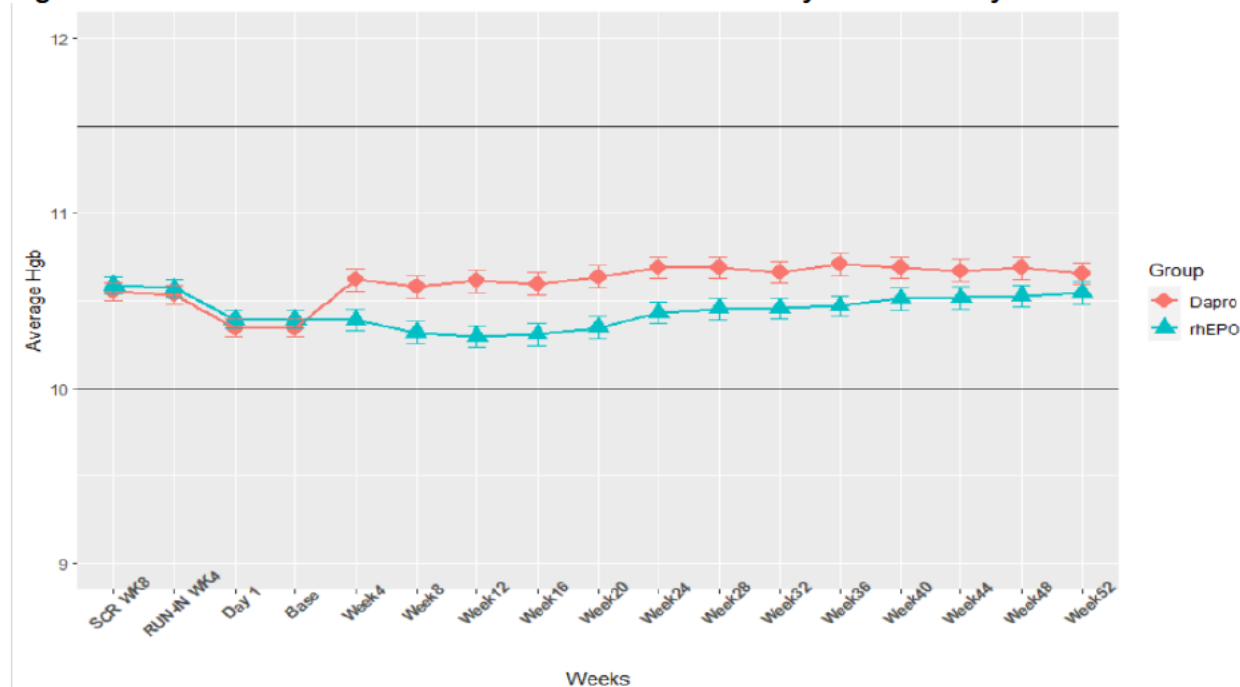
^a Participants with ERI ≥2.0 U/kg/wk/g/L (prior epoetin) or ≥0.008 µg/kg/wk/g/L (prior darbepoetin alfa) or ≥0.01 mg/kg/wk/g/L (prior methoxy-PEG-epoetin), or if treated with the equivalent of ≥450 U/kg/week IV epoetin alfa. ERI is calculated as ESA (e.g., epoetin alfa or other ESAs) dose per week (in U or ug) during the 4-week Screening period divided by Baseline dry weight at Day 1 and then divided by the achieved Day 1 Hb.

Abbreviations: CSR, clinical study report; ERI, ESA resistance index; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; hsCRP, high-sensitivity C-reactive protein; rhEPO, recombinant human erythropoietin

Efficacy Results—Primary Endpoint

The mean Hb at Baseline and over the EP was similar between treatment groups and remained within the Hb analysis range (10 to 11.5 g/dL) during the EP (Weeks 28 to 52) (Figure 4). Also, following a switch to daprodustat or active control (epoetin alfa in hemodialysis participants) Hb remained relatively stable and within the analysis range (10 to 11.5 g/dL) from Baseline through Week 52.

Figure 4. Line Plot of Post-Randomization Observed Hb Data by Visit for Study 200807/ASCEND-D



Source: FDA

Note: Error bars indicate 95% CI around mean

Baseline and visits on or before Day 1 include only pre-treatment values.

Post-randomization values include all available observed and imputed Hb values (on- and off-treatment).

The dashed vertical lines represent the EP (Week 28 to Week 52).

The horizontal reference lines represent the Hb analysis range (10-11.5 g/dL). The Hb target range for dose changes is 10-11 g/dL.

Abbreviations: CI, confidence interval; Dapro, daprodustat; EP, evaluation period; Hb, hemoglobin; Hgb, hemoglobin; rhEPO, recombinant human erythropoietin; SCR, screening

The lower bound of the 95% CI for the treatment difference in Hb from baseline to the EP was greater than the prospectively defined non-inferiority margin of -0.75 g/dL, thus demonstrating non-inferiority of daprodustat to rhEPO (see [Table 12](#)).

Table 12. Analysis of Post-randomization Hb Change From Baseline to the EP (Weeks 28 to 52) for Study 200807/ASCEND-D

	Daprodustat (N=1487)	rhEPO ^c (N=1477)
Participants with all 7 scheduled Hb values during the EP	1068 (72)	1087 (74)
Participants with partial scheduled Hb values during the EP	290 (20)	260 (18)
Participants with no observed Hb values during the EP	123 (8)	125 (8)
Number of participants with baseline and EP Hb, n (%) ^a	1487 (100)	1477 (100)
Baseline Hb (g/dL) mean (SD)	10.35 (0.97)	10.39 (0.98)
EP (Weeks 28-52) Hb (g/dL) mean (SD) ^a	10.65 (0.76)	10.48 (0.81)
Adjusted mean change from baseline (SE) ^b	0.28 (0.02)	0.10 (0.02)
Adjusted mean treatment difference (dapro - darbe) ^b		0.18
Two-sided 95% CI for adjusted mean difference ^b	(0.12, 0.24)	

Source: Sponsor CSR

Note: Post-randomization values include on and off treatment values.

^a Hb values during the EP include both observed and imputed values.

^b Based on an ANCOVA model with terms for treatment, baseline Hb, dialysis type and region

^c Epoetin alfa in HD participants or darbepoetin alfa in PD participants

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; EP, evaluation period; Hb, hemoglobin; HD, hemodialysis; PD, peritoneal dialysis; rhEPO, recombinant human erythropoietin; SD, standard deviation; SE, standard error

Sensitivity Analyses and Supportive Analyses for Hemoglobin Change From Baseline

Additional supportive analyses included an on-treatment analysis using only evaluable Hb values, a per-protocol analysis using only evaluable Hb values, analysis with a shorter EP (Weeks 28 to 36), and an observed case analysis (on- and off- treatment). Results were consistent with the non-inferiority conclusion from the primary analysis (See Section 16.1.1)

Results of the tipping point analysis suggest that the extent of missing data would unlikely impact the conclusions of the primary efficacy analysis. Therefore, missing data in these studies are unlikely to have had an impact on the primary results.

Efficacy Results—Key Secondary Endpoints

Daprodustat did not demonstrate a statistically significant reduction in average monthly IV iron dose from baseline to Week 52 compared to rhEPO (see Table 13). The observed p-value was greater than the rank-associated alpha threshold as prescribed by the Holm-Bonferroni adjustment.

Table 13. Analysis of On-Treatment Average Monthly IV Iron Dose During Day 1 to Week 52 for Study 200807/ASCEND-D

	Daprodustat N=1487	rhEPO ^b N=1477
Number of participants during day 1 to Week 52 (%)	1482 (>99)	1472 (>99)
Baseline monthly IV iron dose (mg) mean (SD)	139 (171)	137 (175)
Average monthly IV iron dose (mg) during Day 1 to Week 52 mean (SD)	91 (133)	100 (149)
Adjusted mean change from Baseline (SE)	91 (3)	100 (3)
Adjusted mean treatment difference (dapro - darbe)		-9.1
Two-sided 95% CI for adjusted mean difference		(-18.4, 0.2)
One-sided p-value		0.027 ^c

Source: Sponsor CSR

Note: Iron data after a participant has received an RBC or whole blood transfusion are excluded from the analysis

^a Based on an ANCOVA model with terms for treatment, baseline Hb, and region. One-sided p-value based on test of null hypothesis: (daprodustat - rhEPO) $\geq$ 0 vs alternative: difference <0.

^b Epoetin alfa in HD participants or darbepoetin alfa in PD participants.

^c The one-sided p-value for on-treatment average monthly IV iron dose was ranked second of the 4 key secondary endpoints in the Holm-Bonferroni multiplicity adjustment. The nominal p-value for the treatment difference in average monthly IV iron dose was not lower than the rank-associated alpha threshold of 0.00833

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; Hb, hemoglobin; HD, hemodialysis; IV, intravenous; PD, peritoneal dialysis; RBC, red blood cell; rhEPO, recombinant human erythropoietin; SD, standard deviation; SE, standard error

The proportion of participants with mean Hb within the analysis range of 10 to 11.5 g/dL during the EP was similar between the treatment groups (see Table 14).

Table 14. Participants With Mean EP Hb Below, Within and Above the Analysis Range Using Evaluable Hb Values for Study 200807/ASCEND-D

	Daprodustat N=1487	rhEPO ^a N=1477
Participants with evaluable Hb during the EP	1238	1247
Mean EP Hb above 11.5 g/dL	132 (11)	88 (7)
Mean EP Hb within 10.0-11.5 g/dL	903 (73)	866 (69)
Mean EP Hb below 10.0 g/dL	203 (16)	293 (23)

Source: Sponsor CSR

^a Epoetin alfa in HD participants or darbepoetin alfa in PD participants

Abbreviations: CSR, clinical study report; EP, evaluation period; Hb, hemoglobin; PD, peritoneal dialysis; rhEPO, recombinant human erythropoietin

Efficacy Results—Secondary Endpoints

A supportive analysis was performed for the primary ITT analysis of post-randomization mean change in Hb between Baseline and over the EP, repeating the analysis using a shorter EP (Week 28 to 36). For each study the lower bound of the 95% CI for the treatment difference was greater than the prospectively defined non-inferiority margin of -0.75 g/dL, thus supporting the overall conclusion of non-inferiority of daprodustat (see [Table 228](#) in appendix [16.1](#)). Results were consistent with the conclusion from the primary analysis.

Additional primary endpoint supportive analyses included an on-treatment analysis using only evaluable Hb values, a PP Population analysis using only evaluable Hb values, a shorter EP (Weeks 28 to 36) analysis using only evaluable Hb values, and a post-randomization (on- and off-treatment) observed case analysis. Results were consistent with the non-inferiority conclusion from the primary analysis ([Table 219](#) in Section [16.1](#)).

The proportion of participants with mean Hb within the analysis range during the EP was higher in the daprodustat arm as compared to rhEPO arm ([Table 15](#)).

Table 15. Participants With Mean EP Hb Below, Within and Above the Analysis Range Using Evaluable Hb Values for Study 200807/ASCEND-D

	Daprodustat N=1487	rhEPO N=1477
Participants with evaluable Hb during the EP	1238	1247
Mean EP Hb above 11.5 g/dL	132 (11)	88 (7)
Mean EP Hb within 10.0-11.5 g/dL	903 (73)	866 (69)
Mean EP Hb below 10.0 g/dL	203 (16)	293 (23)

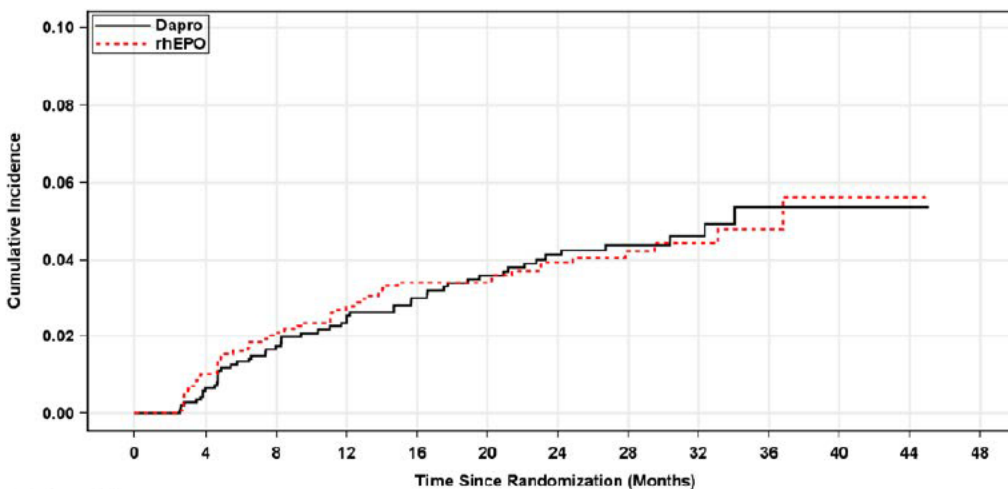
Source: Sponsor CSR

Abbreviations: CSR, clinical study report; EP, evaluation period; Hb, hemoglobin; rhEPO, recombinant human erythropoietin

The proportion of participants stopping randomized treatment due to meeting rescue criteria was small and similar between the treatment groups. Daprodustat did not reduce the risk of stopping randomized treatment due to meeting rescue criteria ([Table 61](#)).

The cumulative incidence for time to stopping randomized treatment due to meeting rescue criteria was similar between the treatment groups ([Table 61](#)).

Figure 5. Kaplan-Meier Plot Time to Stopping Randomized Treatment Due to Meeting Rescue Criteria for Study 200807/ASCEND-D



Subjects at Risk

	0	4	8	12	16	20	24	28	32	36	40	44	48
Dapro	1487	1340	1193	1086	1007	924	832	543	329	119	25	1	0
rhEPO	1477	1333	1208	1103	1017	934	832	559	344	136	31	5	0

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; Dapro, daprodustat; rhEPO, recombinant human erythropoietin

The proportion of participants with a first occurrence of RBC or whole blood transfusion during the on-treatment time period was an exploratory endpoint and was numerically lower in the daprodustat group compared with rhEPO. Participants on daprodustat had a numerically lower risk for time to first occurrence of receiving an on-treatment RBC or whole blood transfusion compared to rhEPO and no statistically testing was performed.

Blood transfusion events to Week 52 are summarized in [Table 16](#). The proportion of participants who received RBC or whole blood transfusions was similar between the treatment groups.

Table 16. On-Treatment Blood Transfusions by Week 52 for Study 200807/ASCEND-D

	Daprodustat N=1487	rhEPO N=1474
Number of participants who received randomized treatment	1482	1474
Number of transfusion events per participant, n (%)		
0	1367 (92)	1314 (89)
1	85 (6)	132 (9)
2	18 (1)	20 (1)
3	7 (<1)	5 (<1)
4	3 (<1)	0
5	1 (<1)	2 (<1)
>5	1 (<1)	1 (<1)

	Daprodustat N=1487	rhEPO N=1474
Number (%) of participants with transfusion events [number of transfusion events] ^a	115 (8) [165]	160 (11) [204]
Total treatment exposure (years)	1270	1277
Overall transfusion events per 100 PY	13	16
Overall transfusion per 100 PY	20	25.5
Overall units per 100 PY	34	37.8

Source: Sponsor CSR

Note: Blood transfusions include RBC and whole blood transfusions.

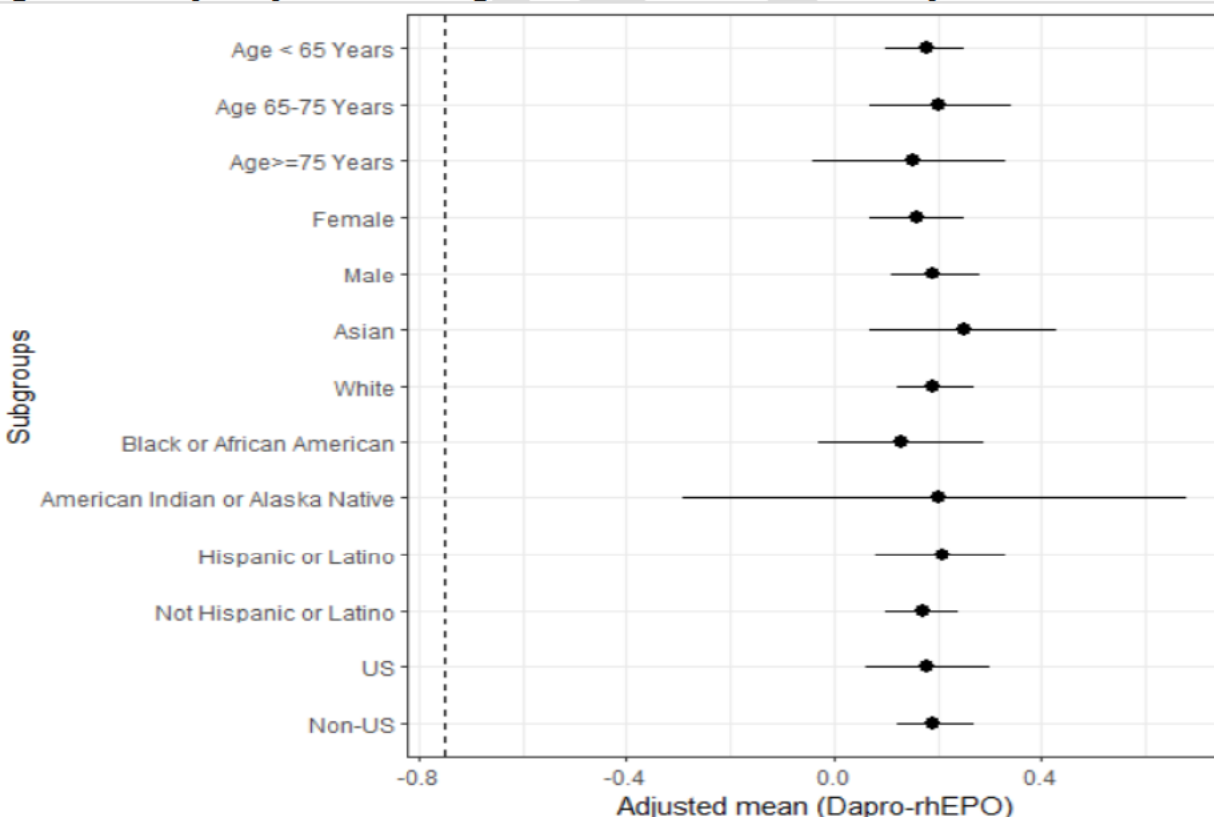
^a RBC and whole blood transfusion events are defined by grouping together transfusion records if the transfusion start/end dates match with or are contained within an Admission/Discharge period. Transfusion records with no matching admission/discharge period are considered to be the same Transfusion Event if the gap between transfusions is 5 days or less.

Abbreviations: CSR, clinical study report; PY, person-year; RBC, red blood cell; rhEPO, recombinant human erythropoietin

Efficacy Results - Subgroups

Overall, the results of the subgroup analyses were consistent with the primary analysis with little or no heterogeneity between subgroups. The adjusted mean differences were consistently >-0.75 for all subgroups (see [Figure 6](#)). In general, the treatment effect of daprodustat compared to rhEPO was generally consistent across all prespecified subgroups, including regional subgroup results.

Figure 6. Primary Analysis of Hb Change From Baseline to the EP for Study 200807/ASCEND-D



Source: FDA reviewer

Abbreviations: Dapro, daprodustat; EP, evaluation period; Hb, hemoglobin; rhEPO, recombinant human erythropoietin; US, United States

6.2.2. Study 200808/ASCEND-ND

6.2.2.1. Design

Overview

To support an indication for the treatment of anemia due to CKD in patients not on dialysis, the Applicant conducted a randomized, Phase 3 Study, 200808/ASCEND-ND, titled “A phase 3 randomized, open-label (sponsor-blind), active controlled, parallel-group, multi-center, event driven study in non-dialysis subjects with anemia associated with chronic kidney disease to evaluate the safety and efficacy of daprodustat compared to darbepoetin alfa.”

Primary Safety and Efficacy Endpoints

Safety: Time to first occurrence of adjudicated MACE (composite of all-cause mortality, nonfatal MI, and nonfatal stroke)

Efficacy: Mean change in Hb between Baseline and EP (Weeks 24 to 52).

Secondary Efficacy Endpoints

- Hb change from Baseline to Week 52
- N (%) responders, defined as having a mean Hb within the Hb analysis range 10 to 11.5 g/dL during EP
- % time Hb in analysis range during the EP
- Time to stopping randomized treatment due to meeting rescue criteria

Exploratory Endpoints

- Number (%) of participants who receive at least one RBC or whole blood transfusions by Week 52 and by end of treatment
- Number of RBC and whole blood transfusions per 100 person years

The key secondary safety endpoints tested for superiority and adjusted for multiplicity included:

- Time to MACE⁴
- Time to first occurrence of MACE or a TEE
- Time to first occurrence of MACE or HHF
- Time to progression of CKD among subjects with baseline $eGFR \geq 15$ mL/min/1.73m² defined as: (a) 40% decline in estimated glomerular filtration rate (eGFR) from baseline (confirmed 4 to 13 weeks later), or (b) ESRD as defined by either initiating chronic dialysis for ≥ 90 days or not initiating chronic dialysis when dialysis is indicated or kidney transplantation

Conditional on both co-primary endpoints ruling out their risk margins, key secondary endpoints would be tested for superiority by comparing the one-sided superiority p-value against the rank-associated alpha derived from the Holm-Bonferroni method.

⁴ Tested for noninferiority as a coprimary endpoint and tested for superiority as a key principal secondary endpoint.

Adjudicated secondary safety endpoints in both trials are listed below. These endpoints are time-to-event endpoints.

- All-cause mortality
- CV mortality
- Fatal or nonfatal MI
- Fatal or nonfatal stroke
- Fatal or nonfatal HHF
- Fatal or nonfatal TEE
- Composite of MACE and HHF⁵
- Composite of CV mortality and nonfatal MI
- Composite of MACE, HHF, and TEE

Study Design

Study 200808/ASCEND-ND was a randomized, open-label (sponsor blind), active-controlled, parallel-group, multi-center, event-driven study in patients with anemia associated with Non-Dialysis (ND)-CKD.

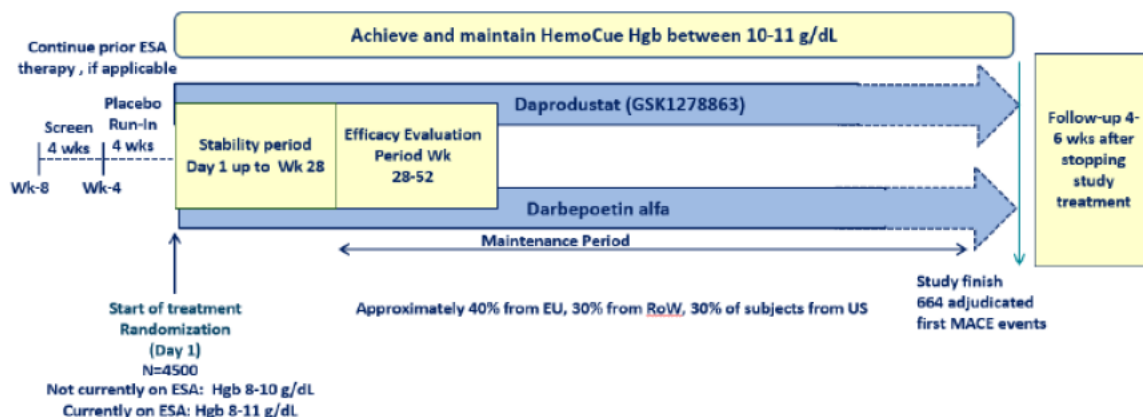
This study comprised four study periods: a 4-week screening period, a 4-week placebo run-in period, a treatment period, and a follow-up period. Prior ESA therapy, if present, continued during the screening and run-in periods.

The Treatment Period consists of (see [Figure 7](#)):

- SP, defined as the period from Day 1 to Week 28 (up to but excluding the Week 28 visit), during which study treatment was dose-titrated to achieve and maintain Hb in the target range.
- MP, defined as the period from the end of the SP (Week 28) to the end of treatment (variable per participant), to assess long term safety and efficacy.
- EP, defined as the period from Week 28 to Week 52, during which Hb efficacy was primarily assessed.
- Alternative EP: Because of the importance of patients not spending undue time with suboptimal Hb levels, a shorter EP, defined as the period from Week 28 to Week 36, was also assessed for Hb efficacy.

⁵ A recurrent event with data on multiple events for analysis

Figure 7. Study 200808/ASCEND-ND Schematic



Source: Sponsor CSR

Abbreviations: CSR, clinical study report; ESA, erythropoiesis-stimulating agents; EU, European Union; Hgb, hemoglobin; RoW, rest of the world; US, United States

In ASCEND-ND, subjects were randomized in a 1:1 ratio to either oral daprodustat or to darbepoetin alfa SC. Daprodustat was dosed daily, while the control darbepoetin alfa was dosed weekly, every 2 weeks, or every 4 weeks, depending on the subject's prior ESA use.

An anemia rescue algorithm, which included a provision for the use of intravenous iron, an RBC transfusion, or both, was provided in the protocols (details are provided in Section 16.4).

Additionally, the protocol required subjects to be iron replete throughout the study – and initiate iron therapy (investigator's choice) if ferritin was less than or equal to 100 ng/mL, transferrin saturation was less than or equal to 20%, or both.

Study Population Inclusion/Exclusion Criteria

Inclusion Criteria

The study recruited male and female (non-pregnant, non-lactating) participants, ≥ 18 years to ≤ 99 years of age with anemia associated with CKD not on dialysis (Kidney Disease Outcomes Quality Initiative [KDOQI] stages 3, 4, or 5 defined by eGFR using the CKD Epidemiology Collaboration [CKD-EPI] formula) and who were either not using ESAs or were current ESA users:

- Group 1 (not using ESAs): No ESA use within the 6 weeks prior to screening and no ESA use between screening and randomization (Day 1).
- Group 2 (ESA users): Use of any approved ESA for the 6 weeks prior to screening and continuing between screening and randomization.

Exclusion Criteria

- On dialysis or clinical evidence of impending need to initiate dialysis within 90 days after study start (Day 1).
- Planned kidney transplant within 52 weeks
- Ferritin ≤ 100 ng/mL (≤ 100 $\mu\text{g/L}$) at screening

- TSAT $\leq$ 20% at screening (If TSAT was 18 to 20%, a retest using a new blood sample could be obtained within 7 days of the final laboratory report; the final retest value must be $>$ 20% to confirm eligibility)
- History of bone marrow aplasia or pure red cell aplasia
- Anemia other than anemia of CKD.
- MI, acute coronary syndrome, stroke, or transient ischemic attack within 4 weeks prior to screening through to randomization (Day 1)
- Bazett's correction of QT interval (QTcB) $>$ 500 msec, or QTcB $>$ 530 msec in participants with bundle branch block at randomization (Day 1)
- Chronic Class IV heart failure
- Uncontrolled hypertension
- Prior (within 2 years) or current history of cancer (with the exception of localized squamous cell or basal cell carcinoma of the skin), or complex kidney cyst (e.g., Bosniak Category II F, III or IV) $>$ 3 cm.; current unstable liver or biliary disease or known hepatic or biliary abnormalities; or gastrointestinal bleeding.
- Participants requiring treatment with a strong inhibitor of CYP2C8 or a strong inducer of CYP2C8 were excluded.

6.2.2.2. Statistical Analysis Plan

Sample Size Determination

The size of this event driven trial was based on the co-primary CV safety objective and determined by a fixed event target of 664 adjudicated first MACE. This provided approximately 90% power to establish non-inferiority assuming a true underlying 3% lower relative risk of MACE in favor of daprodustat compared to darbepoetin alfa (i.e., HR=0.97) and 82% power for the pre-specified non-inferiority margin of 1.25 under the assumption that the true underlying risk of MACE was the same in both arms (i.e., HR=1.00).

For 90% power to test the co-primary non-inferiority Hb hypothesis, with a two-sided 95% CI, pre-specified margin of -0.75 g/dL and a two-sample t-test, a total of 86 evaluable participants per treatment group was required. The planned study size far exceeded this requirement and provided more than 99% power for non-inferiority and a high level of precision to estimate the treatment effect (two-sided 95% CI half width of 0.105 g/dL, assuming 30% of participants would be non-evaluable for efficacy). The largest (most negative) difference between arms that would meet the statistical criterion for non-inferiority would be -0.645 g/dL. If the two-sided 95% CI is completely negative (i.e., lies fully within the range -0.75 to $<$ 0 g/dL) non-inferiority would still be concluded.

Interim Analysis

There were no prospectively defined interim analyses planned to stop the study early for benefit. While the planned futility analysis described below had a small impact on reducing study-wise Type I error rate, there were no plans to adjust the alpha level used for the final analysis.

The IDMC evaluated the co-primary MACE endpoint to assess for futility of achieving non-inferiority at study completion. Pre-specified guidelines governing the decision to continue or

stop the study considered signals for harm, the predictive probability of achieving at least non-inferiority at trial end, and the risk of incorrectly stopping for futility.

Missing Data Handling for Primary Efficacy Endpoint (Hb)

For each missing value between Baseline to Week 52 (inclusive), 200 imputed values for Hb were generated using multiple imputation. The pooled average of these 200 imputed values were used in the summary tables and figures. For co-primary efficacy Hb analysis and the corresponding subgroup analyses using all available observed and imputed Hb values (on and off-treatment), Rubin's rules were used to combine results of the imputed datasets. The MAR assumption was considered appropriate as it is expected that off-treatment Hb values were similar to on-treatment Hb values, since patients would usually take commercially available Darbepoetin medication during such times in order to control their Hb. A tipping point analysis was also performed as a sensitivity analysis for the assumptions of missing data, that is, to mimic MNAR in which the missing observations depend on unobserved values.

Efficacy Analysis for Primary Endpoint

The primary efficacy evaluation was the effect of daprodustat relative to darbepoetin alfa on the change in Hb from Baseline to the average of all values in the EP, regardless of adherence to treatment including interruptions and discontinuation, the use of nonrandomized ESA medication for any reason including rescue therapy or the use of blood transfusions, and assuming participants did not die before the end of the EP.

For each participant, Baseline Hb was the value obtained prior to taking randomized treatment (Day 1 value, or if this was missing, Baseline Hb was the value obtained at the latest non-missing pre-dose assessment on or before the randomization date), and Hb during the EP was determined by calculating the mean of all available and imputed Hb values between Weeks 28 to 52 inclusive regardless of adherence to randomized treatment. Multiple imputation was used to impute missing Hb data, and an ANCOVA model, adjusting for region, current ESA use, Baseline hemoglobin, and treatment, was used to obtain a point estimate, the two-sided 95% CI for the treatment difference (daprodustat-darbepoetin alfa), and an associated one-sided noninferiority test p-value. Non-inferiority was established if the lower limit of the two-sided 95% CI for the treatment difference was greater than the margin of -0.75 g/dL.

Sensitivity Analyses

- Post-randomization Hb (Observed Values Only Analysis): An analysis using available observed Hb values (on and off-treatment) only for post-randomization hemoglobin. Missing Hb data were not imputed.
- Evaluable Hb: An alternative "while on-treatment" estimand using only evaluable Hb data. Missing Hb data were not imputed.
- PP Hb: A PP population analysis based on the evaluable Hb analysis.
- Alternative EP (Week 28-36) Analyses:
 - An alternative EP analysis from Week 28-36 using all available Hb values (on and off treatment). Missing post-Baseline Hb values were imputed.
 - An alternative EP analysis from Week 28-36 using only evaluable Hb values. Missing Hb data were not imputed.

- A tipping point analysis using all available Hb values (on and off-treatment) as a sensitivity for the co-primary estimand and using evaluable Hb values as supportive analysis
- COVID-19 supportive analysis: a plot by calendar date (in quarters) of adjusted treatment difference in mean change in Hb from Baseline using all available Hb values (on and off-treatment) at each time point, to assess the impact of the COVID-19 pandemic.

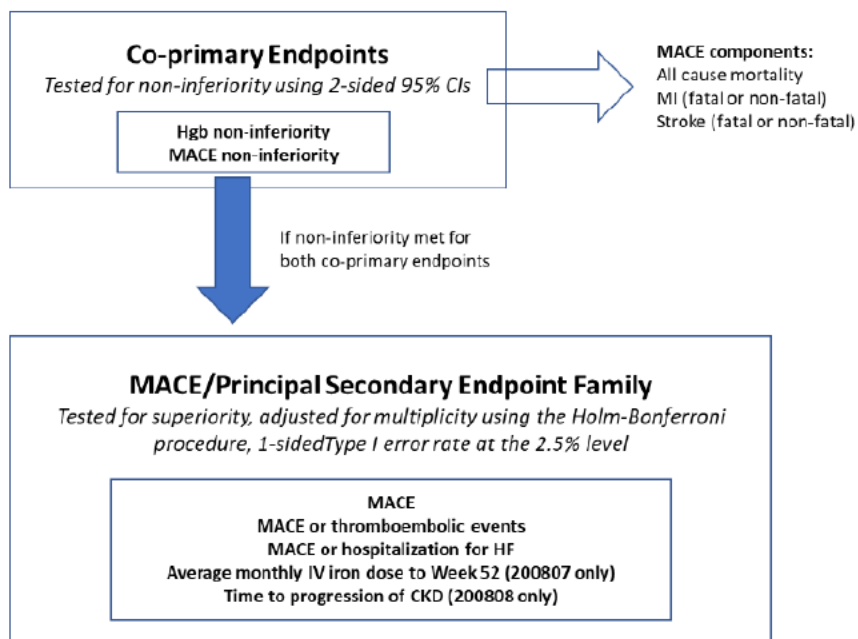
Analyses for Secondary Efficacy Endpoints

- For the mixed model repeated measures (MMRM) analysis of change from baseline in Hb, the non-inferiority (NI) margin used in the co-primary analysis of Hb (-0.75 g/dL) will be used for reference in this comparison, thus generating support for non-inferiority if the lower bound of the two-sided 95% CI is above -0.75 g/dL.
- For the responder analysis, the number and % of responders by treatment group, difference in response rate (daprodustat – darbepoetin alfa) and two-sided 95% CI using Wald method were provided along with the one-sided Cochran-Mantel-Haenszel (CMH) p-value for the treatment group comparison.
- The % time Hb is above, in and below the Hb analysis range (10 to 11.5 g/dL) during the MP and the EP were summarized using mean, standard deviation, minimum, P25, median, P75, and maximum by treatment group.

Multiple Comparisons

The multiplicity strategy used a combination of a gatekeeper approach on the co-primary endpoints, followed by a closed-test multiplicity procedure wrapped around the family of superiority hypotheses consisting of MACE and the key secondary endpoints. [Figure 8](#) illustrates the structure of the statistical testing plan. First, the co-primary endpoints were evaluated for non-inferiority by comparing either the lower bound (Hb) or the upper bound (MACE) of each two-sided 95% CI to the corresponding non-inferiority margin. Conditional on both co-primary endpoints achieving non-inferiority (i.e., passing the gatekeeper), the family of MACE and the key secondary endpoints were formally tested for superiority using the Holm-Bonferroni procedure. The procedure was conducted based on a family-wise Type I error rate set at the one-sided 2.5% level.

Figure 8. Multiplicity Controlled Statistical Testing Plan for Study 200808/ASCEND-ND



Source: Sponsor CSR

Abbreviations: CI, confidence interval; CKD, chronic kidney disease; CSR, clinical study report; IV, intravenous; MACE, major adverse cardiovascular events; MI, myocardial infarction

Subgroup Analysis

Subgroup analyses, including age, sex, region, current ESA use, and race were performed. For both the Hb change from baseline to Weeks 28 to 52 and the Hb responder during EP, the region and current ESA use at randomization subgroups were conducted in a method similar to that described for the primary and secondary ITT analyses.

6.2.2.3. Results of Analyses

Patient Disposition

Participants were considered to have completed the study if they completed all visits through the End of Study visit irrespective of whether they discontinued randomized treatment. Participants were also considered to have completed the study if they died while on study. Of 3872 participants in the ITT population, 3743 (97%) participants completed the study through the End of Study visit or died while on study. A total of 64 (3%) participants in the daprodustat group and 65 (3%) participants in the darbepoetin alfa group discontinued randomized treatment and withdrew from the study (see [Table 17](#)). **There were no differences between the treatment groups in time to withdrawal from the study.**

Table 17. Summary of Subject Status and Reasons for Study Withdrawal for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935	Total N=3872
Overall subject status			
Completed	1873 (97%)	1870 (97%)	3743 (97%)
Withdrawn	64 (3%)	65 (3%)	129 (3%)
Primary reason/sub-reason for study withdrawal			
Study closed/terminated	0	3 (<1%)	3 (<1%)
Lost to follow-up	25 (1%)	29 (1%)	54 (1%)
Withdrew consent	32 (2%)	23 (1%)	55 (1%)
Logistical circumstances	4 (<1%)	5 (<1%)	9 (<1%)
Privacy preferences	19 (<1%)	12 (<1%)	31 (<1%)
Adverse experience	3 (<1%)	0	3 (<1%)
Other subject decision	6 (<1%)	6 (<1%)	12 (<1%)
Investigator site closed	6 (<1%)	10 (<1%)	16 (<1%)
Missing	1 (<1%)	0	1 (<1%)
Outcome of adverse events which led to study withdrawal			
Nonfatal	3 (<1%)	0	3 (<1%)
Fatal	0	0	0

Source: Sponsor CSR
Abbreviations: CSR, clinical study report

Of the 3872 participants in the ITT population, 2417 (62%) completed randomized treatment. The proportion of participants who discontinued randomized treatment was high and similar in the daprodustat group [727 (38%) participants] compared to darbepoetin alfa [726 (38%) participants]. Of the participants who discontinued randomized treatment, 156 (8%) in the daprodustat group and 166 (9%) in the darbepoetin alfa group died while on randomized treatment. The primary reason for discontinuation was similar in both treatment groups (see [Table 18](#)).

Table 18. Summary of Randomized Treatment Status and Discontinuation of Randomized Treatment for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935	Total N=3872
Overall study treatment status			
Never received study treatment	0	2 (<1%)	2 (<1%)
Prematurely discontinued study treatment	727 (38%)	726 (38%)	1453 (38%)
Did not die while taking study treatment	571 (29%)	560 (29%)	1131 (29%)
Died while taking study treatment	156 (8%)	166 (9%)	322 (8%)
Did not prematurely discontinue study treatment	1210 (62%)	1207 (62%)	2417 (62%)

	Daprodustat N=1937	Darbepoetin N=1935	Total N=3872
Overall primary reason/subreason for all study treatment discontinuation:			
Adverse event	254 (13%)	222 (11%)	476 (12%)
Protocol deviation	14 (<1%)	20 (1%)	34 (<1%)
Subject reached protocol-defined stopping criteria	151 (8%)	161 (8%)	312 (8%)
Kidney transplant	55 (3%)	53 (3%)	108 (3%)
Rescue	39 (2%)	64 (3%)	103 (3%)
Pregnancy	1 (<1%)	0	1 (<1%)
Cancer	40 (2%)	34 (2%)	74 (2%)
Subject met liver chemistry stopping criteria	5 (<1%)	2 (<1%)	7 (<1%)
Prohibited medication use	2 (<1%)	0	2 (<1%)
Transition to dialysis	8 (<1%)	8 (<1%)	16 (<1%)
Worsening of kidney function due to ADPKD	1 (<1%)	0	1 (<1%)
Study closed/terminated	0	0	0

Source: Sponsor CSR

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; CSR, clinical study report

Demographic Characteristics and Baseline Characteristics

Except for Baseline Hb which was higher in ESA users compared with ESA non-users, Baseline demographic characteristics, renal characteristics, CV and diabetes characteristics, blood pressure values, and markers of iron metabolism were generally similar between treatment groups and between Baseline ESA users and nonusers. Baseline characteristics are summarized in [Table 19](#) by baseline ESA use.

Table 19. Summary of Demographic Characteristics Overall and by Baseline ESA Use for Study 200808/ASCEND-ND

	Baseline ESA Users		Baseline ESA Non-Users		Overall	
	Daprodustat N=907	Darbepoetin N=903	Daprodustat N=1030	Darbepoetin N=1032	Daprodustat N=1937	Darbepoetin N=1935
Age (years), n	907	903	1030	1032	1937	1935
Mean (SD)	65.4 (14.4)	65.1 (14.1)	64.3 (13.7)	64.7 (13.6)	64.8 (14.0)	64.9 (13.8)
Median	68.0	68.0	66.0	66.0	67.0	67.0
Min, max	19, 94	18, 94	20, 94	22, 98	19, 94	18, 98
Age category, n (%)	907	903	1030	1032	1937	1935
< 65 years	366 (40)	378 (42)	470 (46)	464 (45)	836 (43)	842 (44)
65-<75 years	284 (31)	294 (33)	315 (31)	317 (31)	599 (31)	611 (32)
≥75 years	257 (28)	231 (26)	245 (24)	251 (24)	502 (26)	482 (25)
Gender, n (%)	907	903	1030	1032	1937	1935
Female	534 (59)	507 (56)	568 (55)	564 (55)	1102 (57)	1071 (55)
Male	373 (41)	396 (44)	462 (45)	468	835 (43)	864 (45)
Ethnicity, n (%)	907	903	1030	1032	1937	1935
Hispanic or Latino	225 (25)	255 (28)	205 (20)	212 (21)	430 (22)	467 (24)
Not Hispanic or Latino	682 (75)	648 (72)	825 (80)	820 (79)	1507 (78)	1468 (76)

	Baseline ESA Users		Baseline ESA Non-Users		Overall	
Race, n (%)	907	903	1030	1032	1937	1935
American Indian or Alaskan Native	62 (7)	66 (7)	26 (3)	34 (3)	88 (5)	100 (5)
Asian	244 (27)	242 (27)	281 (27)	295 (29)	525 (27)	537 (28)
Black or African American	52 (6)	44 (5)	131 (13)	141 (14)	183 (9)	185 (10)
Native Hawaiian or other Pacific Islander	2 (<1)	3 (<1)	5 (<1)	4 (<1)	7 (<1)	7 (<1)
White	528 (58)	520 (58)	570 (55)	535 (52)	1098 (57)	1055 (55)
Mixed race	19 (2)	28 (3)	17 (2)	23 (2)	36 (2)	51 (3)
BL weight (kg)	907	903	1030	1032	1937	1935
Mean (SD)	72.9 (18.9)	72.2 (17.5)	75.3 (21.0)	74.8 (20.4)	74.2 (20.1)	73.6 (9.2)
Median	70.4	71.0	71.7	71.4	71.0	71.3
Min, max	35.3, 160.7	35.0, 143.0	36.4, 172.0	34.7, 185.9	35.3, 172.0	34.7, 185.9
BL weight (kg), n (%)	907	903	1030	1032	1937	1935
<75 kg	538 (59)	529 (59)	577 (56%)	586 (57%)	1115 (58)	1115 (58)
≥75 kg	369 (41)	374 (41)	453 (44%)	446 (43%)	822 (42)	820 (42)
BL hsCRP (mg/L)	899	900	1024	1030	1923	1930
Mean (SD)	5.9 (15.3)	6.30 (14.2)	5.2 (10.3)	6.1(13.8)	5.5 (12.9)	6.2 (13.7)
Median	2.0	2.1	2.0	1.9	2.0	2.0
Min, max	0.1, 255.8	0.1, 185.9	0.1, 121.0	0.1, 206.8	0.1, 255.8	0.1, 206.8
BL eGFR, n (mL/min/1.73m ²)	906	903	1030	1032	1936	1935
Mean (SD)	20.1 (11.2)	20.8 (11.2)	20.1 (11.3)	20.6 (10.9)	20.1 (11.3)	20.7 (11.1)
Median	17.0	19.0	17.0	18.0	17.0	18.0
Min, max	2.0, 66.0	3.0, 70.0	2.0, 71.0	3.0, 68.0	2.0, 71.0	3.0, 70.0
BL CKD stage	907	903	1030	1032	1937	1935
Stage 2 (eGFR <60<89 mL/min/1.73m ²)	6 (<1)	6 (<1)	3 (<1)	2 (<1)	9 (<1)	8 (<1)
Stage 3 (eGFR 30-<60 mL/min/1.73m ²)	157 (17)	164 (18)	179 (17)	199 (19)	336 (17)	363 (19)
Stage 4 (eGFR 15-<30 mL/min/1.73m ²)	412 (45)	430 (48)	463 (45)	464 (45)	875 (45)	894 (46)
Stage 5 (eGFR <15 mL/min/1.73m ²)	331 (36)	303 (34)	385 (37)	367 (36)	716 (37)	670 (35)
Missing	1 (<1)	0	0	0	1 (<1)	0
Standardized prior ESA dose (U/week)	907	903	-	-	-	-
Mean (SD)	5472 (5146)	5395 (4559)	-	-	-	-
Median	3944	3921	-	-	-	-
Min, max	102, 55779	307, 41871	-	-	-	-
BL Hb (g/dL)	907	903	1030	1032	1937	1935
Mean (SD)	10.3 (0.9)	10.3 (0.9)	9.5 (0.8)	9.5 (0.8)	9.9 (0.9)	9.9 (1.0)
Median	10.4	10.4	9.5	9.0	9.8	9.9
Min to max	6.6, 13.8	7.2, 15.9	3.6, 12.5	5.7, 14.1	3.6, 13.8	5.7, 15.9

	Baseline ESA Users		Baseline ESA Non-Users		Overall	
BL Hb group (g/dL)	907	903	1030	1032	1937	1935
<9 g/dL	69 (8)	81 (9)	236 (23)	257 (25)	305 (16)	338 (17)
≥9 and <10 g/dL	240 (26)	213 (24)	502 (49)	504 (49)	742 (38)	717 (37)
≥10 and ≤11 g/dL	420 (46)	456 (50)	269 (26)	248 (24)	689 (36)	704 (36)
>11 g/dL	178 (20)	153 (17)	23 (2)	23 (2)	201 (10)	176 (9)

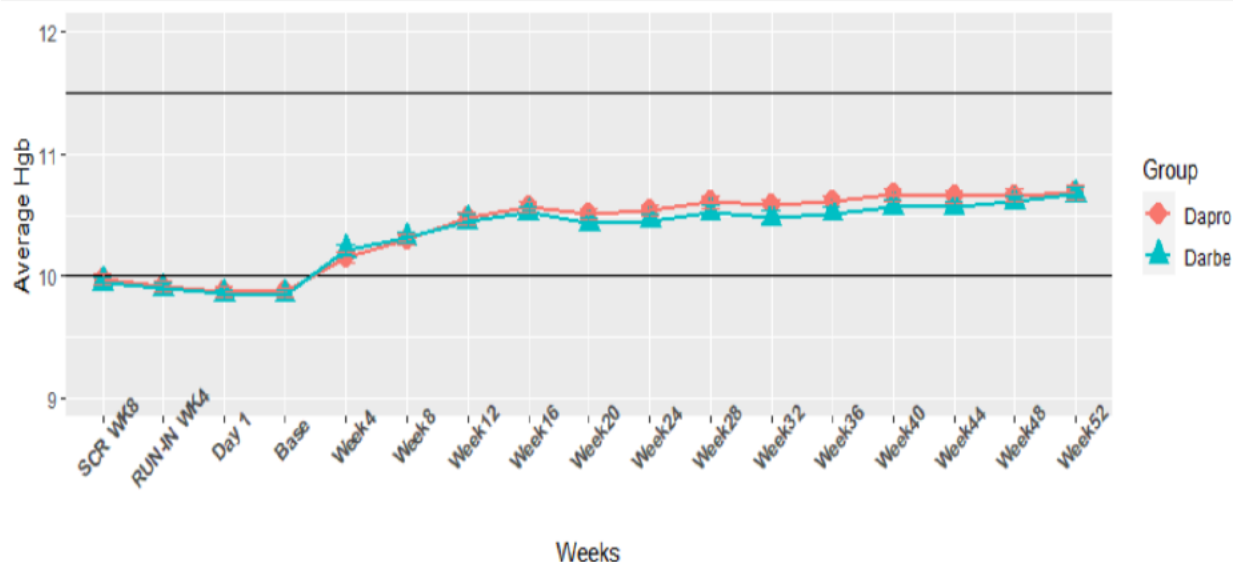
Source: Sponsor CSR report

Abbreviations: BL, baseline; CKD, chronic kidney disease; CSR, clinical study report; eGFR, estimated glomerular filtration rate; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; hsCRP, high-sensitivity C-reactive protein; SD, standard deviation

Efficacy Results—Primary Endpoint

Mean Hb at Baseline and over the EP was similar between treatment groups and remained within the Hb analysis range (10 to 11.5 g/dL) during the EP (Weeks 28 to 52) (See [Figure 9](#)).

Figure 9. Line Plot of Post-Randomization Observed Hb Data by Visit (To Week 52) for Study 200808/ASCEND-ND



Source: FDA

Note: Error bars indicate 95% CI around mean

Baseline and visits on or before Day 1 include only pre-treatment values.

Post-randomization values include all available observed and imputed Hb values (on- and off-treatment).

The dashed vertical lines represent the EP (Week 28 to Week 52).

The horizontal reference lines represent the Hb analysis range (10-11.5 g/dL). The Hb target range for dose changes is 10-11 g/dL.

Abbreviations: CI, confidence interval; Dapro, daprodustat; Darbe, darbepoetin alfa; EP, evaluation period; Hb, hemoglobin; Hgb, hemoglobin; SCR, screening

The proportion of participants with imputations was similar between the treatment groups. Of the 361 (19%) participants in the daprodustat arm and 357 (18%) participants in the darbepoetin alfa arm with no observed Hb values in the EP, the majority (daprodustat 198 [10%] and darbepoetin alfa 206 [11%]) had no values because the end of the study visit occurred before Week 28 ([Table 20](#)). As shown in the table below, daprodustat was non-inferior to darbepoetin alfa because the lower bound of the 95% CI is greater than the pre-specified margin of -0.75.

Table 20. Analysis of Post-Randomization Hb Change From Baseline to the EP (Weeks 28 to 52) for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935
Participants with all 7 scheduled Hb values during the EP	1084 (56)	1079 (56)
Participants with partial scheduled Hb values during the EP	473 (24)	477 (25)
Participants with no observed Hb values during the EP	361 (19)	357 (18)
Number of participants with Baseline and EP Hb, n (%) ^a	1937 (100)	1935 (100)
Baseline Hb (g/dL) mean (SD)	9.87 (0.94)	9.85 (0.95)
EP (Weeks 28-52) Hb (g/dL) mean (SD) ^a	10.59 (0.63)	10.51 (0.71)
Adjusted mean change from Baseline (SE) ^b	0.74 (0.02)	0.66 (0.02)
Adjusted mean treatment difference (dapro-darbe) ^b		0.08
Two-sided 95% CI for adjusted mean difference ^b		(0.03, 0.13)

Source: Sponsor CSR report

^a Post-randomization values include on and off treatment values. Observed and imputed Hb values are used in this analysis. There were no missing baseline values in the analysis.

^b Based on an ANCOVA model with terms for treatment, baseline Hb, current ESA use and region.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; EP, evaluation period; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; SD, standard deviation; SE, standard error

Primary Efficacy Sensitivity Analysis

For the analysis of the co-primary efficacy endpoint, a multiple imputation approach was used to handle missing Hb values. Missing scheduled Hb data were imputed based on the MAR assumption. However, it is important to examine the sensitivity of inferences to departures from the MAR assumption for the monotone missing data. A tipping point analysis was performed as a sensitivity analysis for the assumptions of missing data, that is, to mimic MNAR in which the missing observations depend on unobserved values. Results of the tipping point analysis suggest that the extent of missing data would unlikely impact the conclusions of the co-primary efficacy analysis.

Efficacy Results—Secondary Endpoints

A supportive analysis was performed for the primary ITT analysis of post-randomization mean change in Hb between Baseline and over the EP, repeating the analysis using a shorter EP (Week 28 to 36). For each study the lower bound of the 95% CI for the treatment difference was greater than the prospectively defined non-inferiority margin of -0.75 g/dL, thus supporting the overall conclusion of non-inferiority of daprodustat (see [Table 21](#)). Results were consistent with the conclusion from the primary analysis.

Table 21. Analysis of Post-Randomization Hb Change From Baseline to the Alternative EP (Weeks 28 to 36) for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935
Number of participants with baseline and EP Hb, n(%) ^a	1937(100)	1935 (100)
Baseline Hb (g/dL) mean (SD)	9.87 (0.94)	9.85 (0.95)
EP (Weeks 28-36) Hb (g/dL) mean (SD) ^a	10.56 (0.78)	10.44 (0.86)
Adjusted mean change from baseline (SE) ^b	0.70 (0.03)	0.07 (0.03)
Adjusted mean treatment difference (dapro - darbe) ^b		0.10
Two-sided 95% CI for adjusted mean difference ^b		(0.03, 0.16)

Source: Sponsor CSR

^a Hb values during the EP include both observed and imputed values.

^b Based on an ANCOVA model with terms for treatment, baseline Hb, and region

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; EP, evaluation period; Hb, hemoglobin; SD, standard deviation; SE, standard error

Additional primary endpoint supportive analyses included an on-treatment analysis using only evaluable Hb values, a PP Population analysis using only evaluable Hb values, a shorter EP (Weeks 28 to 36) analysis using only evaluable Hb values, and a post-randomization (on- and off-treatment) observed case analysis. Results were consistent with the non-inferiority conclusion from the primary analysis (see [Table 22](#)).

Table 22. Primary Endpoint Hb Supportive Analyses for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935
Evaluable Hb (Weeks 28 to 52), ITT population		
Number of participants with baseline and EP Hb, n (%) ^a	1491 (77)	1520 (79)
Adjusted mean change from baseline (SE) ^b	0.70 (0.02)	0.64 (0.02)
Adjusted mean treatment difference (dapro - darbe) ^b		0.07
Two-sided 95% CI for adjusted mean difference		(0.01, 0.12)
Evaluable Hb (Weeks 28 to 52), PP population		
Number of participants with baseline and EP Hb, n (%) ^a	982 (53)	1083 (60)
Adjusted mean change from baseline (SE) ^b	0.76 (0.02)	0.67 (0.02)
Adjusted mean treatment difference (dapro - darbe) ^b		0.09
Two-sided 95% CI for adjusted mean difference ^b		(0.04, 0.14)
Evaluable Hb Alternative EP (Weeks 28 to 36), ITT population		
Number of participants with baseline and EP Hb, n (%) ^a	1483 (77)	1503 (78)
Adjusted mean change from baseline (SE) ^b	0.69 (0.02)	0.62 (0.02)
Adjusted mean treatment difference (dapro - darbe) ^b		0.08
Two-sided 95% CI for adjusted mean difference ^b		(0.01, 0.01)
Post-randomization Hb (observed cases only), ITT population		
Number of participants with baseline and EP Hb, n (%)	1576 (81)	1578 (82)
Adjusted mean change from baseline (SE) ^b	0.71 (0.02)	0.63 (0.02)
Adjusted mean treatment difference (dapro - darbe) ^b		0.08
Two-sided 95% CI for adjusted mean difference ^b		(0.03, 0.13)

Source: Sponsor CSR

^a Hb values during the EP include observed values only.

^b Based on an ANCOVA model with terms for treatment, Baseline Hb, and region.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; EP, evaluation period; Hb, hemoglobin; ITT, intent-to-treat; PP, per protocol; SE, standard error

The proportion of participants with mean Hb within the analysis range during the EP was higher in the daprodustat arm as compared to Darbepoetin arm ([Table 23](#)).

Table 23. Participants With Mean EP Hb Below, Within and Above the Analysis Range Using Evaluable Hb Values for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935
Participants with evaluable Hb during the EP	1491	1520
Mean EP Hb above 11.5 g/dL	220 (15)	321 (21)
Mean EP Hb within 10.0-11.5 g/dL	1167 (78)	1063 (70)
Mean EP Hb below 10.0 g/dL	104 (7)	136 (9)

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; EP, evaluation period; Hb, hemoglobin

The proportion of participants stopping randomized treatment due to meeting rescue criteria was small and similar between the treatment groups. Daprodustat did not reduce the risk of stopping randomized treatment due to meeting rescue criteria ([Table 24](#)).

The cumulative incidence for time to stopping randomized treatment due to meeting rescue criteria was similar between the treatment groups ([Figure 10](#)).

Table 24. Analysis of Time to Permanently Stopping Randomized Treatment Due to Meeting Rescue Criteria for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935
Number of participants	1937	1935
Permanently stopping randomized treatment due to meeting rescue criteria	39 (2.0)	64 (3.3)
Censored	1898 (98.0)	1871 (96.7)
Incidence rate per 100 PY (two-sided 95% CI)	1.31 (0.93, 1.79)	2.09 (1.61, 2.67)
Hazard ratio		
Estimate ^a		0.63
Two-sided 95% CI for hazard ratio		(0.42, 0.94)
One-sided p-value ^b		0.01
Absolute rate difference per 100 PY (two-sided 95% CI) ^c	-0.79 (-1.44, -0.13)	

Source: Sponsor CSR

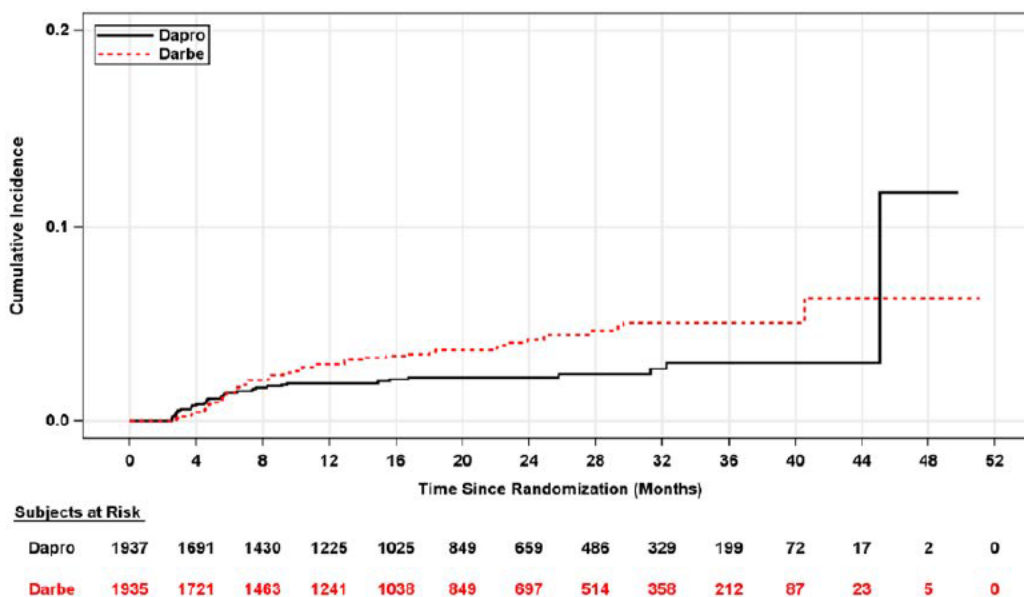
^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, and region. A hazard ratio <1 indicates a lower risk with daprodustat compared to Darbepoetin.

^b One-sided p-value based on Wald test of null hypothesis: (daprodustat/Darbepoetin) ≥1 vs alternative: ratio <1.

^c A rate difference <0 indicates a lower risk with daprodustat compared to Darbepoetin.

Abbreviations: CI, confidence interval; CSR, clinical study report; PY, person-year

Figure 10. Kaplan-Meier Plot Time to Stopping Randomized Treatment Due to Meeting Rescue Criteria for Study 200808/ASCEND-ND



Source: Sponsor CSR
Abbreviations: CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa

The proportion of participants with a first occurrence of RBC or whole blood transfusion during the on-treatment time period was an exploratory endpoint and was numerically lower in the daprodustat group compared with darbepoetin. Participants on daprodustat had a numerically lower risk for time to first occurrence of receiving an on-treatment RBC or whole blood transfusion compared to darbepoetin and no statistically testing was performed ([Table 25](#)).

Table 25. Analysis of Time to First Occurrence of On-Treatment RBC or Whole Blood Transfusion for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935
Number of participants	1937	1935
Permanently stopping randomized treatment due to meeting rescue criteria	247 (12.8)	261 (13.5)
Censored	1690 (87.2)	1672 (86.5)
Incidence rate per 100 PY (two-sided 95% CI)	8.82 (7.76, 9.99)	9.18 (8.10, 10.37)
Hazard Ratio		
Estimate ^a		0.96
Two-sided 95% CI for hazard ratio		(0.80, 1.14)
One-sided p-value ^b		0.32
Absolute rate difference per 100 PY (two-sided 95% CI) ^c	-0.36 (-1.93, 1.21)	

Source: Sponsor CSR
^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, and region. A hazard ratio <1 indicates a lower risk with daprodustat compared to darbepoetin.
^b One-sided p-value based on Wald test of null hypothesis: (daprodustat/darbepoetin) ≥1 vs alternative: ratio <1.
^c A rate difference <0 indicates a lower risk with daprodustat compared to darbepoetin.
Abbreviations: CI, confidence interval; CSR, clinical study report; PY, person-year; RBC, red blood cell

Blood transfusion events to Week 52 are summarized in [Table 26](#). The proportion of participants who received RBC or whole blood transfusions was similar between the treatment groups.

Table 26. On-Treatment Blood Transfusions by Week 52 for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935
Number of participants who received randomized treatment	1937	1933
Number of transfusion events per participant, n (%)		
0	1790 (92)	1775 (92)
1	120 (6)	121 (6)
2	20 (1)	27 (1)
3	4 (<1)	9 (<1)
4	1 (<1)	1 (<1)
5	0	0
>5	2 (<1)	0
Number (%) of participants with transfusion events [number of transfusion events] ^a	147 (8) [194]	158 (8) [206]
Total treatment exposure (years)	1573	1597
Overall transfusion events per 100 PY	12	13
Overall transfusion per 100 PY	17	20
Overall units per 100 PY	26	31

Source: Sponsor CSR

Note: Blood transfusions include RBC and whole blood transfusions.

^a RBC and whole blood transfusion events are defined by grouping together transfusion records if the transfusion start/end dates match with or are contained within an Admission/Discharge period. Transfusion records with no matching admission/discharge period are considered to be the same Transfusion Event if the gap between transfusions is 5 days or less.

Abbreviations: CSR, clinical study report; PY, person-year; RBC, red blood cell

Efficacy Results—Subgroups

A subgroup analysis was performed for the primary ITT analysis of post randomization mean change in Hb between Baseline and over the EP (Weeks 28 to 52) by ESA use at baseline. The analysis by ESA use at Baseline had an interaction p-value of 0.0025, however, mean Hb remained in the analysis range (10 to 11.5 g/dL) during the EP regardless of ESA use at Baseline in both groups (see [Table 27](#)).

Table 27. Subgroup Analysis of Post-Randomization Hb Change From Baseline to the EP (Weeks 28 to 52) by ESA Use at Baseline for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935
ESA non-user		
Number of participants	1030	1032
Number of participants with baseline and EP Hb (%) ^a	1030 (100)	1032 (100)
Baseline Hb (g/dL) mean (SD)	9.49 (0.79)	9.47 (0.78)
EP Hb (g/dL) mean (SD) ^a	10.57 (0.61)	10.40 (0.65)
Adjusted mean change from baseline (SE) ^b	0.78 (0.03)	0.63 (0.03)
Adjusted mean treatment difference (dapro-darbe) ^b		0.16
Two-sided 95% CI ^b		(0.08, 0.23)

	Daprodustat N=1937	Darbepoetin N=1935
ESA user		
Number of participants	907	903
Number of participants with baseline and EP Hb (%) ^a	907 (100)	903 (100)
Baseline Hb (g/dL) mean (SD)	10.29 (0.92)	10.29 (0.93)
EP Hb (g/dL) mean (SD) ^a	10.63 (0.64)	10.63 (0.76)
Adjusted mean change from baseline (SE) ^b	0.68 (0.03)	0.69 (0.03)
Adjusted mean treatment difference (dapro-darbe) ^b		-0.01
Two-sided 95% CI ^b		(-0.08, 0.07)
Sub-group by treatment interaction		0.0025

Source: Sponsor CSR

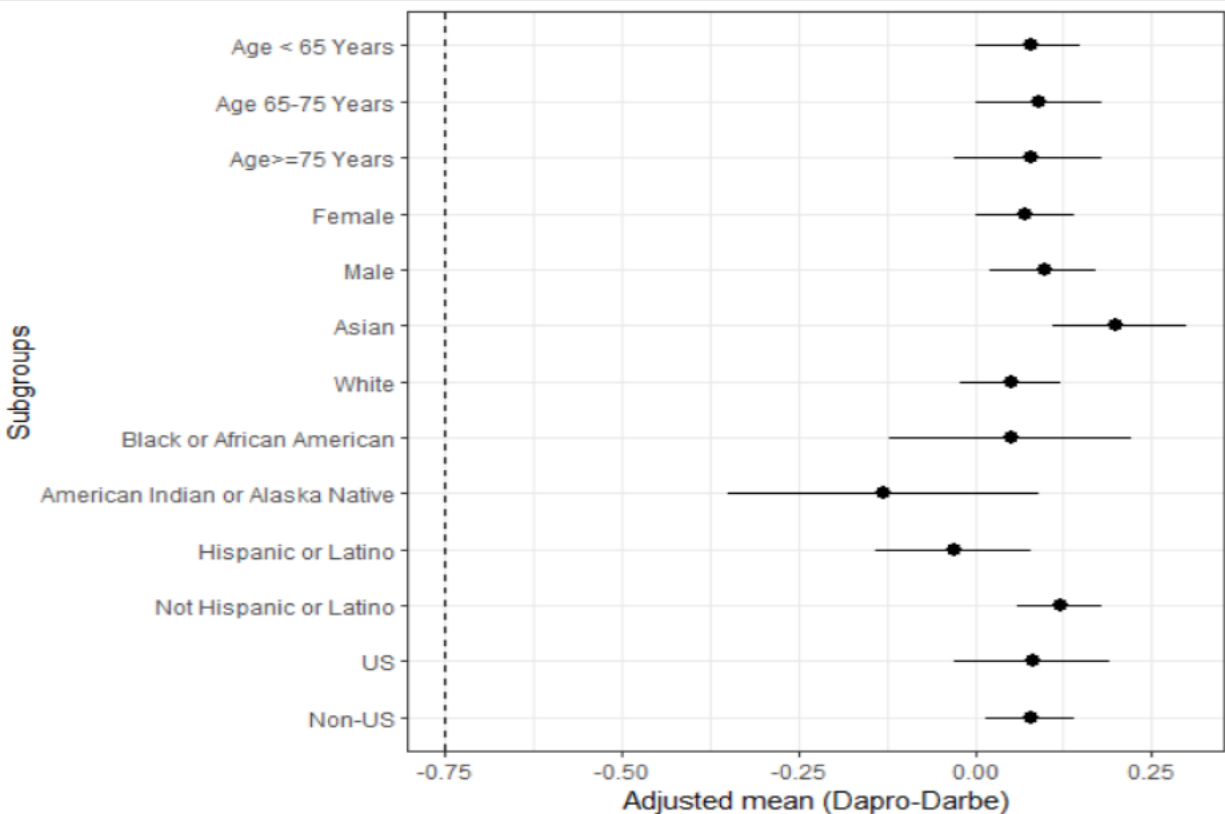
^a Post-randomization values include on and off treatment values. Observed and imputed Hb values are used in this analysis. There were no missing Baseline values in the analysis.

^b Based on an ANCOVA model with terms for treatment, baseline hemoglobin, region, subgroup, and treatment by subgroup interaction.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; EP, evaluation period; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; SD, standard deviation; SE, standard error

Overall, the results of the other subgroup analyses were consistent with the primary analysis with little or no heterogeneity between subgroups. The adjusted mean differences were consistently >-0.75 (see [Figure 11](#)).

Figure 11. Primary Analysis of Hb Change From Baseline to the EP for Study 200808/ASCEND-ND



Source: FDA reviewer

Abbreviations: Dapro, daprodustat; Darbe, darbepoetin alfa; EP, evaluation period; Hb, hemoglobin; US, United States

6.2.3. Study 204837/ASCEND-TD

6.2.3.1. Design

Overview

To support a three times a week dosing regimen in patients on hemodialysis, the Applicant conducted a randomized, Phase 3 Study, 204837/ASCEND-TD, titled “A Phase 3 randomized, double-blind, active-controlled, parallel-group, multi-center study in hemodialysis participants with anemia of chronic kidney disease to evaluate the efficacy, safety and pharmacokinetics of three-times weekly dosing of daprodustat compared to recombinant human erythropoietin, following a switch from recombinant human erythropoietin or its analogues.”

Primary Efficacy Endpoint

Mean change in Hb between Baseline and EP (Weeks 28 to 52)

Key Secondary Efficacy Endpoint

Average monthly IV iron dose (mg)/participant to Week 52

Secondary Efficacy Endpoints

- Hb change from Baseline to Week 52
- N (%) responders, defined as mean Hb within the Hb analysis range 10 to 11.5 g/dL during EP
- % time Hb in analysis range during the EP
- Time to stopping randomized treatment due to meeting rescue criteria

Study Design

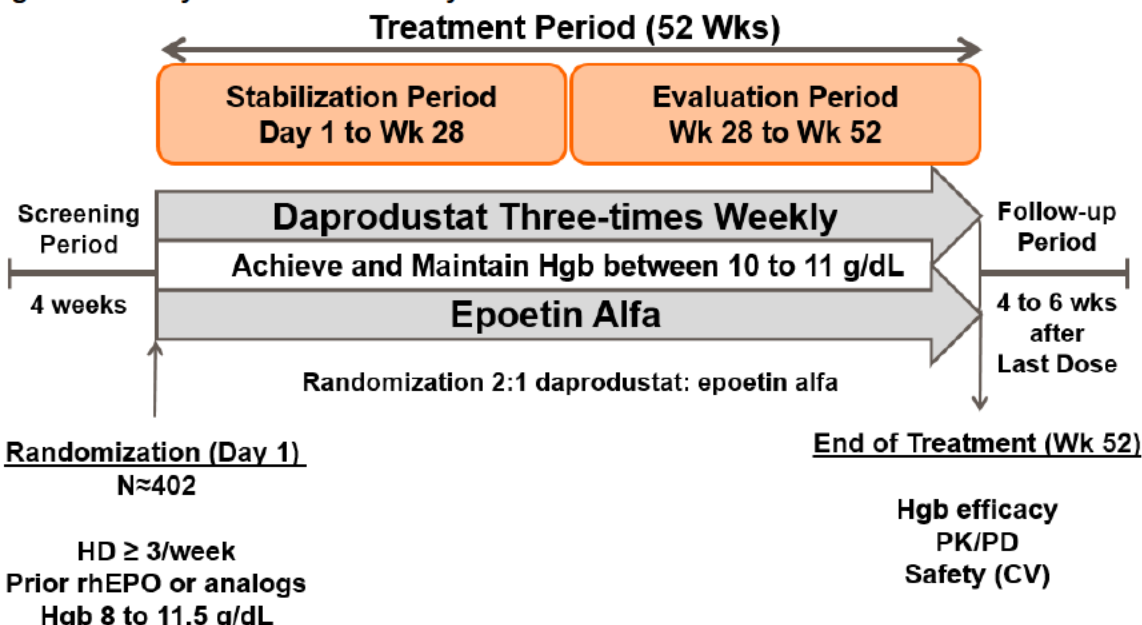
Study 204837/ASCEND-TD was a randomized, double-blind, active-controlled, double-dummy, parallel-group, multi-center study in hemodialysis-dependent participants, including combination methods such as hemodiafiltration or hemofiltration with hemodialysis, who had anemia of CKD and were being treated with rhEPO or its analogues.

This study comprised a 4-week Screening Period, a 52-week Treatment Period and a Follow-up Period ([Figure 12](#)).

The 52-week Treatment Period consists of:

- SP, defined as the period from Day 1 to Week 28 (up to but excluding the Week 28 visit), during which study treatment was dose-titrated to achieve and maintain Hb in the target range.
- EP, defined as the period from Week 28 to Week 52, to assess long-term efficacy and safety. Throughout the trial, including during the EP, randomized treatment was titrated (in a double-blind fashion) according to a prespecified algorithm.

Figure 12. Study Schematic for Study 204837/ASCEND-TD



Source: Sponsor CSR

Abbreviations: CSR, clinical study report; CV, coefficient of variation; HD, hemodialysis; Hgb, hemoglobin; PK/PD, pharmacokinetic/pharmacodynamic; rhEPO, recombinant human erythropoietin

Randomization was stratified by region and participants were randomized in a 2:1 ratio to either:

- Daprodustat TIW + saline IV injection (placebo), or;
- Epoetin alfa once weekly or TIW (depending on dose level) + placebo tablets.

Study Population Inclusion/Exclusion Criteria

Inclusion Criteria

The study recruited male and female (non-pregnant, non-lactating) participants, aged ≥ 18 years to ≤ 99 years of age with anemia of CKD who were being treated with rhEPO or its analogues. Participants were hemodialysis-dependent (including hemofiltration or hemodiafiltration) for greater than 90 days and receiving hemodialysis (in-center) ≥ 3 times per week. Hb eligibility range is defined as:

- Week -4: Hb 8 to 11.5 g/dL (5 to 7.4 mmol/L); If Hb is 11.6 to 11.9 g/dL (7.2 to 7.4 mmol/L), up to two retests are allowed; the retest value must be between 8 to 11.5 g/dL (5 to 7.1 mmol/L).
- Day 1: Hb 8 to 11 g/dL (5 to 6.8 mmol/L) and receiving at least the minimum rhEPO or analogue dose; Hb > 11 g/dL to 11.5 g/dL (6.8 to 7.1 mmol/L) and receiving greater than the minimum rhEPO or analogue dose

Exclusion Criteria

- Planned kidney transplant within 52 weeks
- Ferritin ≤ 100 ng/mL (≤ 100 $\mu\text{g/L}$) at screening
- TSAT $\leq 20\%$ at screening

- History of bone marrow aplasia or pure red cell aplasia
- Anemia other than anemia of CKD.
- MI, acute coronary syndrome, stroke, or transient ischemic attack within 4 weeks prior to screening through to randomization (Day 1)
- QTcB >500 msec, or QTcB >530 msec in participants with bundle branch block at randomization (Day 1)
- Chronic Class IV heart failure
- Uncontrolled hypertension
- Prior or current history of cancer; liver disease or known hepatic or biliary abnormalities; or gastrointestinal bleeding.
- Participants requiring treatment with strong inhibitor of CYP2C8 or a strong inducer of CYP2C8 were excluded.

6.2.3.2. Statistical Analysis Plan

Sample Size Determination

Approximately 402 participants were planned to be randomized in a 2:1 ratio to daprodustat TIW or rhEPO (268 to the daprodustat TIW arm and 134 to the rhEPO arm), to provide at least 100 participants exposed to daprodustat for one year. Participants were treated to achieve and maintain Hb between 10 and 11 g/dL. The expected difference in mean Hb change from baseline and the EP, between arms, was 0 g/dL and the anticipated between participant standard deviation (SD) was 1.5 g/dL, based on historical rhEPO trials and daprodustat clinical trial experience to date. With a prespecified non-inferiority margin of -0.75 g/dL, a two-sample t-test and assuming that up to approximately 10% of participants had no Hb values measured during the EP, 402 randomized participants would provide >90% power to test the primary hypothesis.

With 402 randomized participants, it was anticipated that the difference in mean Hb change from baseline between arms would be estimated with a precision of 0.33 g/dL (half width of the two-sided 95% CI) and the largest (most negative) difference between arms that would meet the statistical criterion for non-inferiority would be -0.42 g/dL.

Interim Analysis

No interim analyses were performed.

Missing Data

For the analysis of the primary efficacy endpoint, a multiple imputation approach was used to handle missing Hb values. Both intermittent missing and monotone missing scheduled Hb data were imputed based on the MAR assumption. However, it is important to examine the sensitivity of inferences to departures from the MAR assumption for the monotone missing data. A tipping point analysis was performed as a sensitivity analysis for the assumptions of missing data, that is, to mimic MNAR in which the missing observations depend on unobserved values.

Primary Efficacy Analysis

The primary analysis of post-randomization mean change in Hb between baseline and over the EP (Weeks 28 to 52) for the ITT population was analyzed using an ANCOVA model adjusted for treatment, baseline Hb and region.

Sensitivity Analyses

- An alternative “while on-treatment” estimand using only evaluable Hb data. Missing Hb data were not imputed.
- PP population analysis based on the evaluable Hb analysis.
- A tipping point analysis using all available Hb values (on and off treatment) as a sensitivity analysis for the primary estimand. Missing post-baseline Hb values were imputed.
- A tipping point analysis using only evaluable Hb values. Missing Hb data were not imputed.
- An alternative EP analysis from Week 28 to 36 using all available Hb values (on and off treatment). Missing post-baseline Hb values were imputed.
- An alternative EP analysis from Week 28 to 36 using only evaluable Hb values. Missing Hb data were not imputed.

Secondary Efficacy Analyses

An ANCOVA model was used to compare the difference in average monthly IV iron dose per participant between arms, adjusting for treatment, baseline monthly IV iron dose and region.

A least square mean estimates and two-sided 95% CI for the treatment difference are provided, together with the one-sided superiority test p-value.

The secondary analysis of Hb change from baseline to Week 52 Hb was tested for noninferiority using the -0.75 g/dL margin used in the primary analysis. A MMRM approach was used with an unstructured covariance matrix to compare the difference in means between arms. The model was fitted to Hb data collected post-baseline up to Week 52, excluding values collected during the SP (Day 1 to Week 28). The model included factors for treatment, time, region, baseline Hb and the baseline Hb by time and treatment by time interaction terms.

For the Hb responder analysis, responders were participants with a mean Hb during the EP that fell within the Hb analysis range of 10 to 11.5 g/dL. A CMH chi-squared test, adjusting for treatment and region, was used to compare the number and % of responders between the treatment groups.

For the analysis of time to stopping study treatment due to meeting rescue criteria, a Cox proportional hazards model, adjusting for treatment and region, was used to generate the hazard ratio and associated 95% CI for the treatment group comparison. A one-sided superiority p-value was generated using a Wald test. The incidence rate per 100 person-years (PY) and associated 95% CI for each treatment group, as well as the absolute rate difference per 100 PY and associated 95% CI were also provided.

Multiple Comparison

The primary endpoint was tested first for non-inferiority, using the lower limit of the two-sided 95% CI. Conditional on achieving statistical significance (i.e., establishing non-inferiority) the single key secondary endpoint was tested for superiority using a one-sided 2.5% significance level. This two-step hierarchical strategy preserved the study-wise Type I error rate at a one-sided 2.5% level.

The additional secondary/exploratory endpoints, if tested, were not adjusted for multiplicity. A nominal one-sided 2.5% significance level was applied per test.

Subgroup Analysis

Post hoc subgroup analyses, including age, sex, region, and race were performed for the primary ITT analysis of post-randomization mean change in Hb between baseline and over the EP (Weeks 28 to 52).

6.2.3.3. Results of Analyses

Patient Disposition

Participants were considered to have completed the study if they remained on study to Week 52 and completed the Week 52 visit irrespective of whether they discontinued randomized treatment. Participants were also considered to have completed the study if they died while on study. Of 407 participants in the ITT population, 404 (>99%) participants completed the study to Week 52 or died while on study. One (<1%) participant in the daprodustat TIW group and 2 (1%) participants in the epoetin alfa group discontinued randomized treatment and withdrew from the study (see [Table 28](#)).

Table 28. Summary of Subject Status and Reasons for Withdrawal for Study 204837/ASCEND-TD

	Daprodustat N=270	rhEPO N=137	Total N=407
Overall subject status			
Completed	269 (>99%)	135 (99%)	404 (>99%)
Withdrawn	1 (<1%)	2 (1%)	3 (<1%)
Primary reason/sub-reason for study withdrawal			
Adverse event	0	0	0
Protocol deviation	0	0	0
Study closed/terminated	0	0	0
Lost to follow-up	0	0	0
Investigator site closed	0	0	0
Investigator discretion	0	0	0
Withdrew consent	1 (<1%)	2 (<1%)	3 (<1%)
Logistical circumstances	0	0	0
Privacy preferences	1 (<1%)	2 (<1%)	3 (<1%)
Adverse experience	0	0	0
Other subject decision	0	0	0
Outcome of adverse events which led to study withdrawal			
Nonfatal	0	0	0
Fatal	0	0	0

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; rhEPO, recombinant human erythropoietin

Of the 407 participants in the ITT population, 289 (71%) remained on randomized treatment until Week 52 as scheduled. The proportion of participants who discontinued randomized treatment was similar in the daprodustat group [78 (29%) participants] compared to epoetin alfa [39 (28%) participants]. The primary reasons for discontinuation were similar in both treatment groups (see [Table 29](#)).

Table 29. Summary of Randomized Treatment Status and Reasons for Discontinuation of Randomized Treatment for Study 204837/ASCEND-TD

	Daprodustat N=270	rhEPO N=137	Total N=407
Overall study treatment status			
Never received study treatment	0	1 (<1%)	1 (<1%)
Prematurely discontinued study treatment	78 (29%)	39 (28%)	117 (29%)
Did not die while taking study treatment	71 (26%)	36 (26%)	107 (26%)
Died while taking study treatment	7 (3%)	3 (2%)	10 (2%)
Did not prematurely discontinue study treatment	192 (71%)	97 (71%)	289 (71%)
Overall primary reason/subreason for all study treatment discontinuation:			
Adverse event	28 (10%)	11 (8%)	39 (10%)
Protocol deviation	0	0	0
Subject reached protocol-defined stopping criteria	23 (9%)	14 (10%)	37 (9%)
Subject had renal transplant	12 (4%)	9 (7%)	21 (5%)
Rescue	6 (2%)	3 (2%)	9 (2%)
Pregnancy	0	1 (<1%)	1 (<1%)
Subject met GSK defined liver chemistry stopping criteria	0	0	0
Cancer	4 (1%)	1 (<1%)	5 (1%)
Need for chronic use of prohibited medication	0	0	0
Change in dialysis modality	1 (<1%)	0	1 (<1%)
Reduction in dialysis frequency to less than 3 times per week	0	0	0
Study closed/terminated	0	0	0
Lost to follow-up	0	0	0
Investigator discretion	4 (1%)	3 (2%)	7 (2%)
Decision by subject or proxy	23 (9%)	11 (8%)	34 (8%)
Subject reported lack of efficacy	2 (<1%)	1 (<1%)	3 (<1%)
Preference for another treatment regimen	3 (1%)	1 (<1%)	4 (<1%)
Burden of study adherence	3 (1%)	2 (1%)	5 (1%)
Geographical relocation	7 (3%)	2 (1%)	9 (2%)
Stopped dialysis	0	0	0

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; GSK, glycogen synthase kinase; rhEPO, recombinant human erythropoietin

Demographic Characteristics and Baseline Characteristics

Baseline demographic characteristics, and Hb levels were generally similar between treatment groups for each of the active-controlled dialysis studies.

Baseline demographic characteristics and other Baseline characteristics are summarized in [Table 30](#) and [Table 31](#), respectively.

Table 30. Summary of Demographic Characteristics for Study 204837/ASCEND-TD

	Daprodustat TIW N=270	Epoetin N=137
Age (years), n	270	136
Mean (SD)	59.4 (14.2)	55.8 (15.3)
Median	60.0	56.0
Min, max	21, 90	22, 87
Age category, n (%)	270	137
<65 years	167 (62)	96 (70)
65 - 74 years	66 (24)	24 (18)
≥75 years	37 (14)	16 (12)
Missing	0	1 (<1)
Gender, n (%)	270	137
Female	121 (45)	56 (41)
Male	149 (55)	81 (59)
Ethnicity, n (%)	270	137
Hispanic or Latino	69 (26)	33 (24)
Not Hispanic or Latino	198 (73)	104 (76)
Missing	3 (1)	0
Race, n (%)	270	137
American Indian or Alaska native	1 (<1)	1 (<1)
Asian	20 (7)	9 (7)
Black or African American	49 (18)	32 (23)
Native Hawaiian or other Pacific Islander	1 (<1)	0
White	195 (72)	94 (69)
Mixed race	1 (<1)	1 (<1)
Missing	3 (1)	0
Baseline weight (kg)	270	137
Mean (SD)	76.5 (19.0)	77.5 (19.3)
Median	74.0	75.4
Min, max	36.1, 138.3	38.5, 131.3
Baseline weight group, n (%)	270	137
<75 kg	143 (53)	68 (50)
≥75 kg	127 (47)	69 (50)
Missing	0	0
Baseline hsCRP	266	136
Mean (SD)	8.9 (14.5)	11.8 (16.4)
Median	4.3	4.7
Min, max	0.2, 119.6	0.2, 97.5

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; hsCRP, high-sensitivity C-reactive protein; SD, standard deviation; TIW, three times a week

Table 31. Summary of Baseline Characteristics for Study 204837/ASCEND-TD

	Daprodustat TIW N=270	Epoetin N=137
Time on dialysis at screening, n (%)	270	137
<2 years	82 (30)	42 (31)
≥2 and <5 years	95 (35)	52 (38)
≥5 years	93 (34)	43 (31)
Dialysis type at screening, n (%)	270	137
Hemodialysis conventional	237 (88)	120 (88)
Hemofiltration/hemodiafiltration	33 (12)	17 (12)
Peritoneal dialysis	0	0

	Daprodustat TIW N=270	Epoetin N=137
Standardized prior ESA dose (U/week)	270	137
Mean (SD)	8462 (6283)	7958 (6635)
Median	6297	5943
Min, max	464, 43995	200, 44941
rhEPO hyporesponder, n (%) ^a	270	137
No	233 (86)	116 (85)
Yes	37 (14)	21 (15)
Missing	0	0
Baseline Hb (g/dL)	270	137
Mean (SD)	10.4 (0.8)	10.6 (0.9)
Median	10.5	10.7
Min, max	8.1, 13.4	5.3, 12.3
Baseline Hb group, n (%)	270	137
<9 g/dL	15 (6)	6 (4)
≥9 and <10 g/dL	51 (19)	20 (15)
≥10 and ≤11 g/dL	141 (52)	60 (44)
>11 g/dL	63 (23)	51 (37)

Source: Sponsor CSR

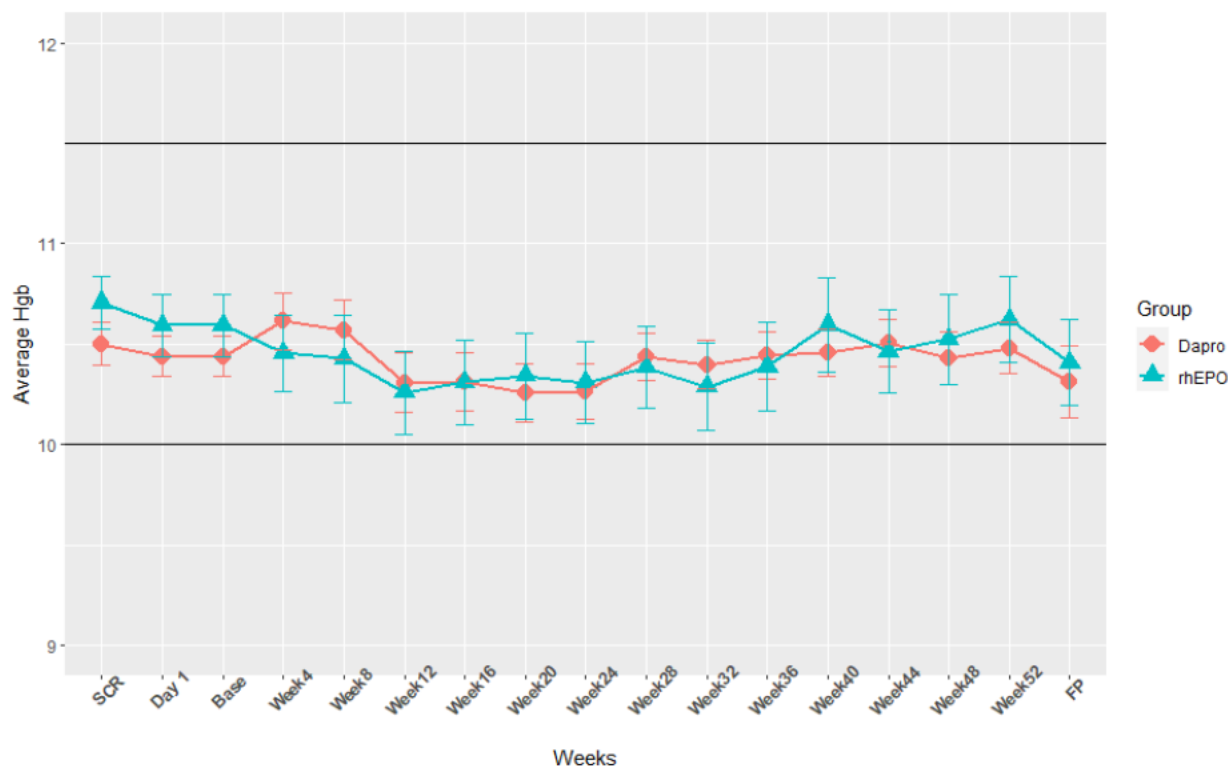
^a Participants with ERI ≥2.0 U/kg/wk/g/L (prior epoetin) or ≥0.008 µg/kg/wk/g/L (prior darbepoetin alfa) or ≥0.01 mg/kg/wk/g/L (prior methoxy-PEG-epoetin), or if treated with the equivalent of ≥450 U/kg/week IV epoetin alfa. ERI is calculated as ESA (e.g., epoetin alfa or other ESAs) dose per week (in U or ug) during the 4-week Screening period divided by Baseline dry weight at Day 1 and then divided by the achieved Day 1 Hb.

Abbreviations: CSR, clinical study report; ERI, ESA resistance index; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; rhEPO, recombinant human erythropoietin; SD, standard deviation; TIW, three times a week

Efficacy Results—Primary Endpoint

For Study 204837/ASCEND-TD, mean Hb at Baseline and over the EP was similar between treatment groups and remained within the Hb analysis range (10 to 11.5 g/dL) during the EP (Weeks 28 to 52) (Figure 13). Also, Hb remained relatively stable and within the analysis range (10 to 11.5 g/dL) from Baseline through Week 52.

Figure 13. Line Plot of Post-Randomization Observed Hb Data by Visit for Study 204837/ASCEND-TD



Source: FDA

Note: Error bars indicate 95% CI around mean

Baseline and visits on or before Day 1 include only pre-treatment values.

Post-randomization values include all available observed and imputed Hb values (on- and off-treatment).

The dashed vertical lines represent the EP (Week 28 to Week 52).

The horizontal reference lines represent the Hb analysis range (10-11.5 g/dL). The Hb target range for dose changes is 10-11 g/dL.

Abbreviations: CI, confidence interval; EP, evaluation period; Hb, hemoglobin; SCR, screening

For study 204837/ASCEND-TD the lower bound of the 95% CI for the treatment difference of -0.21 was greater than the prospectively defined non-inferiority margin of -0.75 g/dL, thus demonstrating non-inferiority of daprodustat (see [Table 32](#)).

Table 32. Analysis of Post-Randomization Hb Change From Baseline to the EP (Weeks 28 to 52) for Study 204837/ASCEND-TD

	Daprodustat (N=270)	Epoetin (N=137)
Participants with all 7 scheduled Hb values during the EP	186 (69)	92 (67)
Participants with partial scheduled Hb values during the EP	66 (24)	36 (26)
Participants with no observed Hb values during the EP	18 (7)	9 (7)
Number of participants with baseline and EP Hb, n (%) ^a	270 (100)	137 (100)
Baseline Hb (g/dL) mean (SD)	10.44 (0.83)	10.59 (0.93)
EP (Weeks 28-52) Hb (g/dL) mean (SD) ^a	10.45 (0.55)	10.51 (0.85)
Adjusted mean change from baseline (SE) ^b	-0.04 (0.05)	0.02 (0.07)
Adjusted mean treatment difference (dapro - rhEPO) ^b		-0.05
Two-sided 95% CI for adjusted mean difference ^b		(-0.21, 0.10)

Source: Sponsor CSR

Note: Post-randomization values include on and off treatment values.

^a Hb values during the EP include both observed and imputed values.

^b Based on an ANCOVA model with terms for treatment, and region

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; EP, evaluation period; Hb, hemoglobin; rhEPO, recombinant human erythropoietin; SD, standard deviation; SE, standard error

Sensitivity Analysis

For the analysis of the Hb co-primary efficacy endpoint, a multiple imputation approach was used to handle missing Hb values. Missing scheduled Hb data were imputed based on the MAR assumption. However, it is important to examine the sensitivity of inferences to departures from the MAR assumption for the monotone missing data. A tipping point analysis was performed as a sensitivity analysis for the assumptions of missing data, that is, to mimic MNAR in which the missing observations depend on unobserved values.

Results of the tipping point analysis suggest that the extent of missing data would unlikely impact the conclusions of the primary/co-primary efficacy analysis. Therefore, missing data in these studies are unlikely to have had an impact on the primary results.

Efficacy Results—Key Secondary Endpoints

Daprodustat did not demonstrate a statistically significant reduction in average monthly IV iron dose to Week 52 compared to Epoetin (see [Table 33](#)).

Table 33. Analysis of On-Treatment Average Monthly IV Iron Dose During Day 1 to Week 52 for Study 204837/ASCEND-TD

	Daprodustat (N=270)	Epoetin (N=137)
Number of participants during day 1 to Week 52 (%)	270 (100)	136 (>99)
Baseline monthly IV iron dose (mg) mean (SD)	186 (282)	175 (178)
Average monthly IV iron dose (mg) during Day 1 to Week 52 mean (SD)	98 (187)	100 (210)
Adjusted mean change from Baseline (SE)	97 (11)	102 (16)
Adjusted mean treatment difference (dapro - Darbe)		-5
Two-sided 95% CI for adjusted mean difference		(-42, 33)
One-sided p-value ^a		0.40

Source: Sponsor CSR

Note: Iron data after a participant has received an RBC or whole blood transfusion is excluded from the analysis

^a Based on an ANCOVA model with terms for treatment, baseline Hb, dialysis type and region. One-sided p-value based on test of null hypothesis: (daprodustat - rhEPO) ≥ 0 vs alternative: difference < 0 .

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; Hb, hemoglobin; IV, intravenous; RBC, red blood cell; rhEPO, recombinant human erythropoietin; SD, standard deviation; SE, standard error

Efficacy Results—Secondary Endpoints

Because of the importance of patients not spending undue time with suboptimal Hb levels, a supportive analysis was performed for the primary ITT analysis of post-randomization mean change in Hb between Baseline and over the EP, repeating the analysis using a shorter EP (Week 28 to 36). For each study the lower bound of the 95% CI for the treatment difference was greater than the prospectively defined non-inferiority margin of -0.75 g/dL, thus supporting the overall conclusion of non-inferiority of daprodustat (see [Table 34](#)). Results were consistent with the conclusion from the primary analysis.

Table 34. Analysis of Post-Randomization Hb Change From Baseline to the Alternative EP (Weeks 28 to 36) for Study 204837/ASCEND-TD

	Daprodustat (N=270)	Epoetin (N=137)
Number of participants with baseline and EP Hb, n(%) ^a	270(100)	137 (100)
Baseline Hb (g/dL) mean (SD)	10.44 (0.83)	10.59 (0.93)
EP (Weeks 28-36) Hb (g/dL) mean (SD) ^a	10.43 (0.72)	10.38 (0.94)
Adjusted mean change from baseline (SE) ^b	-0.05 (0.05)	-0.12 (0.08)
Adjusted mean treatment difference (dapro - rhEPO) ^b		0.07
Two-sided 95% CI for adjusted mean difference ^b	(-0.12, 0.25)	

Source: Sponsor CSR

^a Hb values during the EP include both observed and imputed values.

^b Based on an ANCOVA model with terms for treatment, Baseline Hb, and region

Abbreviations: ANCOVA, analysis of covariance; CSR, clinical study report; Dapro, daprodustat; EP, evaluation period; Hb, hemoglobin; rhEPO, recombinant human erythropoietin; SD, standard deviation; SE, standard error

Additional primary endpoint supportive analyses included an on-treatment analysis using only evaluable Hb values, a PP population analysis using only evaluable Hb values, a shorter EP (Weeks 28 to 36) analysis using only evaluable Hb values, and a post-randomization (on- and off-treatment) observed case analysis. Results were consistent with the non-inferiority conclusion from the primary analysis ([Table 35](#)).

Table 35. Primary Endpoint Hb Supportive Analyses for Study 204837/ASCEND-TD

	Daprodustat TIW N=270	Epoetin N=137
Evaluable Hb (Weeks 28 to 52), ITT population		
Number of participants with baseline and EP Hb, n (%) ^a	215 (80)	107 (78)
Adjusted mean change from baseline (SE) ^b	-0.08 (0.05)	-0.06 (0.07)
Adjusted mean treatment difference (dapro - Epoetin) ^b		-0.02
Two-sided 95% CI for adjusted mean difference	(-0.18, 0.14)	
Evaluable Hb (Weeks 28 to 52), PP population		
Number of participants with baseline and EP Hb, n (%) ^a	143 (53)	82 (60)
Adjusted mean change from baseline (SE) ^b	-0.08 (0.05)	-0.03 (0.07)
Adjusted mean treatment difference (dapro - Epoetin) ^b		-0.06
Two-sided 95% CI for adjusted mean difference ^b	(-0.22, 0.11)	
Evaluable Hb alternative EP (Weeks 28 to 36), ITT population		
Number of participants with baseline and EP Hb, n (%) ^a	213 (79)	105 (77)
Adjusted mean change from baseline (SE) ^b	-0.09 (0.06)	-0.14 (0.08)
Adjusted mean treatment difference (dapro - Epoetin) ^b		0.04
Two-sided 95% CI for adjusted mean difference ^b	(-0.15, 0.23)	

	Daprodustat TIW N=270	Epoetin N=137
Post-randomization Hb (observed cases only), ITT population		
Number of participants with baseline and EP Hb, n (%)	252 (93)	128 (93)
Adjusted mean change from baseline (SE) ^b	-0.01 (0.05)	0.04 (0.07)
Adjusted mean treatment difference (dapro - Epoetin) ^b		-0.06
Two-sided 95% CI for adjusted mean difference ^b		(-0.22, 0.10)

Source: Sponsor CSR

^a Hb values during the EP include observed values only.

^b Based on an ANCOVA model with terms for treatment, baseline Hb, and region.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; EP, evaluation period; Hb, hemoglobin; ITT, intent-to-treat; PP, per protocol; SE, standard error; TIW, three times a week

The proportion of participants with mean Hb within the analysis range during the EP was higher in the daprodustat arm as compared to Epoetin arm ([Table 36](#)).

Table 36. Participants With Mean EP Hb Below, Within and Above the Analysis Range Using Evaluable Hb Values for Study 204837/ASCEND-TD

	Daprodustat N=270	Epoetin N=137
Participants with evaluable Hb during the EP	215	107
Mean EP Hb above 11.5 g/dL	3 (1)	9 (8)
Mean EP Hb within 10.0-11.5 g/dL	172 (80)	68 (64)
Mean EP Hb below 10.0 g/dL	40 (19)	30 (28)

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; EP, evaluation period; Hb, hemoglobin

The proportion of participants stopping randomized treatment due to meeting rescue criteria was small and similar between the treatment groups. Daprodustat did not reduce the risk of stopping randomized treatment due to meeting rescue criteria ([Table 37](#)).

The cumulative incidence for time to stopping randomized treatment due to meeting rescue criteria was similar between the treatment groups ([Figure 14](#)).

Table 37. Analysis of Time to Permanently Stopping Randomized Treatment Due to Meeting Rescue Criteria for Study 204837/ASCEND-TD

	Daprodustat N=270	Epoetin N=137
Number of participants	270	137
Permanently stopping randomized treatment due to meeting rescue criteria	6 (2.2)	3 (2.2)
Censored	264 (97.8)	134 (97.8)
Incidence rate per 100 PY (two-sided 95% CI)	2.68 (0.98,5.84)	2.66 (0.55,7.77)
Hazard ratio		
Estimate ^a		1.06
Two-sided 95% CI for hazard ratio		(0.26, 4.22)
One-sided p-value ^b		0.53
Absolute rate difference per 100 PY (two-sided 95% CI) ^c		0.02 (-3.67, 3.72)

Source: Sponsor CSR

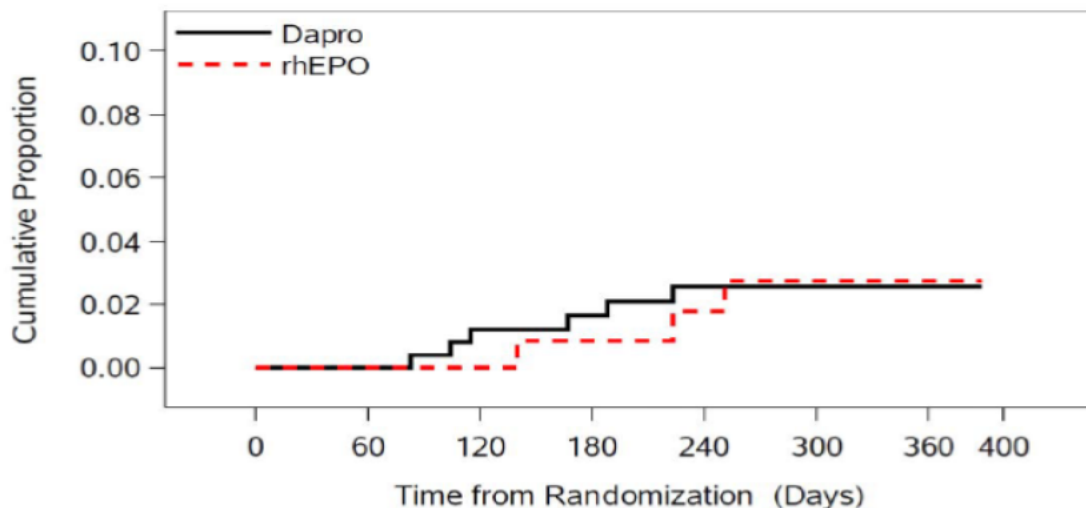
^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, and region. A hazard ratio <1 indicates a lower risk with daprodustat compared to rhEPO.

^b One-sided p-value based on Wald test of null hypothesis: (daprodustat/rhEPO) ≥1 vs alternative: ratio <1.

^c A rate difference <0 indicates a lower risk with daprodustat compared to rhEPO.

Abbreviations: CI, confidence interval; CSR, clinical study report; PY, person-year; rhEPO, recombinant human erythropoietin

Figure 14. Kaplan-Meier Plot Time to Stopping Randomized Treatment Due to Meeting Rescue Criteria for Study 204837/ASCEND-TD



Subjects at risk

	0	60	120	180	240	300	360	400
Dapro	270	256	241	223	202	198	167	0
rhEPO	137	129	121	112	104	99	88	0

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; Dapro, daprodustat; year, rhEPO, recombinant human erythropoietin

The proportion of participants with a first occurrence of RBC or whole blood transfusion during the on-treatment time period was an exploratory endpoint and was numerically lower in the daprodustat group compared with Epoetin. Participants on daprodustat had a numerically lower risk for time to first occurrence of receiving an on-treatment RBC or whole blood transfusion compared to Epoetin, but no statistical testing was performed on this exploratory endpoint ([Table 38](#)).

Table 38. Analysis of Time to First Occurrence of On-Treatment RBC or Whole Blood Transfusion for Study 204837/ASCEND-TD

	Daprodustat N=270	Epoetin N=137
Number of participants	270	136
Permanently stopping randomized treatment due to meeting rescue criteria	21 (7.8)	16 (11.8)
Censored	249 (92.2)	120 (88.2)
Incidence rate per 100 PY (two-sided 95% CI)	9.64 (5.97, 14.73)	15.01 (8.58, 24.37)
Hazard ratio		
Estimate ^a		0.61
Two-sided 95% CI for hazard ratio		(0.32, 1.18)
Absolute rate difference per 100 PY (two-sided 95% CI) ^c	-5.37 (-13.80, 3.06)	

Source: Sponsor CSR

^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, and region. A hazard ratio <1 indicates a lower risk with daprodustat compared to rhEPO.

^b One-sided p-value based on Wald test of null hypothesis: (daprodustat/rhEPO) ≥1 vs alternative: ratio <1.

^c A rate difference <0 indicates a lower risk with daprodustat compared to rhEPO.

Abbreviations: CI, confidence interval; CSR, clinical study report; PY, person-year; RBC, red blood cell; rhEPO, recombinant human erythropoietin

Blood transfusion events to Week 52 are summarized in [Table 39](#). The proportion of participants who received RBC or whole blood transfusions was similar between the treatment groups.

Table 39. On-treatment Blood Transfusions by Week 52 for Study 204837/ASCEND-TD

	Daprodustat N=270	Epoetin N=137
Number of participants who received randomized treatment	270	136
Number of transfusion events per participant, n (%)		
0	249 (92)	120 (88)
1	14 (5)	12 (9)
2	7 (3)	3 (2)
3	0	1 (<1)
4	0	0
5	0	0
>5	0	0
Number (%) of participants with transfusion events [number of transfusion events] ^a	21 (8) [28]	16 (12) [21]
Total treatment exposure (years)	223.18	112.64
Overall transfusion events per 100 PY	12.54	18.64
Overall transfusion per 100 PY	15.68	35.51
Overall units per 100 PY	22.40	52.38

Source: Sponsor CSR

Note: Blood transfusions include RBC and whole blood transfusions.

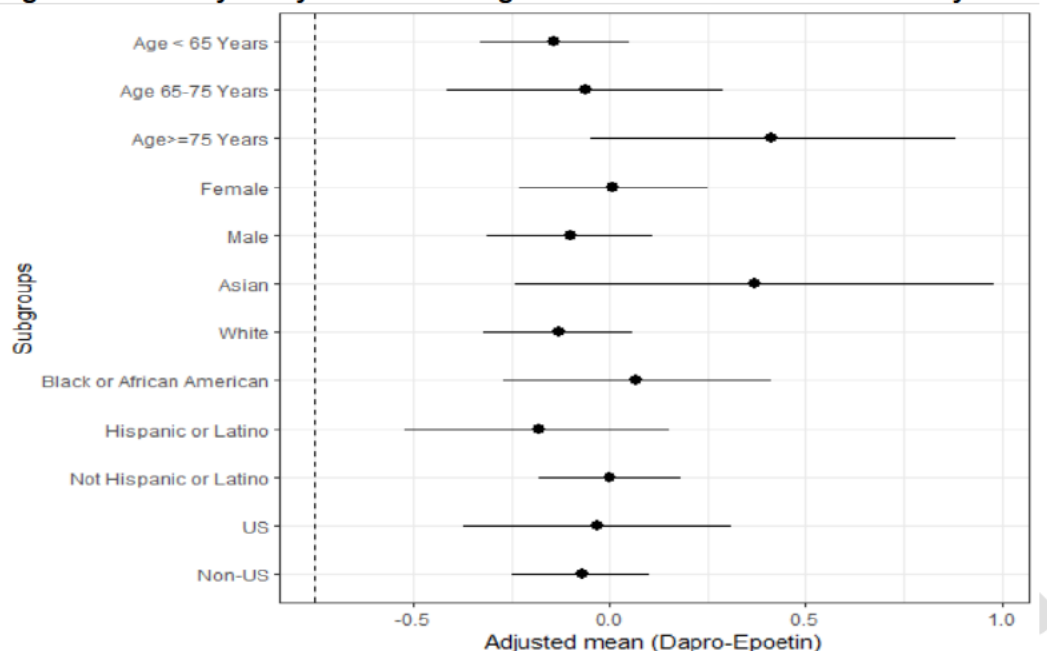
^a RBC and whole blood transfusion events are defined by grouping together transfusion records if the transfusion start/end dates match with or are contained within an Admission/Discharge period. Transfusion records with no matching admission/discharge period are considered to be the same Transfusion Event if the gap between transfusions is 5 days or less.

Abbreviations: CSR, clinical study report; PY, person- year; RBC, red blood cell

Efficacy Results—Other Subgroups

Overall, the results of the subgroup analyses were consistent with the primary analysis with little or no heterogeneity between subgroups. The adjusted mean differences were consistently >0.75 for all subgroups (see [Figure 15](#)).

Figure 15. Primary Analysis of Hb Change From Baseline to the EP for Study 204837/ASCEND-TD



Source: FDA reviewer

Abbreviations: Dapro, daprodustat; EP, evaluation period; Hb, hemoglobin; US, United States

6.2.4. Study 201410/ASCEND-ID

6.2.4.1. Design

Overview

(b) (4) Applicant conducted a randomized, Phase 3 Study, 201410/ASCEND-ID, titled “A 52-week open-label (sponsor-blind), randomized, active-controlled, parallel-group, multi-center study to evaluate the efficacy and safety of daprodustat compared to recombinant human erythropoietin in subjects with anemia associated with chronic kidney disease who are initiating dialysis.”

Primary Efficacy Endpoint

Mean change in Hb between Baseline and EP (Weeks 28 to 52)

Key Secondary Efficacy Endpoint

Average monthly IV iron dose (mg)/participant to Week 52

Secondary Efficacy Endpoints

- Hb change from Baseline to Week 52
- N (%) responders, defined as mean Hb within the Hb analysis range 10 to 11.5 g/dL during EP
- % time Hb in analysis range during the EP
- Time to stopping randomized treatment due to meeting rescue criteria

Study Design

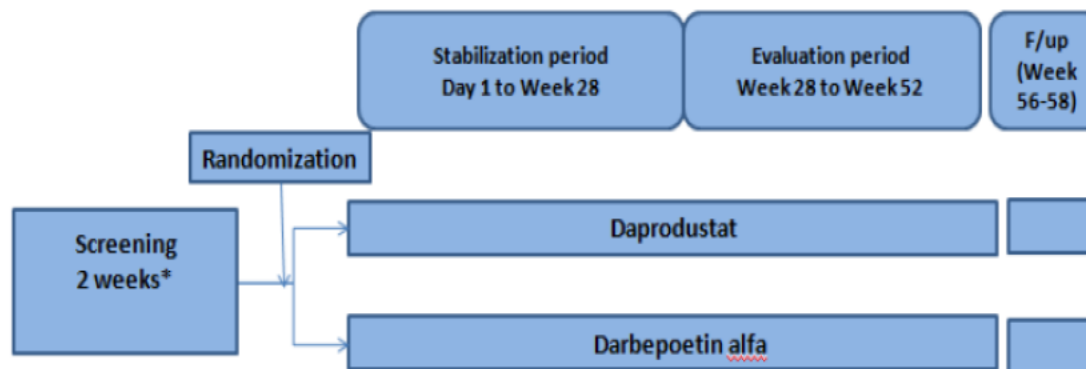
Study 201410/ASCEND-ID was a Phase 3, randomized, open-label (sponsor blind), active-controlled, parallel-group, multi-center study in participants with anemia associated with CKD who were initiating dialysis. The study comprised 3 periods: a screening period of 2 weeks (which could be extended by an additional 4 weeks for kidney ultrasound examination, IV iron supplementation and/or vitamin B12 treatment as needed), a 52-week active treatment period, and a follow-up period of 4 to 6 weeks ([Figure 16](#)).

Participants were stratified by dialysis type to either hemodialysis, or peritoneal dialysis and by whether their dialysis start was planned or unplanned (urgent). Following stratification, participants were randomized 1:1 to receive daprodustat or darbepoetin alfa. During the treatment period, iron therapy was supplied and administered locally.

The treatment period consisted of:

- The SP, defined as the period from Day 1 to Week 28 during which randomized treatment was dose titrated to achieve the appropriate Hb target of 10.0 to 11.0 g/dL.
- The EP, defined as the period from the end of the stabilization period (Week 28) to Week 52, to assess safety and efficacy. If required, randomized treatment could be dose titrated to achieve or maintain Hb level in the target range.

Figure 16. Study Schematic 201410/ASCEND-ID



* Screening period may be extended by an additional 4 weeks for ultrasound examination, IV iron supplementation and/or vitamin B12 treatment as needed.

Source: Sponsor CSR
Abbreviations: CSR, clinical study report; F/up, follow-up; IV, intravenous

Study Population Inclusion/Exclusion Criteria

Key Inclusion Criteria

- Age (confirmed at screening): 18 to 99 years of age inclusive, male and female (non-pregnant, non-lactating) participants.
- Dialysis: participants were planning to start chronic dialysis within the next 6 weeks (from the date of the screening visit) OR had started and received dialysis (as specified below) for end-stage renal disease for a maximum of ≤ 90 days immediately prior to randomization and were not expected to stop dialysis during the duration of the trial:

- hemodialysis: ≥ 2 times/week.
- Peritoneal Dialysis: ≥ 4 times/week including incremental schedule; participants on continuous ambulatory peritoneal dialysis and automated peritoneal dialysis were eligible.
- Hb concentration as measured by HemoCue (range inclusive): 8.0 to 10.5 g/dL (5.0 to 6.5 mmol/L) at screening and 8.0 to 11.0 g/dL (5.0 to 6.8 mmol/L) at randomization.

Key Exclusion Criteria

- Planned living-related or living-unrelated kidney transplant during the study.
- Ferritin ≤ 100 ng/mL (≤ 100 $\mu\text{g/L}$) at screening or after IV iron supplementation.
- TSAT $\leq 20\%$ at screening or after IV iron supplementation.
- History of bone marrow aplasia or pure red cell aplasia.
- Other causes of anemia.
- Use of any ESA treatment within 8 weeks prior to screening except for limited use as part of dialysis initiation (see the Protocol, Section 5.2 for details).
- MI, acute coronary syndrome, stroke, or transient ischemic attack: ≤ 10 weeks prior to screening through to randomization (Day 1).
- Heart failure.
- Current uncontrolled hypertension.
- QTcB (Day 1): QTcB > 500 msec, or QTcB > 530 msec in participants with bundle branch block.
- Previous or current history of malignancy; unstable liver disease; or gastrointestinal (GI) bleeding.
- Use of strong cytochrome P450 CYP2C8 inhibitors or strong CYP2C8 inducers.
- Any prior treatment with daprodustat for > 30 days.

6.2.4.2. Statistical Analysis Plan

Sample Size Determination

The size of this study was determined based on the following criteria: (1) to be sufficient to meet the International Council for Harmonisation (ICH) E1 guideline for participant exposure, number, and duration; and (2) to provide at least 90% power to test the primary non-inferiority hypothesis with a 2-sided 95% CI.

With a pre-specified non-inferiority margin of -0.75 g/dL and a 2-sided 95% CI, approximately 300 randomized participants (150 per treatment group) were deemed sufficient to provide $> 90\%$ power to test the primary hypothesis and to provide safety data on at least 100 participants exposed to daprodustat for 1 year.

Interim Analysis

The IDMC periodically received unblinded safety reports containing, at a minimum, clinical endpoints (whether adjudicated or pending adjudication) and serious adverse events (SAEs) from an independent statistical data analysis center while the study was ongoing. There were no prospectively defined interim analyses planned to stop the study early for Hb efficacy or futility.

Missing Data

For the analysis of the co-primary efficacy endpoint, a multiple imputation approach was used to handle missing Hb values. Both intermittent missing and monotone missing scheduled Hb data were imputed based on the MAR assumption. However, it is important to examine the sensitivity of inferences to departures from the MAR assumption for the monotone missing data. A tipping point analysis was performed as a sensitivity analysis for the assumptions of missing data, that is, to mimic MNAR in which the missing observations depend on unobserved values.

Primary Efficacy Analysis

The primary Hb efficacy analysis assessed the estimand defined as the comparative treatment effect in mean Hb change between Baseline and EP (i.e., Weeks 28 to 52 inclusive) in all randomized participants i.e., the ITT population. An ANCOVA model adjusted for randomization stratification factors (dialysis type and dialysis start manner), Baseline Hb, and treatment was used. All Hb data (on- and off-treatment) recorded during the EP was included in the primary efficacy analysis, regardless of adherence to randomized treatment including interruptions and discontinuations, the use of non-randomized ESA medication for any reason including rescue therapy, or the use of blood transfusions. Multiple imputation was applied in the case of missing Hb data.

Participants were analyzed according to the treatment to which they were randomized using an ANCOVA model. The analysis tested whether daprodustat was non-inferior to darbepoetin alfa. The non-inferiority margin was pre-defined as -0.75 g/dL, determined based upon a combination of clinical judgment, statistical reasoning, and regulatory guidance for designing non-inferiority trials. Non-inferiority would be established if the lower limit of the 2-sided 95% CI for the treatment difference in mean Hb change between Baseline and EP (daprodustat - darbepoetin alfa) was greater than 0.75 g/dL.

Sensitivity Analyses

- An alternative “while on-treatment” estimand using only evaluable Hb data. Missing Hb data were not imputed.
- PP population analysis based on the on-treatment evaluable Hb analysis.
- A tipping point analysis using all available Hb values (on- and off-treatment) as a sensitivity analysis for the primary estimand. Missing post-baseline Hb values were imputed.
- A tipping point analysis similar to the one described above was performed as a sensitivity analysis for this estimand using evaluable Hb values only.
- An alternative EP analysis from Weeks 28 to 36 using all available Hb values (on and off treatment). Missing post-baseline Hb values were imputed.
- An alternative EP analysis from Weeks 28 to 36 using only evaluable Hb values. Missing Hb data were not imputed.
- A post-hoc analysis, which included all available observed post-randomization hemoglobin values (on and off-treatment), evaluated the mean change in hemoglobin between Baseline to the evaluation period (Week 28 to 52).

Secondary Efficacy Analyses

The planned key secondary efficacy analysis was the on-treatment average monthly IV iron dose (mg)/participant from Baseline to Week 52 excluding IV iron use following a participant's first RBC or whole blood transfusion and was based on the ITT population. An ANCOVA model was used to compare the difference in average monthly IV iron dose per participant between treatment groups, adjusting for: treatment; Baseline monthly IV iron dose; dialysis type; and dialysis start manner. The number and percentage of participants with Baseline IV iron use, on treatment EP IV iron use, and on-treatment IV iron use to Week 52 was summarized by treatment.

The secondary analysis of Hb change from baseline to Week 52 Hb was tested for non-inferiority using the -0.75 g/dL margin used in the primary analysis. A MMRM approach was used with an unstructured covariance matrix to compare the difference in means between groups. The model included factors for treatment, time, prognostic randomization factors (dialysis type and dialysis start manner), Baseline Hb, and the Baseline Hb by time and treatment by time interaction terms. This analysis was performed using all available Hb values (on- and off-treatment) and separately using evaluable Hb values only.

For the analysis of % time Hb in range (10.0 to 11.5 g/dL) during the EP (Weeks 28 to 52), the method by Rosendaal was used to calculate the percentage of time (days) a participant's Hb was below, within and above the Hb analysis range during the EP. Non-inferiority with the margin of -0.75 g/dL was assessed using the Hodges-Lehmann estimate of the median treatment difference and associated 95% CI, comparing the percentage of time in range between treatment groups. Superiority was assessed using the 1-sided p-value from a van Elteren test (stratified Wilcoxon rank sum test). The stratified Mann-Whitney estimator stratified by dialysis type and dialysis start manner, and 95% CI for the probability of daprodustat achieving a greater percentage of time with Hb in the analysis range than darbepoetin alfa provided a supportive treatment comparison estimate for the van Elteren test.

For the Hb responder analysis, responders were participants with a mean Hb during the EP that fell within the Hb analysis range of 10.0 to 11.5 g/dL. A Cochran-Mantel-Haenszel chi-squared test, adjusting for treatment, dialysis type, and dialysis start manner, was used to compare the number and % of responders between the treatment groups.

For the analysis of time to stopping randomized treatment due to meeting rescue criteria, a Cox proportional hazards model, adjusting for treatment, dialysis start type, and dialysis start manner (planned or unplanned) was used to generate the hazard ratio and associated 95% CI for the treatment group comparison. A 1-sided superiority p-value was generated using a Wald test. The incidence rate per 100 PY and associated 95% CI for each treatment group, as well as the absolute rate difference per 100 PY and associated 95% CI were also provided.

Multiple Comparison

The primary endpoint was tested first for non-inferiority, using the lower limit of the 2-sided 95% CI. Conditional on achieving statistical significance (i.e., passing the primary gate by establishing non-inferiority) the single key secondary endpoint was tested for superiority using a 1-sided 2.5% significance level. This 2-step hierarchical strategy was implemented to preserve the study-wise Type I error rate at a 1-sided 2.5% significance level.

The additional secondary/exploratory endpoints were not to be adjusted for multiplicity – a nominal 1-sided 2.5% significance level was applied per test.

Subgroup Analysis

Subgroup analyses, including age, sex, and race were performed for the primary ITT analysis of post-randomization mean change in Hb between Baseline and over the EP (Weeks 28 to 52). Subgroup analysis were performed using all available observed and imputed Hb values (on and off-treatment). Rubin’s rules were used to combine results of the imputed datasets.

6.2.4.3. Results of Analyses

Patient Disposition

Participants were considered to have completed the study if they remained on study to Week 52 and completed the Week 52 visit irrespective of whether they discontinued randomized treatment. Participants were also considered to have completed the study if they died while on study. Of 312 participants in the ITT population, 306 (98%) participants completed the study to Week 52 or died while on study. 2 (1%) participants in the daprodustat group and 4 (3%) participants in the darbepoetin alfa group discontinued randomized treatment and withdrew from the study ([Table 40](#)).

Table 40. Summary of Subject Status and Reasons for Withdrawal for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155	Total N=312
Overall subject status			
Completed	155 (99%)	151 (97%)	306 (98%)
Withdrawn	2 (1%)	4 (3%)	6 (2%)
Primary reason/sub-reason for study withdrawal			
Study closed/terminated	0	0	0
Lost to follow-up	0	1 (<1%)	1 (<1%)
Withdrew consent	2 (1%)	3 (2%)	5 (2%)
Logistical circumstances	0	1 (<1%)	1 (<1%)
Privacy preferences	1 (<1%)	1 (<1%)	2 (<1%)
Adverse experience	0	0	0
Other subject decision	1 (<1%)	1 (<1%)	2 (<1%)
Investigator site closed	0	0	0
Outcome of adverse events which led to study withdrawal			
Nonfatal	0	0	0
Fatal	0	0	0

Source: Sponsor CSR

Abbreviations: CSR, clinical study report

Of the 312 participants in the ITT population, 228 (73%) remained on randomized treatment until Week 52 as scheduled. The proportion of participants who discontinued randomized treatment was similar in the daprodustat group [45 (29%) participants] compared to darbepoetin alfa [39 (25%) participants]. The primary reason for discontinuation was generally similar in both treatment groups except for discontinuation due to an adverse event (AE). More participants in the daprodustat group [19 participants (12%)] discontinued randomized treatment due to an AE than in the darbepoetin alfa group [9 participants (6%)] ([Table 41](#)).

Table 41. Summary of Randomized Treatment Status and Reasons for Discontinuation of Randomized Treatment for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155	Total N=312
Overall study treatment status			
Never received study treatment	0	0	0
Prematurely discontinued study treatment	45 (29%)	39 (25%)	84 (27%)
Did not die while taking study treatment	34 (22%)	33 (21%)	67 (21%)
Died while taking study treatment	11 (7%)	6 (4%)	17 (5%)
Did not prematurely discontinue study treatment	112 (71%)	116 (75%)	228 (73%)
Overall primary reason/subreason for all study treatment discontinuation			
Adverse event	19 (12%)	9 (6%)	28 (9%)
Protocol deviation	0	6 (4%)	6 (2%)
Subject reached protocol-defined stopping criteria	8 (5%)	9 (6%)	17 (5%)
Kidney transplant	2 (1%)	3 (2%)	5 (2%)
Rescue	5 (3%)	5 (3%)	10 (3%)
Pregnancy	0	0	0
Cancer	1 (<1%)	1 (<1%)	2 (<1%)
Subject met liver chemistry stopping criteria	0	0	0
Prohibited medication use	0	0	0
Study closed/terminated	0	0	0
Lost to follow-up	0	1 (<1%)	1 (<1%)

Source: Sponsor CSR

Abbreviations: CSR, clinical study report

Demographic Characteristics and Baseline Characteristics

Baseline demographic characteristics and Hb levels were generally similar between treatment groups for each of the active-controlled dialysis studies.

Baseline demographic characteristics and other baseline characteristics are summarized in [Table 42](#) and [Table 43](#), respectively.

Table 42. Summary of Demographic Characteristics for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155
Age (years), n	157	155
Mean (SD)	53.7 (14.3)	55.8 (15.7)
Median	52.0	56.0
Min, max	22, 84	20, 86
Age category, n (%)	157	155
<65 years	119 (76)	110 (71)
65 - 74 years	22 (14)	28 (18)
≥75 years	16 (10)	17 (11)
Missing	0	0
Gender, n (%)	157	155
Female	61 (39)	57 (37)
Male	96 (61)	98 (63)
Ethnicity, n (%)	157	155
Hispanic or Latino	50 (32)	50 (32)
Not Hispanic or Latino	107 (68)	105 (68)
Missing	0	0

	Daprodustat N=157	Darbepoetin N=155
Race, n (%)	157	155
American Indian or Alaska native	5 (3)	2 (1)
Asian	26 (17)	31 (20)
Black or African American	16 (10)	13 (8)
Native Hawaiian or other Pacific Islander	0	0
White	110 (70)	107 (69)
Mixed race	0	2 (1)
Missing	0	0
Baseline weight (kg)	155	153
Mean (SD)	76.0 (18.9)	77.20 (21.7)
Median	75.0	74.0
Min, max	45.0, 136.8	37.4, 173.2
Baseline weight group, n (%)	157	155
<75 kg	77 (49)	82 (53)
≥75 kg	78 (50)	71 (46)
Missing	2 (1)	2 (1)
Baseline hsCRP	155	152
Mean (SD)	10.3 (23.1)	10.83 (23.1)
Median	2.8	3.7
Min, max	0.1, 173.9	0.2, 163.1

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; hsCRP, high-sensitivity C-reactive protein; SD, standard deviation

Table 43. Summary of Baseline Characteristics for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155
Dialysis type at screening, n (%)	157	155
Hemodialysis conventional	125 (80)	119 (77)
Hemofiltration/hemodiafiltration	1 (<1)	7(5)
Peritoneal dialysis	31 (20)	29 (19)
Baseline Hb (g/dL)	157	155
Mean (SD)	9.5 (1.0)	9.5 (1.0)
Median	9.4	9.5
Min, max	5.9, 12.3	6.4, 14.6
Baseline Hb group, n (%)	157	155
<9 g/dL	54 (34)	41 (26)
≥9 and <10 g/dL	52 (33)	69 (45)
≥10 and ≤11 g/dL	44 (28)	42 (27)
>11 g/dL	7 (4)	3 (2)

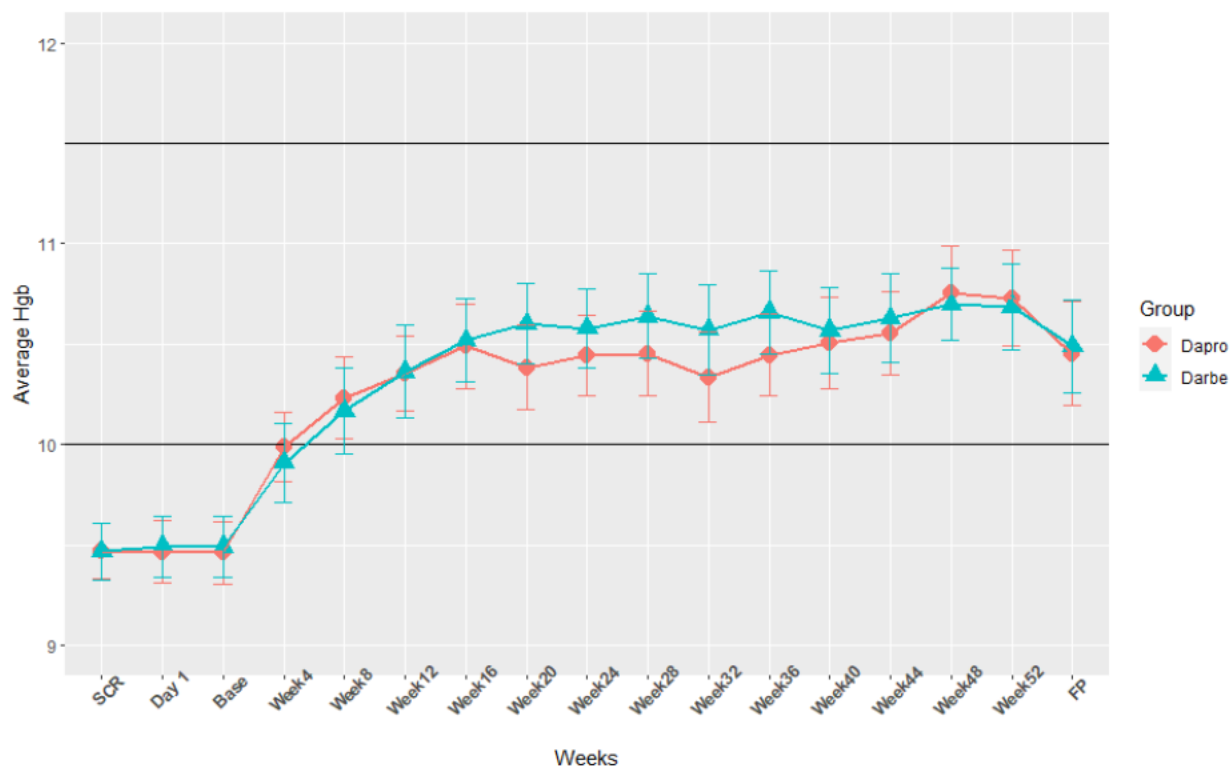
Source: Sponsor CSR

Abbreviations: CSR, clinical study report; Hb, hemoglobin; SD, standard deviation

Efficacy Results—Primary Endpoint

Mean Hb at Baseline and over the EP was similar between treatment groups and remained within the Hb analysis range (10 to 11.5 g/dL) during the EP (Weeks 28 to 52) (Figure 17). Also, mean Hb was below the analysis range at baseline and increased during the first 12 weeks of randomized treatment (daprodustat or darbepoetin alfa). Thereafter Hb remained stable and within the analysis range (10 to 11.5 g/dL) through Week 52.

Figure 17. Line Plot of Post-Randomization Observed Hb Data by Visit for Study 201410/ASCEND-ID



Source: Sponsor CSR

Note: Error bars indicate 95% CI around mean

Baseline and visits on or before Day 1 include only pre-treatment values.

Post-randomization values include all available observed and imputed Hb values (on- and off-treatment).

The dashed vertical lines represent the EP (Week 28 to Week 52).

The horizontal reference lines represent the Hb analysis range (10-11.5 g/dL). The Hb target range for dose changes is 10-11 g/dL.
Abbreviations: CSR, clinical study report; EP, evaluation period; Hb, hemoglobin; Hgb, hemoglobin; SCR, screening; SD, standard deviation FP, Full Period

The lower bound of the 95% CI for the treatment difference of -0.34 was greater than the prospectively defined non-inferiority margin of 0.75 g/dL, thus demonstrating non-inferiority of daprodustat (see [Table 44](#)).

Table 44. Analysis of Post-Randomization Hb Change From Baseline to the EP (Weeks 28 to 52) for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155
Participants with all 7 scheduled Hb values during the EP	108 (69)	114 (74)
Participants with partial scheduled Hb values during the EP	31 (20)	27 (17)
Participants with no observed Hb values during the EP	17 (11)	14 (9)
Number of participants with baseline and EP Hb, n (%) ^a	157 (100)	155 (100)
Baseline Hb (g/dL) mean (SD)	9.46 (1.00)	9.49 (0.97)
EP (Weeks 28-52) Hb (g/dL) mean (SD) ^a	10.49 (0.97)	10.60 (0.92)
Adjusted mean change from baseline (SE) ^b	1.02 (0.09)	1.12 (0.09)
Adjusted mean treatment difference (dapro - Darbe) ^b		-0.10
Two-sided 95% CI for adjusted mean difference ^b		(-0.34, 0.14)

Source: Sponsor CSR

Note: Post-randomization values include on and off treatment values.

^a Hb values during the EP include both observed and imputed values.

^b Based on an ANCOVA model with terms for treatment, baseline Hb, dialysis start manner

Abbreviations: ANCOVA, analysis of covariance; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; EP, evaluation period; Hb, hemoglobin; SD, standard deviation; SE, standard error

Sensitivity Analysis

For the analysis of the Hb co-primary efficacy endpoint in the active controlled studies, a multiple imputation approach was used to handle missing Hb values. Missing scheduled Hb data were imputed based on the MAR assumption. However, it is important to examine the sensitivity of inferences to departures from the MAR assumption for the monotone missing data. A tipping point analysis was performed as a sensitivity analysis for the assumptions of missing data, that is, to mimic MNAR in which the missing observations depend on unobserved values.

Results of the tipping point analysis suggest that the extent of missing data would unlikely impact the conclusions of the Hb co-primary efficacy analysis. Therefore, missing data in these studies are unlikely to have had an impact on the primary results.

Efficacy Results—Key Secondary Endpoints

Daprodustat did not demonstrate a statistically significant reduction in average monthly IV iron dose to Week 52 compared to darbepoetin alfa (see [Table 45](#)).

Table 45. Analysis of On-Treatment Average Monthly IV Iron Dose During Day 1 to Week 52 for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155
Number of participants during day 1 to Week 52 (%)	156 (99)	154 (99)
Baseline monthly IV iron dose (mg) mean (SD)	160 (270)	181 (210)
Average monthly IV iron dose (mg) during Day 1 to Week 52 mean (SD)	142 (161)	128 (137)
Adjusted mean change from baseline (SE)	145 (11)	125 (11)
Adjusted mean treatment difference (dapro - darbe)		19
Two-sided 95% CI for adjusted mean difference		(-11, 50)
One-sided p-value ^a		0.89

Source: Sponsor CSR

Note: Iron data after a participant has received an RBC or whole blood transfusion is excluded from the analysis

^a Based on an ANCOVA model with terms for treatment, baseline monthly IV iron dose, dialysis; One-sided p-value based on test of null hypothesis: (daprodustat - Darbe) ≥ 0 vs alternative: difference < 0 .

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; IV, intravenous; RBC, red blood cell; SD, standard deviation; SE, standard error

Efficacy Results—Secondary Endpoints

Because of the importance of patients not spending undue time with suboptimal Hb levels, a supportive analysis was performed for the primary ITT analysis of post-randomization mean change in Hb between Baseline and over the EP, repeating the analysis using a shorter EP (Week 28 to 36). For each study the lower bound of the 95% CI for the treatment difference was greater than the prospectively defined non-inferiority margin of -0.75 g/dL, thus supporting the overall non-inferiority results of daprodustat (see [Table 46](#)). Results were consistent with the conclusion from the primary analysis.

Table 46. Analysis of Post-Randomization Hb Change From Baseline to the Alternative EP (Weeks 28 to 36) for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155
Number of participants with baseline and EP Hb, n (%) ^a	157 (100)	155 (100)
Baseline Hb (g/dL) mean (SD)	9.46 (1.00)	9.49 (0.97)
EP (Weeks 28-36) Hb (g/dL) mean (SD) ^a	10.38 (1.10)	10.62 (1.09)
Adjusted mean change from Baseline (SE) ^b	0.91 (0.10)	1.14 (0.10)
Adjusted mean treatment difference (dapro - rhEPO) ^b		-0.23
Two-sided 95% CI for adjusted mean difference ^b		(-0.50, -0.04)

Source: Sponsor CSR

^a Hb values during the EP include both observed and imputed values.

^b Based on an ANCOVA model with terms for treatment, baseline Hb, dialysis start manner (201410/ASCEND-ID)

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; EP, evaluation period; Hb, hemoglobin; rhEPO, recombinant human erythropoietin; SD, standard deviation; SE, standard error

Additional primary endpoint supportive analyses included an on-treatment analysis using only evaluable Hb values, a PP Population analysis using only evaluable Hb values, a shorter EP (Weeks 28 to 36) analysis using only evaluable Hb values, and a post-randomization (on- and off-treatment) observed case analysis. Results were consistent with the non-inferiority conclusion from the primary analysis (see [Table 47](#)).

Table 47. Primary Endpoint Hb Supportive Analyses for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155
Evaluable Hb (Weeks 28 to 52), ITT population		
Number of participants with baseline and EP Hb, n (%) ^a	133 (85)	133 (86)
Adjusted mean change from baseline (SE) ^b	0.97 (0.08)	1.17 (0.08)
Adjusted mean treatment difference (dapro - darbe) ^b		-0.20
Two-sided 95% CI for adjusted mean difference		(-0.43, 0.04)
Evaluable Hb (Weeks 28 to 52), PP population		
Number of participants with baseline and EP Hb, n (%) ^a	77 (100)	101 (100)
Adjusted mean change from baseline (SE) ^b	1.14 (0.1)	1.26 (0.09)
Adjusted mean treatment difference (dapro - darbe) ^b		-0.12
Two-sided 95% CI for adjusted mean difference ^b		(-0.38, 0.14)
Evaluable Hb Alternative EP (Weeks 28 to 36), ITT population		
Number of participants with baseline and EP Hb, n (%) ^a	132 (84)	131 (85)
Adjusted mean change from baseline (SE) ^b	0.92 (0.09)	1.21 (0.09)
Adjusted mean treatment difference (dapro - darbe) ^b		-0.28
Two-sided 95% CI for adjusted mean difference ^b		(-0.54, -0.02)

	Daprodustat N=157	Darbepoetin N=155
Post-randomization Hb (observed cases only), ITT population		
Number of participants with baseline and EP Hb, n (%)	140 (89)	141 (91)
Adjusted mean change from baseline (SE) ^b	0.98 (0.09)	1.10 (0.09)
Adjusted mean treatment difference (dapro - darbe) ^b		-0.13
Two-sided 95% CI for adjusted mean difference ^b		(-0.37, 0.12)

Source: Sponsor CSR

^a Hb values during the EP include observed values only.

^b Based on an ANCOVA model with terms for treatment, baseline Hb, dialysis start manner (201410/ASCEND-ID).

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; EP, evaluation period; Hb, hemoglobin; ITT, intent-to-treat; SE, standard error

The proportion of participants with mean Hb within the analysis range during the EP was similar between the treatment groups (Table 48).

Table 48. Participants With Mean EP Hb Below, Within and Above the Analysis Range Using Evaluable Hb Values for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155
Participants with evaluable Hb during the EP	133	133
Mean EP Hb above 11.5 g/dL	34 (26)	28 (21)
Mean EP Hb within 10.0-11.5 g/dL	86 (65)	87 (65)
Mean EP Hb below 10.0 g/dL	13 (10)	18 (14)

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; EP, evaluation period; Hb, hemoglobin

The proportion of participants stopping randomized treatment due to meeting rescue criteria was small and similar between the treatment groups. Daprodustat did not reduce the risk of stopping randomized treatment due to meeting rescue criteria (Table 49).

The cumulative incidence for time to stopping randomized treatment due to meeting rescue criteria was similar between the treatment groups.

Table 49. Analysis of Time to Permanently Stopping Randomized Treatment Due to Meeting Rescue Criteria for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155
Number of participants	157 (100)	155 (100)
Permanently stopping randomized treatment due to meeting rescue criteria	5 (3)	5 (3)
Censored	152 (97)	150 (97)
Incidence rate per 100 PY (two-sided 95% CI)	3.73 (1.21,8.71)	3.62 (1.17,8.44)
Hazard ratio		
Estimate ^a		1.06
Two-sided 95% CI for hazard ratio		(0.31, 3.66)
One-sided p-value ^b		0.53
Absolute rate difference per 100 PY (two-sided 95% CI) ^c	0.11 (-4.44, 4.67)	

Source: Sponsor CSR

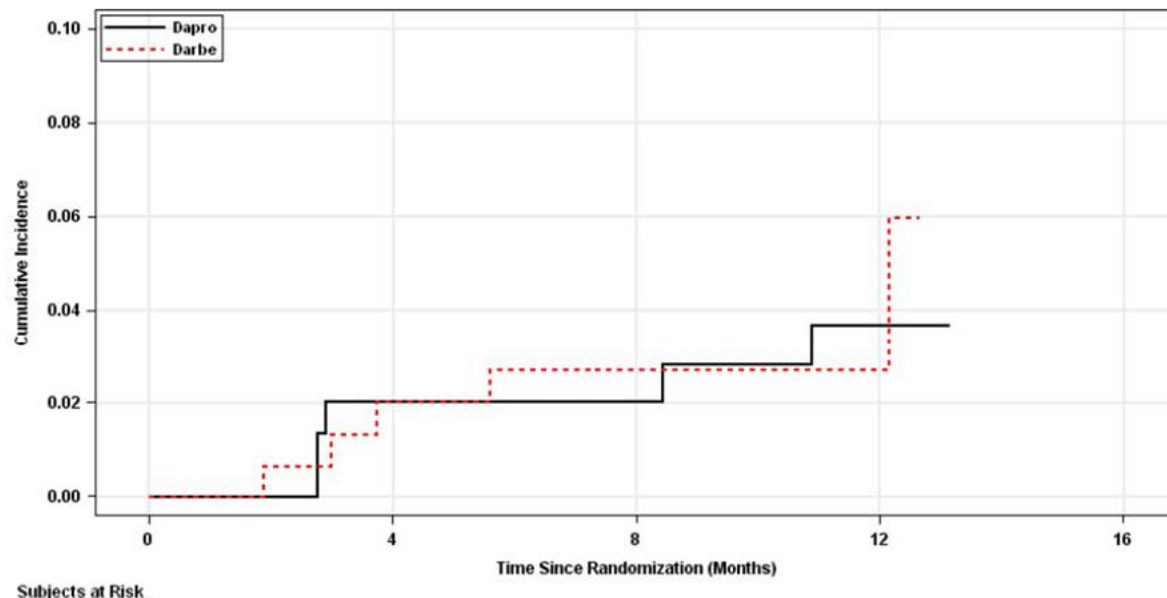
^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, dialysis start manner, and dialysis type. A hazard ratio <1 indicates a lower risk with daprodustat compared to rhEPO.

^b One-sided p-value based on Wald test of null hypothesis: (daprodustat/rhEPO) ≥1 vs alternative: ratio <1.

^c A rate difference <0 indicates a lower risk with daprodustat compared to rhEPO.

Abbreviations: CI, confidence interval; CSR, clinical study report; PY, person-year; rhEPO, recombinant human erythropoietin

Figure 18. Kaplan-Meier Plot Time to Stopping Randomized Treatment Due to Meeting Rescue Criteria for Study 201410/ASCEND-ID



Subjects at Risk

	0	4	8	12	16
Dapro	157	141	125	39	0
Darbe	155	144	131	43	0

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa

The proportion of participants with a first occurrence of RBC or whole blood transfusion during the on-treatment time period was similar in the daprodustat and darbepoetin alfa groups. Participants on daprodustat had a numerically lower risk for time to first occurrence of receiving an on-treatment RBC or whole blood transfusion compared to darbepoetin alfa, but the reduction in risk was not statistically significant (Table 50).

Table 50. Analysis of Time to First Occurrence of On-Treatment RBC or Whole Blood Transfusion for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155
Number of participants	157	155
First occurrence of on-treatment RBC or whole blood transfusion, n (%)	18 (11.5)	21 (13.5)
Censored, n (%)	139 (88.5)	134 (86.5)
Incidence rate per 100 PY (two-sided 95% CI)	13.91 (8.24, 21.98)	15.94 (9.87, 24.37)
Hazard ratio		
Estimate ^a		0.88
Two-sided 95% CI for hazard ratio		(0.47, 1.66)
One-sided p-value ^b		0.35
Absolute rate difference per 100 PY (two-sided 95% CI) ^c		-2.03 (-11.40, 7.34)

Source: Sponsor CSR

^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, dialysis start manner

^b One-sided p-value based on Wald test of null hypothesis: (daprodustat/darbepoetin) ≥ 1 vs alternative: ratio < 1 .

^c A rate difference < 0 indicates a lower risk with daprodustat compared to darbepoetin.

Abbreviations: CI, confidence interval; CSR, clinical study report; PY, person-year; RBC, red blood cell

Blood transfusion events to Week 52 are summarized in [Table 51](#). The proportion of participants who received RBC or whole blood transfusions was similar between the treatment groups.

Table 51. On-treatment Blood Transfusions by Week 52 for Study 201410/ASCEND-ID

	Daprodustat N=157	Darbepoetin N=155
Number of participants who received randomized treatment	157	155
Number of transfusion events per participant, n (%)		
0	139 (89)	134 (86)
1	17 (11)	17 (11)
2	1 (<1)	0
3	0	3 (2)
4	0	0
5	0	0
>5	0	1 (<1)
Number (%) of participants with transfusion events [number of transfusion events] a	18 (11) [19]	21 (14) [32]
Total treatment exposure (years)	134.0	138.2
Overall transfusion events per 100 PY	14.2	23.1
Overall transfusion per 100 PY	22.4	36.9
Overall units per 100 PY	38.8	48.5

Source: Sponsor CSR

Note: Blood transfusions include RBC and whole blood transfusions.

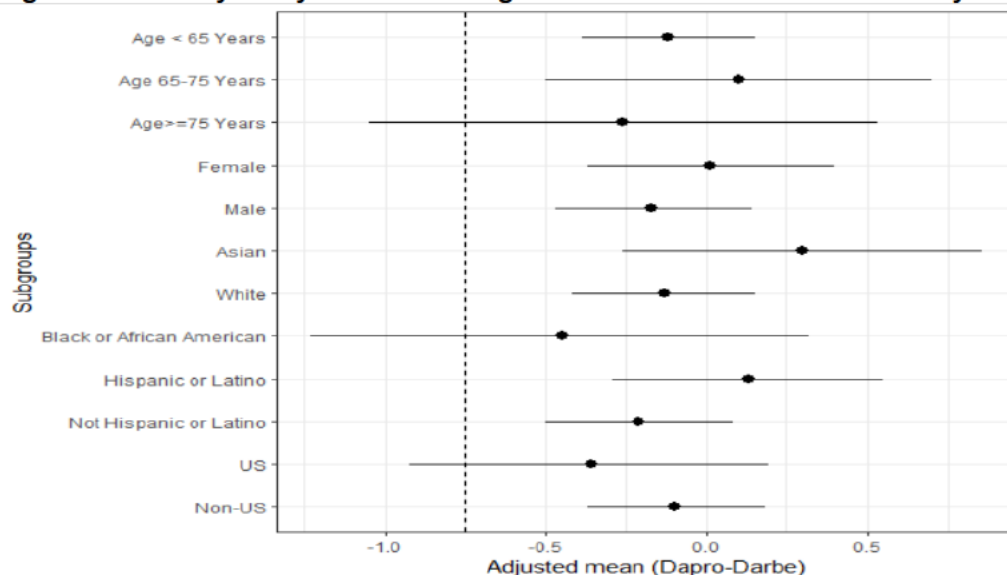
^a RBC and whole blood transfusion events are defined by grouping together transfusion records if the transfusion start/end dates match with or are contained within an Admission/Discharge period. Transfusion records with no matching admission/discharge period are considered to be the same Transfusion Event if the gap between transfusions is 5 days or less.

Abbreviations: CSR, clinical study report; PY, person-year; RBC, red blood cell

Efficacy Results—Other Subgroups

Overall, the results of the subgroup analyses were consistent with the primary analysis with little or no heterogeneity between subgroups. The adjusted mean differences were consistently > -0.75 for most of the subgroups except for Age ≥ 75 and Black or African American (see [Figure 19](#)). Because of small sample sizes in these subgroups, which is reflected by their very large confidence intervals, results should be interpreted with caution.

Figure 19. Primary Analysis of Hb Change From Baseline to the EP for Study 201410/ASCEND-ID



Source: FDA reviewer
Abbreviations: Dapro, daprodustat; Darbe, darbepoetin alfa; EP, evaluation period; Hb, hemoglobin; US, United States

6.2.5. Study 205270/ASCEND NHQ

6.2.5.1. Design

Overview

The Applicant conducted a randomized, Phase 3 Study, 205270/ASCEND NHQ, titled “A 28-week, randomized, double-blind, placebo-controlled, parallel-group, multi-center, study in recombinant human erythropoietin (rhEPO) naïve non-dialysis participants with anemia associated with chronic kidney disease to evaluate the efficacy, safety, and effects on quality of life of daprodustat compared to placebo.”

Primary Efficacy Endpoint

Mean change in Hb between Baseline and EP (Weeks 24 to 28)

Key Secondary Efficacy Endpoints

% of participants having a Hb increase of ≥ 1.0 g/dL from Baseline to EP (Weeks 24 to 28)
Mean change in SF-36 v2.0 Vitality domain between Baseline and Week 28

Secondary Efficacy Endpoints

- Hb change from Baseline to Week 28
- N (%) responders, defined as mean Hb within the Hb analysis range 11 to 12 g/dL during EP
- % time Hb in analysis range during the EP
- Time to stopping randomized treatment due to meeting rescue criteria

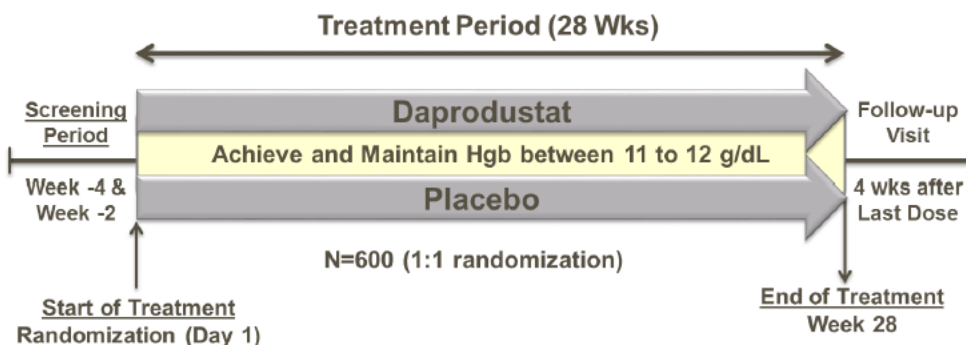
- Mean change from baseline by domain and overall symptom score on the Chronic Kidney Disease-Anemia (CKD-AQ) symptom questionnaire
- Change from baseline in the Patient Global Impression of Severity (PGI-S)

Study Design

This was a 28-week, randomized, double-blind, placebo-controlled, parallel-group, multi-center Phase 3 study in participants with anemia associated with CKD who were not on dialysis. This study enrolled participants who had a Hb of 8.5 to 10.0 g/dL, inclusive, as measured by a point-of-care Hb analyzer (HemoCue). These participants had limited history of IV iron administration and were rhEPO naïve prior to Screening and Randomization.

The study included a 4-week Screening period, a 28-week Treatment period, and a 4-week Follow-up period ([Figure 20](#)). The total duration of study participation for each participant was approximately 36 weeks. Rescreening was allowed up to 2 additional times.

Figure 20. Study Schematic (Study 205270/ASCEND-NHQ)



Source: Sponsor's CSR
Abbreviations: CSR, clinical study report; Hgb, hemoglobin

Participants were randomized 1:1 to receive either daprodustat or matching placebo tablets. Daprodustat and placebo doses for participants were titrated based upon HemoCue Hb. Dose modifications for these participants followed a protocol-specified dose adjustment algorithm to achieve and maintain Hb within the target range of 11.0 to 12.0 g/dL, inclusive. Dose changes were made programmatically in a blinded fashion by the IRT system in order to ensure that investigators and participants stayed blinded to the study treatment and the dose adjustments.

A rescue algorithm was provided to minimize participants having an inadequate response to the treatment for their anemia for an extended period of time and to enable rescue therapy to be provided to the participants based on local clinical practice ([Section 16.4](#)).

A Clinical Events Committee adjudicated any event suspected to be MACE.

Study Population

Participants were eligible for inclusion in this study only if all of the protocol-specified criteria applied at Screening (Week -4 and Week -2) and Randomization (Day 1), unless otherwise specified. Key inclusion and exclusion criteria are summarized below.

Key Inclusion and Exclusion Criteria

Inclusion Criteria

- Male or female participants ≥ 18 years of age, at the time of signing the informed consent.
- CKD confirmed at Screening: Kidney Disease Outcomes Quality Initiative (KDOQI) CKD stages 3, 4, or 5 defined by estimated glomerular filtration rate (eGFR) using the CKD Epidemiology Collaboration (CKD-EPI) formula.
- Stable HemoCue Hb from 8.5 to 10.5, inclusive, at the Screening visit (Week -4) and from 8.5 to 10.0 g/dL, inclusive, at Randomization (Day 1).
- Treatment with up to one IV iron dose within the 8 weeks prior to Screening and no IV iron use between the Screening visit and Randomization (Day 1). If needed, stable oral iron supplementation was permitted ($< 50\%$ change in overall dose maintenance and no change in type of iron prescribed doses in the 4 weeks prior to Day 1 Randomization visit).

Exclusion Criteria

- Dialysis or clinical evidence of impending need to initiate dialysis within 180 days after Randomization (Day 1).
- Planned living-related or living-unrelated kidney transplant within 28 weeks after Randomization (Day 1).
- Low values of ferritin or transferrin saturation (TSAT) at Screening.
- History of rhEPO or rhEPO analogue use within the 8 weeks prior to Screening and rhEPO use between Screening and Randomization (Day 1).
- History of transfusion within the 8 weeks prior to Screening and transfusion between Screening and Randomization (Day 1).
- History of bone marrow aplasia or pure red cell aplasia.
- Megaloblastic anemia (untreated pernicious anemia and folate deficiency), thalassemia major, sickle cell disease, or myelodysplastic syndrome.
- Evidence of actively bleeding gastric, duodenal, or esophageal ulcer disease or clinically significant GI bleeding ≤ 8 weeks prior to Screening through Randomization (Day 1).
- QTcB > 500 msec or QTcB > 530 msec in participants with Bundle Branch Block. There was no QTc exclusion for participants with a predominantly paced rhythm.
- For participants residing in Italy, QTcB exclusion criteria were replaced with QTcF criteria: QTcF > 470 ms (females) and > 450 ms (males) pre-dose prior to Randomization (Day 1) and presence of second or third degree AV block.
- Malignancy within 2 years of Screening through Randomization (Day 1), currently receiving treatment for cancer, or had a known complex kidney cyst > 3 cm.
- Any prior treatment with daprodustat for > 30 days.
- Ferric citrate use within 4 weeks prior to Randomization (Day 1).
- Significant CV or hepatic disease.

6.2.5.2. Statistical Analysis Plan

Approximately 600 participants were planned to be randomized (300 per arm) to receive daprodustat or placebo, who would be treated to achieve and maintain Hb between 11 and

12 g/dL. The primary efficacy endpoint was the mean change in Hb between baseline and over the EP (mean of all available [on and off treatment] Hb values for Week 24 through Week 28, inclusive, including any unscheduled Hb values that were taken during this time period). For the primary endpoint, the expected difference in mean Hb change from baseline and the EP, between arms, was 1.0 g/dL and the anticipated between participant standard deviation was 1.5 g/dL, based on historical rhEPO trials and daprodustat clinical trial experience to date. For additional details on the statistical analysis plan refer to Section [16.1.3](#).

6.2.5.3. Results of Analyses

Patient Disposition

Participants were considered to have completed the study if they completed the 28 Week treatment period and the follow-up visit irrespective of whether they discontinued randomized treatment. Participants were also considered to have completed the study if they died while on study. Of 614 participants in the ITT population, 590 (96%) participants completed the study to Week 28 or died while on study. Seven participants (2%) in the daprodustat group and 17 participants (6%) in the placebo group discontinued randomized treatment and withdrew from the study ([Table 52](#)).

Table 52. Summary of Subject Status and Reasons for Study Withdrawal (Study 205270/ASCEND-NHQ)

	Placebo N=307	Daprodustat N=307	Total N=614
Overall subject status			
Never treated	0	0	0
Completed	290 (94%)	300 (98%)	590 (96%)
Ongoing	0	0	0
Withdrawn	17 (6%)	7 (2%)	24 (4%)
Primary reason/subreason for study withdrawal			
Adverse event	5 (2%)	3 (<1%)	8 (1%)
Protocol deviation	0	0	0
Study closed/terminated	0	0	0
Lost to follow-up	7 (2%)	2 (<1%)	9 (1%)
Investigator site closed	0	0	0
Investigator discretion	1 (<1%)	0	1 (<1%)
Withdrew consent	4 (1%)	2 (<1%)	6 (<1%)
Logistical circumstances	0	0	0
Privacy preferences	0	0	0
Adverse experience	0	0	0
Other subject decision	4 (1%)	2 (<1%)	6 (<1%)
Outcome of adverse events which led to study withdrawal			
Nonfatal	5 (2%)	3 (<1%)	8 (1%)
Fatal	0	0	0

Source: Sponsor CSR
Abbreviations: CSR, clinical study report

Of the 614 participants in the ITT population, 465 (76%) completed randomized treatment. The proportion of participants who discontinued randomized treatment was lower in the daprodustat group [53 (17%) participants] compared to placebo [96 (31%) participants]. The primary reasons for discontinuation were generally similar in both treatment groups with the exceptions of

‘participant reached protocol-defined stopping criteria (rescue)’ and ‘decision by proxy’ which were higher in the placebo group (Table 53).

Table 53. Summary of Subject Status and Reasons for Discontinuation of Study Treatment (Study 205270/ASCEND-NHQ)

	Placebo N=307	Daprodustat N=307	Total N=614
Overall study treatment status			
Never received study treatment	0	0	0
Prematurely discontinued study treatment	96 (31%)	53 (17%)	149 (24%)
Did not die while taking study treatment	94 (31%)	53 (17%)	147 (24%)
Died while taking study treatment	2 (<1%)	0	2 (<1%)
Did not prematurely discontinue study treatment	211 (69%)	254 (83%)	465 (76%)
Overall Primary reason/subreason for all study treatment discontinuation:			
Adverse event	24 (8%)	22 (7%)	46 (7%)
Protocol deviation	1 (<1%)	0	1 (<1%)
Subject reached protocol-defined stopping criteria	41 (13%)	14 (5%)	55 (9%)
Subject had renal transplant	0	1 (<1%)	1 (<1%)
Transition to dialysis	11 (4%)	9 (3%)	20 (3%)
Rescue	26 (8%)	2 (<1%)	28 (5%)
Pregnancy	0	0	0
Cancer event	1 (<1%)	0	1 (<1%)
Need for chronic use of prohibited medication	0	1 (<1%)	1 (<1%)
Multiple	2 (<1%)	1 (<1%)	3 (<1%)
Study closed/terminated	0	0	0
Lost to follow-up	1 (<1%)	1 (<1%)	2 (<1%)
Investigator discretion	4 (1%)	6 (2%)	10 (2%)
Decision by subject or proxy	25 (8%)	10 (3%)	35 (6%)
Investigator site closed	0	0	0
Overall primary reason/subreason for study treatment discontinuation for subjects that did not die while taking study treatment:			
Adverse event	22 (7%)	22 (7%)	44 (7%)

Source: Sponsor CSR
Abbreviations: CSR, clinical study report

Table 54 shows the distribution of missing Hb measures over time, which was higher in the placebo arm as compared to the daprodustat arm, especially after Week 4.

Table 54. Distribution of Patients With Missing Hb Measures Over Time (Study 205270/ASCEND-NHQ)

	Placebo N=307	Daprodustat N=307
Baseline	0	0
Week 2	9 (3%)	9 (2.7%)
Week 4	17 (5.5%)	13 (4.3%)
Week 8	37 (12%)	20 (6.5%)
Week 12	51 (16.6%)	27 (8.8%)
Week 16	60 (20%)	33 (10.7%)
Week 20	75 (24.4%)	40 (13%)
Week 24	85 (27.7%)	50 (16.2%)
Week 28	92 (30%)	60 (20%)

Source: FDA reviewer

Demographic Characteristics and Baseline Characteristics

Baseline demographic characteristics, renal characteristics, Hb levels, CV and diabetes characteristics, blood pressure values, and markers of iron metabolism were generally similar between treatment groups.

Baseline demographic characteristics and Baseline renal function and Hb levels are summarized in [Table 55](#) and [Table 56](#), respectively.

Table 55. Summary of Demographic Characteristics (Study 205270/ASCEND-NHQ)

	Placebo N=307	Daprodustat N=307
Age (years), n	307	307
Mean (SD)	66.6 (12.9)	65.3 (13.4)
Median	67	66
Min to max	22 to 91	23 to 89
Age category, n (%)	307	307
<65 years	121 (39)	135 (44)
65-<75 years	96 (31)	82 (27)
≥75 years	90 (29)	90 (29)
Gender, n (%)	307	307
Female	178 (58)	176 (57)
Male	129 (42)	131 (43)
Ethnicity, n (%)	307	307
Hispanic or Latino	103 (34)	104 (34)
Not Hispanic or Latino	204 (66)	203 (66)
Race, n (%)	307	307
Black or African American	47 (15)	44 (14)
American Indian or Alaska native	34 (11)	34 (11)
Asian	28 (9)	30 (10)
Native Hawaiian or other Pacific Islander	1 (<1)	0
White	195 (64)	197 (64)
Mixed race	2 (<1)	2 (<1)
Baseline weight (kg), n (%)	307	307
<75 kg	150 (49)	157 (51)
≥75 kg	157 (51)	150 (49)
Baseline hsCRP (mg/L), n	300	302
Mean (SD)	7.0 (12.6)	7.2 (14.8)
Median	2.8	2.3
Min to max	0.2 to 126.4	0.2 to 118.3

Source: Sponsor CSR

Abbreviations: CSR, clinical study report; hsCRP, high-sensitivity C-reactive protein; SD, standard deviation

Table 56. Summary of Baseline Renal Function and Hb Levels (Study 205270/ASCEND-NHQ, ITT Population)

	Placebo N=307	Daprodustat N=307
Baseline CKD stage		
Stage 2 (eGFR ≥60<90 mL/min/1.73m ²)	2 (<1)	3 (<1)
Stage 3 (eGFR 30-<60 mL/min/1.73m ²)	87 (28)	92 (30)
Stage 4 (eGFR 15-<30 mL/min/1.73m ²)	137 (45)	139 (45)
Stage 5 (eGFR <15 mL/min/1.73m ²)	81 (26)	73 (24)

	Placebo N=307	Daprodustat N=307
Baseline Hb (g/dL)	307	307
Mean (SD)	9.7 (0.7)	9.7 (0.6)
Median	9.7	9.8
Min to max	7.9 to 14.2	6.9 to 12.1
Baseline Hb group 1, n (%)	307	307
<9 g/dL	46 (15)	28 (9)
≥9 and <10 g/dL	151 (49)	168 (55)
≥10 and ≤11 g/dL	99 (32)	106 (35)
>11 g/dL	11 (4)	5 (2)
Baseline Hb group 2, n (%)	307	307
<8.5 g/dL	5 (2)	5 (2)
≥8.5 and <9 g/dL	41 (13)	23 (7)
≥9 and ≤10 g/dL	172 (56)	194 (63)
>10 g/dL	89 (29)	85 (28)

Source: Sponsor CSR

Group1: Hb subgroup categories in which the Hb target range was 10-11 g/dL.

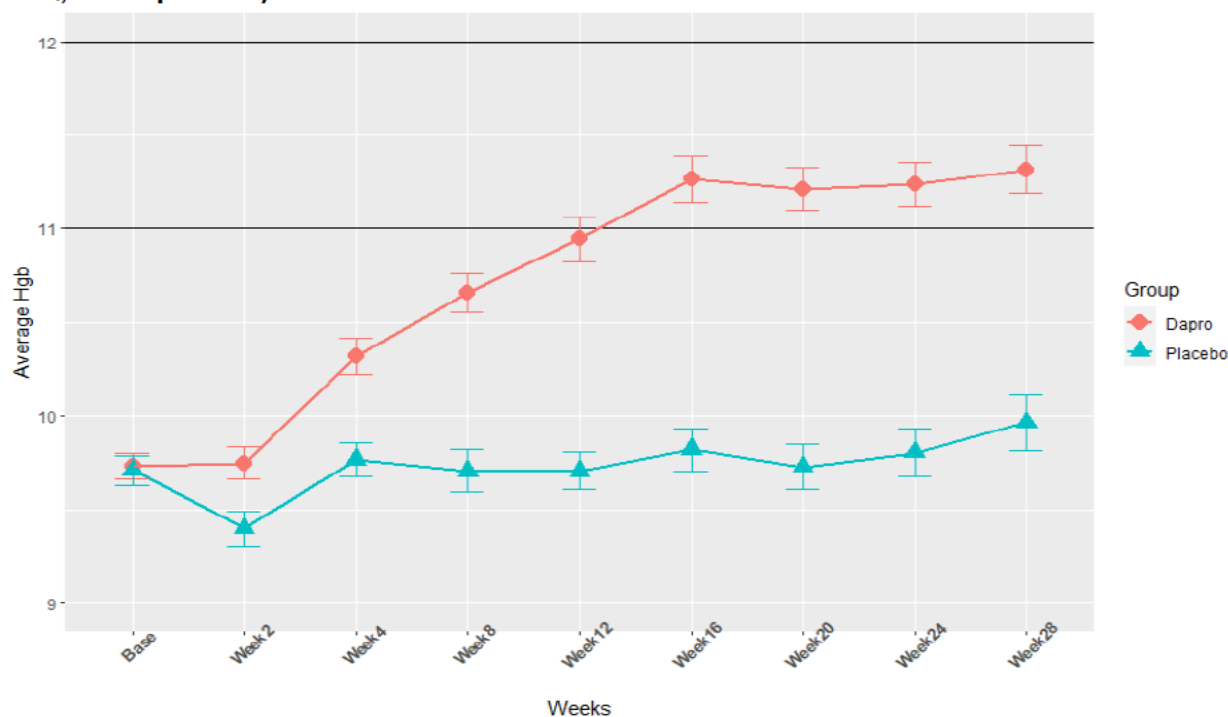
Group2: Hb subgroup categories in which the Hb target range was 11-12 g/dL.

Abbreviations: CKD, chronic kidney disease; CSR, clinical study report; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; ITT, intent-to-treat; SD, standard deviation

Efficacy Results—Primary Endpoint

Mean Baseline Hb was similar between the treatment groups (See [Figure 21](#)). In the daprodustat group, increases in mean Hb were observed within 4 weeks of initiating treatment and continued through Week 28; mean Hb values were in the Hb analysis range (11 to 12 g/dL) during the EP (Week 24 to Week 28). Numerically small changes in mean Hb were seen in the placebo group from Baseline through Week 28 and mean Hb remained below the analysis range.

Figure 21. Line Plot of Post-Randomization Observed Hb Data by Visit 28 (Study 205270/ASCEND-NHQ, ITT Population)



Source: Sponsor CSR

Note: Error bars indicate 95% CI; Baseline and visits on or before Day 1 include only pre-treatment values; The dashed vertical lines represent the EP (Week 24 to Week 28); Post-randomization values include all available observed and imputed Hb values; The horizontal reference lines represent the Hb analysis and target range (10-12 g/dL).

Abbreviations: CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; EP, evaluation period; Hb, hemoglobin; Hgb, hemoglobin; ITT, intent-to-treat

For the primary ITT analysis of mean change in Hb between Baseline and over the EP (mean over Weeks 24 to 28) daprodustat was superior to placebo. The adjusted mean treatment difference (daprodustat - placebo) was 1.40 g/dL (95% CI: 1.23, 1.56; one-sided p value <0.0001) (see [Table 57](#)).

Table 57. Primary Analysis of Post-Randomization Hb Change From Baseline to the EP (Weeks 24 to 28) for Study 205270/ASCEND-NHQ; ITT Population

	Placebo N=307	Daprodustat N=307
Participants with all scheduled Hb values during the EP	239 (78)	259 (84)
Participants with partial scheduled Hb values during the EP	27 (9)	12 (4)
Participants with no observed Hb values during the EP	41 (13)	36 (12)
Participants with Baseline and EP Hb ^a , n (%)	307 (100)	307 (100)
Baseline Hb (g/dL) mean (SD)	9.71 (0.73)	9.73 (0.64)
EP (Weeks 24-28) Hb (g/dL) mean (SD) ^a	9.91 (0.95)	11.31 (0.88)

	Placebo N=307	Daprodustat N=307
Adjusted mean change from baseline (SE) ^b	0.19 (0.06)	1.58 (0.06)
Adjusted mean treatment difference (dapro-placebo) ^b		1.40
Two-sided 95% CI for adjusted mean difference ^b		1.23, 1.56
One-sided superiority p-value ^{b,c}		<0.0001

Source: Sponsor CSR

Note: Post-randomization Hb measurements (which includes on and off treatment values) are included in this display.

^a Hb values during the EP include both observed and imputed values

^b Based on an ANCOVA model with terms for treatment, baseline Hb, and region. One-sided p-value based on test of null hypothesis: (daprodustat - placebo) ≤ 0 vs alternative: difference > 0.

^c Superiority for the difference in the primary Hb endpoint between the daprodustat and placebo treatment groups was established if the one-sided p-value is less than 0.025.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; EP, evaluation period; Hb, hemoglobin; ITT, intent-to-treat; SD, standard deviation; SE, standard error

Supportive analyses were conducted without imputation for missing Hb data, which included an on-treatment analysis using only evaluable Hb values and a post-randomization (on- and off-treatment) observed case analysis. Results were consistent with the superiority conclusion from the primary analysis (p value <0.0001) (see [Table 58](#)).

Table 58. Primary Endpoint Hb Without Imputation for Study 205270/ASCEND-NHQ (ITT Population)

	Placebo N=307	Daprodustat N=307
Evaluable Hb (Weeks 24 to 28), ITT population		
Participants with baseline and EP Hb ^a , n (%)	217 (71)	252 (82)
Adjusted mean change from baseline (SE) ^b	0.08 (0.06)	1.55 (0.06)
Adjusted mean treatment difference (dapro-placebo) ^b		1.47
Two-sided 95% CI for adjusted mean difference ^b		1.32, 1.63
One-sided superiority p-value ^{b,c}		<0.0001
Post-randomization Hb (observed cases only), ITT population		
Participants with baseline and EP Hb ^a , n (%)	266 (87)	272 (89)
Adjusted mean change from baseline (SE) ^b	0.12 (0.06)	1.52 (0.06)
Adjusted mean treatment difference (dapro-placebo) ^b		1.40
Two-sided 95% CI for adjusted mean difference ^b		1.23, 1.57
One-sided superiority p-value ^{b,c}		<0.0001

Source: Sponsor CSR

Note: Evaluable values are on-treatment values that are not taken within the 8 weeks following a red blood cell or whole blood transfusion or a post-randomization non-randomized ESA treatment.

Note: Post-randomization Hb measurements include on and off treatment values.

^a Hb values during the EP include observed values only.

^b Based on an ANCOVA model with terms for treatment, baseline Hb, and region. One-sided p-value based on test of null hypothesis: (daprodustat - placebo) ≤ 0 vs alternative: difference > 0.

^c Superiority for the difference in the primary Hb endpoint between the daprodustat and placebo treatment groups was established if the one-sided p-value is less than 0.025.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; EP, evaluation period; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; ITT, intent-to-treat; SE, standard error

Based on the disposition distribution, the placebo arm had higher dropout as compared to the treatment arm. Thus, we were concerned that the MAR might not be valid. To study the robustness of the primary efficacy results, we requested that the Applicant perform a tipping point analysis. This analysis showed that the extent of missing data is unlikely to have had an impact on the conclusions of the primary Hb analysis and SF-36 v2.0 Vitality domain analysis.

Efficacy Results—Key Secondary Endpoints

The analysis of proportion of participants with a ≥ 1 g/dL increase in Hb between Baseline and over the EP (Weeks 24 to 28) showed that daprodustat was superior to placebo. The difference in response rate (daprodustat-placebo) was 0.56 (95% CI: 0.49, 0.63; one sided $p < 0.0001$) ([Table 59](#)).

Table 59. Analysis of Participants With Post-Randomization Hb Increase of ≥ 1.0 g/dL From Baseline to the EP for Study 205270/ASCEND-NHQ (ITT Population)

	Placebo N=307	Daprodustat N=307
Participants with baseline and EP Hb ^a , n (%)	307 (100)	307 (100)
Participants with Hb increase of ≥ 1.0 g/dL from baseline to EP ^a , n (%)	54 (18)	235 (77)
Difference in response rate (dapro-placebo)		0.56
Two-sided 95% CI for difference in response rate		0.49, 0.63
One-sided p-value ^{b,c}		<0.0001

Source: Sponsor CSR

Note: Post-randomization values include on and off treatment values.

^a Hb values during the EP include both observed and imputed values

^b Treatment group comparisons are based on a Cochran-Mantel-Haenszel test adjusted for treatment group, and region. One-sided p-value based on test of null hypothesis: (daprodustat - Placebo) ≤ 0 vs alternative: difference > 0 .

^c Superiority for the difference between the daprodustat and placebo treatment groups was established if the one-sided p-value is less than 0.025.

Abbreviations: CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; EP, evaluation period; Hb, hemoglobin; ITT, intent-to-treat

Sensitivity analysis was conducted using only evaluable Hb data. The difference in response rate (daprodustat-placebo) was 0.65 (95% CI: 0.58, 0.72; one sided $p < 0.0001$). Results were supportive of the analysis of proportion of participants with a ≥ 1 g/dL increase in Hb between Baseline and over the EP (Weeks 24 to 28) using all Hb values.

The SF-36 v2.0 Vitality domain was scored on a 0-100 scale (refer to [Section 16.1](#) for more details on the SF-36 v2.0 Vitality domain). Daprodustat was superior to placebo in mean change in SF-36 Vitality domain-score between Baseline and Week 28 with a mean difference of 5.4 (one-sided $p = 0.0005$) ([Table 60](#)). Although statistical significance has been reached, this small difference between the two arms raised a concern of clinical meaningfulness (refer to [Section 6.3.2](#) for more details).

Table 60. SF-36 v2.0 Vitality Domain-Score Change From Baseline at Week 28, 0-100 Scoring for Study 205270/ASCEND-NHQ (ITT Population)

	Placebo N=307	Daprodustat N=307
0-100 Scoring		
Number of participants with baseline and Week 28 SF-36	307 (100)	307 (100)
Vitality domain-score ^b , n (%)		
Baseline SF-36 v2.0 Vitality domain-score mean (SD) ^b	52.2 (21.1)	50.7 (21.2)
Week 28 SF-36 v2.0 Vitality domain-score mean (SD) ^b	53.8 (17.0)	58.4 (18.3)
Adjusted mean SF-36 v2.0 Vitality domain change from baseline at Week 28 (SE) ^a	1.9 (1.2)	7.3 (1.1)
Adjusted mean treatment difference (dapro-placebo) ^a		5.4
Two-sided 95% CI for adjusted mean difference ^a		2.2, 8.6
One-sided p-value ^{a,c}		0.0005

Source: Sponsor CSR

^a Based on an ANCOVA model with terms for treatment, baseline score, and region. One-sided p-value based on test of null hypothesis: (daprodustat-placebo) ≤ 0 vs alternative: difference > 0.

^b SF-36 v2.0 Vitality domain data includes imputed values.

^c Superiority for the difference between the daprodustat and placebo treatment groups was established if the one-sided p-value is less than 0.025.

Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; ITT, intent-to-treat; SD, standard deviation; SE, standard error

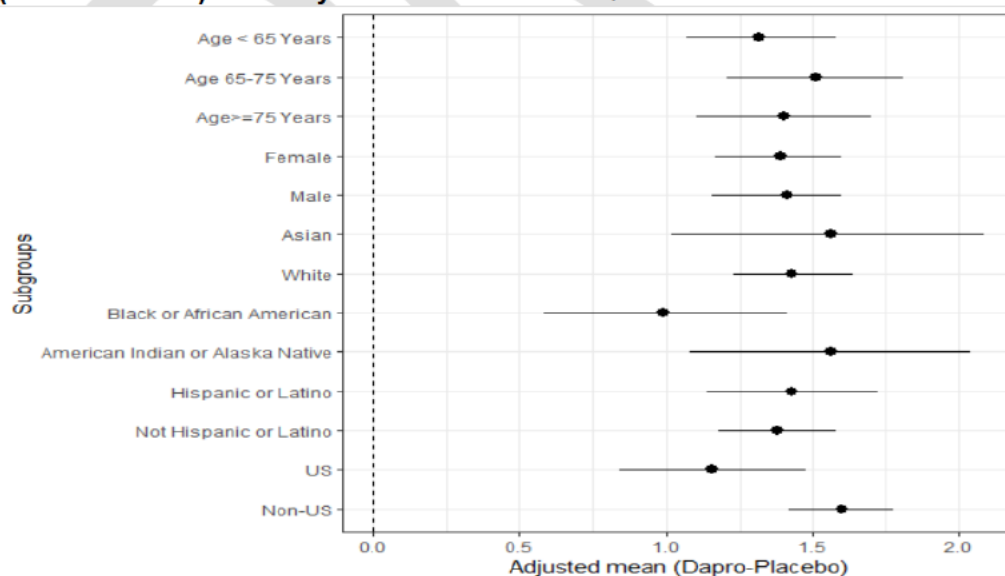
Efficacy Results-Secondary Endpoints

For discussion on the efficacy results for the secondary endpoints, refer to Section [16.1.3](#).

Efficacy Results—Subgroups

Overall, the results of the subgroup analyses were consistent with the analysis results based on the entire patient population with little or no heterogeneity between subgroups. The adjusted mean differences were consistently >0 (see [Figure 22](#)).

Figure 22. Subgroup Analysis for the Primary Endpoint of Hb Change From Baseline to the EP (Weeks 24 to 28) for Study 205270/ASCEND-NHQ

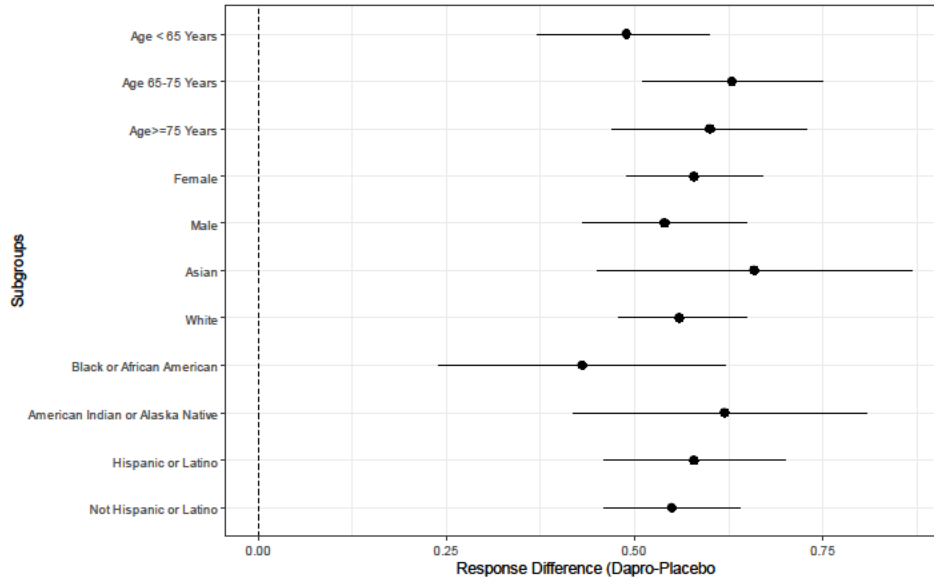


Source: FDA reviewer

Abbreviations: EP, evaluation period; Hb, hemoglobin; US, United States

For the key secondary endpoint Hb Increase of ≥ 1.0 g/dL from Baseline to the EP, the response differences were consistently >0 (see [Figure 23](#)).

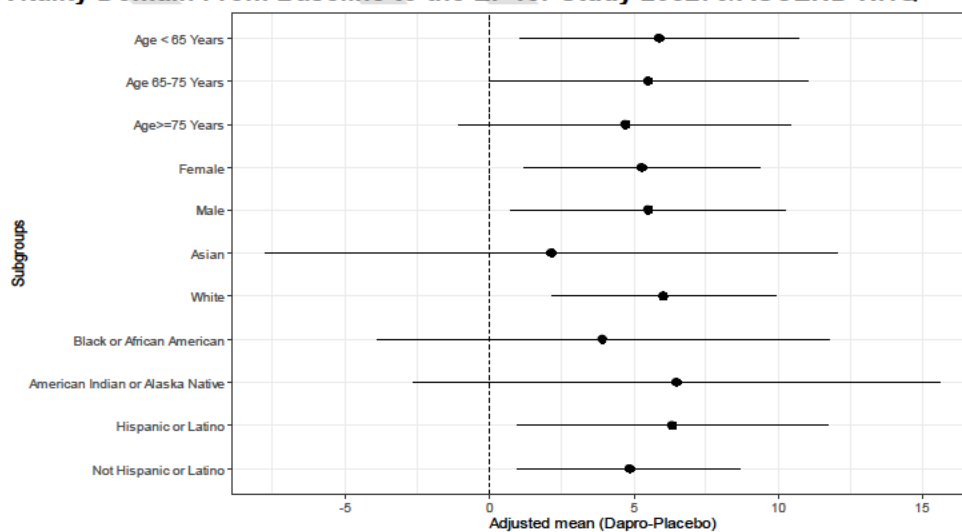
Figure 23. Subgroup Analysis Results for Key Secondary Endpoint of Hb Increase of ≥ 1.0 g/dL From Baseline to the EP for Study 205270/ASCEND-NHQ



Source: FDA reviewer
 Abbreviations: EP, evaluation period; Dapro, daprodustat; Hb, hemoglobin

However, for the key secondary endpoint change in SF-36 v2.0 Vitality domain from baseline to the EP, the adjusted mean differences were not consistently > 0 for all subgroups (see [Figure 24](#)). This could be because of small sample size in each of these subgroups.

Figure 24. Subgroup Analysis Results for Key Secondary Endpoint of Change in SF-36 v2.0 Vitality Domain From Baseline to the EP for Study 205270/ASCEND-NHQ



Source: FDA reviewer
 Abbreviations: EP, evaluation period; Dapro, daprodustat

6.3. Key Efficacy Review Issues

6.3.1. Use of Rescue Therapy in the ASCEND Program

Issue

Impact of Rescue Therapy Use on the Non-Inferiority Efficacy Conclusion of Daprodustat

Background

Hb response and reduction in transfusion need have been accepted endpoints for establishing clinical benefit in patients with anemia of CKD. The ASCEND-D and ASCEND-ND trials used Hb response at the primary efficacy period of 28-52 weeks as the primary efficacy endpoint. The pre-specified non-inferiority Hb comparisons used -0.75 g/dL as the non-inferiority margin, which was appropriately justified based on previous applications for drugs intended to treat anemia of CKD and statistical reasoning. An evaluation of the time to stopping randomized treatment due to meeting rescue criteria was evaluated as a secondary efficacy endpoint in the ASCEND-D and ASCEND-ND study to further evaluate the robustness of the efficacy results.

Assessment

ASCEND-D

The cumulative incidence for time to stopping randomized treatment due to meeting rescue criteria was similar between the treatment groups (Table 61).

Table 61. Analysis of Time to Permanently Stopping Randomized Treatment Due to Meeting Rescue Criteria for Study 200807/ASCEND-D

	Daprodustat N=1487	rhEPO ^d N=1477
Number of participants	1487	1477
Permanently stopping randomized treatment due to meeting rescue criteria	53 (3.6)	53 (3.6)
Censored	1434 (96.4)	1424 (96.4)
Incidence rate per 100 PY (two-sided 95% CI)	1.95 (1.46, 2.56)	1.93 (1.45, 2.52)
Hazard Ratio		
Estimate ^a		1.04
Two-sided 95% CI for hazard ratio		(0.71, 1.52)
One-sided p-value ^b		0.58
Absolute rate difference per 100 PY (two-sided 95% CI) ^c		0.02 (-0.72, 0.76)

Source: Sponsor CSR

^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, dialysis type and region. A hazard ratio <1 indicates a lower risk with daprodustat compared to rhEPO.

^b One-sided p-value based on Wald test of null hypothesis: (daprodustat/rhEPO) ≥1 vs alternative: ratio <1.

^c A rate difference <0 indicates a lower risk with daprodustat compared to rhEPO.

^d Epoetin alfa in HD participants or darbepoetin alfa in PD participants.

Abbreviations: CI, confidence interval; CSR, clinical study report; HD, hemodialysis; PD, peritoneal dialysis; PY, person-year; rhEPO, recombinant human erythropoietin

Participants on daprodustat had a numerically lower risk for time to first occurrence of receiving an on-treatment RBC or whole blood transfusion compared to rhEPO. However, the observed

reduction in risk was not statistically significant and this endpoint was not pre-specified in the testing hierarchy (Table 62).

Table 62. Analysis of Time to First Occurrence of On-Treatment RBC or Whole Blood Transfusion for Study 200807/ASCEND-D

	Daprodustat N=1487	rhEPO ^d N=1477
Number of participants	1482	1477
First occurrence of on-treatment RBC or whole blood transfusion ^a	233 (15.7)	270 (18.3)
Censored	1249 (84.3)	1204 (81.7)
Incidence rate per 100 PY (two-sided 95% CI)	9.30 (8.14, 10.57)	10.87 (9.62, 12.25)
Hazard Ratio		
Estimate ^a		0.86
Two-sided 95% CI for hazard ratio		(0.72, 1.02)
One-sided p-value ^b		0.04
Absolute rate difference per 100 PY (two-sided 95% CI) ^c		-1.57 (-3.34, 0.19)

Source: Sponsor CSR

^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, and region. A hazard ratio <1 indicates a lower risk with daprodustat compared to rhEPO.

^b One-sided p-value based on Wald test of null hypothesis: (daprodustat/rhEPO) ≥1 vs alternative: ratio <1.

^c A rate difference <0 indicates a lower risk with daprodustat compared to rhEPO.

^d Epoetin alfa in HD participants or darbepoetin alfa in PD participants.

Abbreviations: CI, confidence interval; CSR, clinical study report; HD, hemodialysis; PD, peritoneal dialysis; PY, person-year; RBC, red blood cell; rhEPO, recombinant human erythropoietin

ASCEND-ND

The proportion of participants stopping randomized treatment due to meeting rescue criteria was 2% in the daprodustat arm compared to 3% participants in the darbepoetin alfa arm. One subject met the rescue criteria but did not discontinue randomized treatment (Table 63).

The daprodustat arm showed an observed reduced risk of stopping randomized treatment due to meeting rescue criteria compared to the darbepoetin alfa arm (nominally significant one-sided p=0.01) (Table 64), but this endpoint was not pre-specified as a major efficacy endpoint under the testing hierarchy and, therefore, was not included in the study-wise type I error control.

Table 63. Summary of Analysis of Time to Permanently Stopping Randomized Treatment Due to Meeting Rescue Criteria for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935
Number of participants	1937	1935
Permanently stopping randomized treatment due to meeting rescue criteria; n (%)	39 (2.0)	64 (3.3)
Censored; n (%)	1898 (98.0)	1871 (96.7)
Incidence rate per 100 PY (two-sided 95% CI)	1.31 (0.93, 1.79)	2.09 (1.61, 2.67)

	Daprodustat N=1937	Darbepoetin N=1935
Hazard ratio (HR)		
Estimate ^a		0.63
Two-sided 95% CI for HR		(0.42, 0.94)
One-sided p-value ^b		0.01
Absolute rate difference per 100 PY (two-sided 95% CI) ^c	-0.79 (-1.44, -0.13)	

Source: Sponsor CSR

^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, current ESA use, and region. A hazard ratio <1 indicates a lower risk with Dapro compared to Darbe.

^b One-sided p-value based on Wald test of null hypothesis: (Dapro/Darbe) ≥1 vs alternative: ratio <1.

^c A rate difference <0 indicates a lower risk with Dapro compared to Darbe.

Abbreviations: CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; ESA, erythropoiesis-stimulating agents; PY, person-year

The percentage of participants with a first occurrence of RBC or whole blood transfusion during the on-treatment time period was similar between the treatment groups ([Table 64](#)).

Table 64. Summary of Analysis of Time to First Occurrence of On-Treatment RBC or Whole Blood Transfusion for Study 200808/ASCEND-ND

	Daprodustat N=1937	Darbepoetin N=1935
Number of participants	1937	1933
First occurrence of RBC or whole blood transfusion during the time period for on-treatment cardiovascular events	247 (12.8)	261 (13.5)
Censored	1690 (87.2)	1672 (86.5)
Incidence rate per 100 PY (two-sided 95% CI)	8.82 (7.76, 9.99)	9.18 (8.10, 10.37)
Hazard ratio (HR)		
Estimate ^a		0.96
Two-sided 95% CI for HR		(0.806, 1.141)
One-sided p-value ^b		0.32
Absolute rate difference per 100 PY (two-sided 95% CI) ^c	-0.36 (-1.93, 1.21)	

Source: Sponsor CSR

^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, current ESA use at randomization, and region. A hazard ratio <1 indicates a lower risk with Dapro compared to Darbe.

^b One-sided p-value based on Wald test of null hypothesis: (Dapro/Darbe) ≥1 vs alternative: ratio <1.

^c A rate difference <0 indicates a lower risk with Dapro compared to Darbe.

Abbreviations: CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Darbe, darbepoetin alfa; ESA, erythropoiesis-stimulating agents; PY, person-year; RBC, red blood cell

Conclusion

The above results support the non-inferiority conclusion of the efficacy of daprodustat on Hb. We expect the similarity in Hb response between daprodustat and the ESA control arms to translate into similar reductions in the need for RBC transfusions, an accepted clinical benefit for treatments for anemia of CKD, because of avoiding the procedure and its attendant risks.

6.3.2. Lack of Clinical Meaningfulness on Key Secondary Endpoint of SF-26 V2.0 Vitality Domain Score in ASCEND-NHQ

Issue

Lack of Clinical Meaningfulness on Key Secondary Endpoint of SF-36 v2.0 Vitality domain score in ASCEND-NHQ.

- Sub-Issue 1: The Applicant's proposed 6-point improvement threshold in the 0-100 scaled SF-36 v2.0 Vitality domain score did not represent a clinically meaningful within-patient improvement.
- Sub-Issue 2: A meaningful treatment effect was not demonstrated upon review of the data from the secondary PRO endpoint of change from baseline to Week 28 in the 0-100 scaled SF-36 v2.0 Vitality domain score.

Background

Sub-Issue 1

The Applicant proposed a 6-point reduction (improvement) in the 0-100 scaled SF-36 v2.0 Vitality domain score as a clinically meaningful within-patient change based on literature review and distribution-based and anchor-based methodologies. During the IND phase (IND 101291), we had advice regarding the Applicant's approach to deriving the threshold(s) for meaningful within-patient score change for the SF-36 v2.0 Vitality domain (i.e., identified several limitations with using a literature review to derive threshold(s) for meaningful within-patient score change, recommended using an anchor-based analysis supplemented with both empirical cumulative distribution function [eCDF] and probability density function [PDF] curves). Ultimately, we concluded at that time that there was insufficient evidence to support that a 6-point improvement in the SF-36 v2.0 Vitality domain score is a meaningful within-patient change. For additional details, refer to the Final Written Response dated December 4, 2019.

Sub-Issue 2

Although statistically significant results were reported on the key secondary endpoint of change from baseline to Week 28 in the 0-100 scaled SF-36 v2.0 Vitality domain score, the difference between the daprodustat and placebo means was small. Minimal magnitude of changes was observed at the item- and domain-level of the SF-36 v2.0 Vitality domain-score, using both raw and transformed score scales.

Assessment

Sub-Issue 1

As part of the NDA, the Applicant submitted the "Patient-Reported Outcome Evidence Dossier for SF-36 Vitality domain and CKD-AQ in anemia due to Chronic Kidney Disease (CKD)" Version 4.0 dated August 31, 2021 (hereafter referred to as the SF-36 PRO Dossier), which included findings from the literature review and anchor-based analyses.

Literature Review

FDA reviewed the Applicant's summary report of literature review (Appendix 16 of the SF-36 PRO Dossier) and noted much of the cited literature is based on approaches that are not aligned with FDA guidance (e.g., use of distribution-based methods, between-group differences). Based on FDA Guidance for Industry Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims, sponsors should use anchor-based methods⁶ to directly incorporate patients' perspective to help interpret the clinical meaningfulness of PRO endpoints. Other approaches, such as distribution-based approaches using an effect size or a standard deviation, or model-based approaches, are inappropriate as a primary method to determine clinical meaningfulness as they do not directly take into account the patient perspective. FDA concluded that the Applicant's literature review is not adequate (b) (4)

Anchor-Based Analyses

In response to the FDA's recommendation in the IND phase, the Applicant conducted anchor-based analyses using data from Studies ASCEND-ND and ASCEND-ID and derived a meaningful within-patient change threshold range of 6- to 21-point improvement (see [Figure 25](#)) based on 4 patient-reported anchor scales: PGI-S scale, Patient Global Impression of Change (PGI-C) scale, and items 1 and 2 from the CKD-AQ. The Applicant used the PGI-S and the PGI-C scales as the primary anchors and CKD-AQ items 1 and 2 as supportive anchors in the quantitative anchor-based analyses. Refer to [Table 227](#) in Section [16.1](#) for a summary of the anchor scales.

Figure 25. Excerpt of Text From Applicant's SF-36 PRO Dossier

- **+ 6 points:** this threshold is based on changes observed in participants who had improved by +1 point on the PGI-S between Day 1 and Week 28 and changes observed among participants who reported being "very much improved" on the PGI-C at Week 28. +1 point improvements on the CKD-AQ Item 1 and 2 were 7.7 and 4.7 respectively and a moderate improvement on the PGI-C was 4.5. This threshold is also supported by consideration of mean and median definitions of meaningful change as reported in the research literature ([Appendix 16](#)).
- **+ 12 points:** this threshold is based on changes observed in participants who had improved by +2 points on the PGI-S between Day 1 and Week 28.
- **+ 18 points:** this threshold is based on participants who had improved by +2 points on CKD-AQ Item 2 (low energy) between Day 1 and Week 28.
- **+21 points:** this threshold is based on participants who had improved by +2 points on CKD-AQ Item 1 (tiredness) between Day 1 and Week 28

Source: Applicant's SF-36 PRO Dossier.

Abbreviations: CKD-AQ, Chronic Kidney Disease Anemia Questionnaire; PGI-C, Patient Global Impression of Change; PGI-S, Patient Global Impression of Severity

⁶ Anchor scale(s) are used as external criteria to define patients who have or have not experienced a meaningful change in their condition, with the change in PRO score observed in these sets of patients referenced in the interpretation of within-patient change scores.

FDA identified limitations in the PGI-S and PGI-C anchor scales that limit the interpretability of the results of the anchor-based analyses. Specifically, the concepts measured in the SF-36 v2.0 Vitality domain (tiredness, weakness, full of life, energy) were not aligned with the concepts measured in the PGI-S and PGI-C anchor scales (overall CKD symptoms). Due to these limitations, FDA focused on the Applicant's anchor-based analyses using items 1 and 2 from the CKD-AQ, which measure tiredness and energy that are better aligned with the SF-36 v2.0 Vitality domain. As such, FDA considered the range of 18- to 21-point improvement appropriate based on the Applicant's anchor-based analyses results. Refer to the full COA review by the Division of Clinical Outcome Assessment (DCOA) for additional details on the adequacy of the anchor scales.

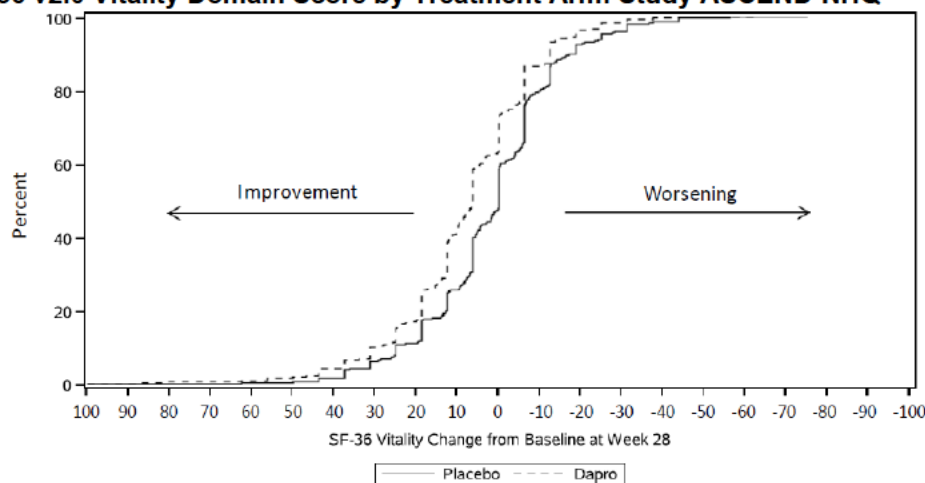
Of note, while PRO data (e.g., SF-36 v2.0 Vitality domain, CKD-AQ items 1 and 2) were collected in Studies ASCEND-ND and ASCEND-ID, the open-label study design of these two studies was a limitation to the interpretation of PRO data and subsequently the evaluation of clinically meaningful-within patient change, as it is unknown whether patients' knowledge of treatment assignment may lead to systematic overestimation or underestimation of the treatment effect, the magnitude of which is currently unknown. Therefore, FDA requested additional anchor-based analyses using Study ASCEND-NHQ data, which suggested a threshold range of 12.5- to 18.75-point improvement in the SF-36 v2.0 Vitality domain score as clinically meaningful to patients. Of note, the Hb target in ASCEND-NHQ was higher than the other studies (11 to 12 g/dL, in contrast to 10 to 11 g/dL, which reflects current labeling for ESAs). The relevance of any efficacy conclusions to a population treated to a lower target is unclear.

Refer to Section [16.1](#) of this review for more discussion on the anchor-based analyses.

Sub-Issue 2

FDA reviewed the Applicant's eCDF curves to further evaluate the observed changes in the SF-36 v2.0 Vitality domain-score. The eCDF plot below ([Figure 26](#)) displays a continuous view of the cumulative proportion of patients reporting any amount of change from baseline to Week 28 in the 0-100 scaled SF-36 v2.0 Vitality domain score between treatment arms of ASCEND-NHQ. Note that a positive change (>0) to the left represents an improvement in the score and a negative change (<0) to the right represents a worsening in the score. There is not a clear and consistent separation between daprodustat and placebo arms within the range of 12- to 21-point improvement, which encompasses all threshold ranges derived using data from Studies ASCEND-ND, ASCEND-ID and ASCEND-NHQ.

Figure 26. eCDF, Change From Baseline at Week 28 for On-Treatment Observed and Imputed SF-36 v2.0 Vitality Domain Score by Treatment Arm: Study ASCEND-NHQ



Source: Applicant's response to information request dated Sep 19, 2022.
Abbreviations: Dapro, daprodustat; eCDF, empirical Cumulative Distribution Function

The Applicant additionally provided the response rate of patients achieving different levels of improvement for each arm, and the difference in response rates between arms of ASCEND-NHQ. [Table 65](#) shows the Applicant's post-hoc responder analysis results using different cutoffs. At the thresholds of ≥ 12 , ≥ 18 , and ≥ 21 , the treatment difference was 12%, 8% and 6%, respectively, and the corresponding 95% confidence intervals include 0 at the thresholds of ≥ 18 and ≥ 21 . These modest to small treatment differences were not considered as representative of a clinically meaningful improvement to patients.

Table 65. Proportion of Patients Who Achieved Meaningful Improvement at Week 28 From Baseline in SF-36 v2.0 Vitality Domain Score at Various Thresholds: Study ASCEND-NHQ

Threshold	Daprodustat N=307	Placebo N=307	Diff. From Treatment (95% CI)
≥ 12	121 (39%)	77 (25%)	12% (3%, 21%)
≥ 18	78 (25%)	54 (18%)	8% (0%, 16%)
≥ 21	52 (17%)	34 (11%)	6% (-1%, 14%)

Source: FDA reviewer table based on Applicant's Study 205270 CSR Efficacy Data Source Tables-Post Hoc. Table 2.141 corresponds to a threshold of 12, Table 2.137 corresponds to a threshold of 18, and Table 2.138 corresponds to a threshold of 21.
Abbreviations: CI, confidence interval

When looking at the distribution of SF-36 v2.0 Vitality domain-score by visit, the difference between arms of change from baseline in the raw scores (ranges between 4 and 20; [Table 67](#)) and 0-100 scaled ([Table 66](#)) SF-36 v2.0 Vitality domain scores were both minimal. Specifically, the mean change observed in the raw domain scores ([Table 67](#)) for the daprodustat arm reflected less than one response category change on each item (e.g., a mean change from 12.1 to 13.3 in the raw score would represent a 0.3-category change on each of the 4 items under the assumption of equal weight).

Table 66. Distribution of On-Treatment SF-36 v2.0 Vitality Domain Score by Visit, 0-100 Scoring: Study ASCEND-NHQ

Treatment	N	Planned		n	Missing n	Mean	SD	Min.	P25	Median	P75	Max.
		Relative	Time									
Placebo	307	Baseline		294	13 (4.2%)	52.020	21.4225	0.00	37.500	50.000	68.750	100.00
		Week 8		250	57 (18.6%)	54.025	20.6619	0.00	43.750	56.250	68.750	100.00
		Week 12		234	73 (23.8%)	55.796	20.6996	0.00	43.750	56.250	75.000	100.00
		Week 28		194	113 (36.8%)	54.220	19.1428	0.00	43.750	56.250	68.750	100.00
Dapro	307	Baseline		293	14 (4.6%)	50.619	21.5271	0.00	37.500	50.000	62.500	100.00
		Week 8		251	56 (18.2%)	56.350	21.6793	0.00	43.750	56.250	75.000	100.00
		Week 12		251	56 (18.2%)	55.951	20.6815	0.00	43.750	56.250	75.000	100.00
		Week 28		213	94 (30.6%)	58.187	20.3501	0.00	43.750	56.250	75.000	100.00

Source: Table 69.309001 in the Applicant's response to information request dated August 5, 2022.
Abbreviations: Dapro, daprodustat; SD, standard deviation

Table 67. Distribution of On-Treatment SF-36 v2.0 Vitality Domain Score by Visit, Raw Scoring: Study ASCEND-NHQ

Treatment	N	Planned		n	Missing n	Mean	SD	Min.	P25	Median	P75	Max.
		Relative	Time									
Placebo	307	Baseline		294	13 (4.2%)	12.3	3.43	4	10.0	12.0	15.0	20
		Week 8		250	57 (18.6%)	12.6	3.31	4	11.0	13.0	15.0	20
		Week 12		234	73 (23.8%)	12.9	3.31	4	11.0	13.0	16.0	20
		Week 28		194	113 (36.8%)	12.7	3.06	4	11.0	13.0	15.0	20
Dapro	307	Baseline		293	14 (4.6%)	12.1	3.44	4	10.0	12.0	14.0	20
		Week 8		251	56 (18.2%)	13.0	3.47	4	11.0	13.0	16.0	20
		Week 12		251	56 (18.2%)	13.0	3.31	4	11.0	13.0	16.0	20
		Week 28		213	94 (30.6%)	13.3	3.26	4	11.0	13.0	16.0	20

Source: Table 69.309002 in the Applicant's response to information request dated August 5, 2022.
Abbreviations: Dapro, daprodustat; SD, standard deviation

Conclusion

The Applicant's proposed 6-point improvement threshold in the 0-100 scaled SF-36 v2.0 Vitality domain score did not represent a clinically meaningful within-patient improvement. Statistically significant results were reported on the key secondary endpoint of change from baseline to Week 28 in the 0-100 scaled SF-36 v2.0 Vitality domain-score in ASCEND-NHQ. However, FDA determined that these results did not substantiate a clinically meaningful improvement of daprodustat compared to placebo due to the minimal magnitude of changes observed at the item- and domain-level (using raw and transformed score scales), and modest to small between arm differences in response rates. In addition, the Hb target of 11 to 12 g/dL studied in ASCEND-NHQ is higher than the Hb target used in ASCEND-ND and ASCEND-D (i.e., higher than the target Hb used to establish the efficacy and safety of daprodustat for the approved use), further calling into question the relevance of the findings in ASCEND-NHQ.

6.1. Efficacy Summary

After conducting a thorough evaluation of the ASCEND program, we agree with the Applicant that non-inferiority to an ESA comparator, based on hemoglobin response, was demonstrated in the DD, incident dialysis (ID), and NDD populations, and the DD population using three times a week dosing. We agree superiority was achieved against placebo for Hb in ASCEND-NHQ, but we do not agree a clinically important benefit was demonstrated on patient reported outcomes (See Section 6.3.2).

Our non-inferiority conclusion was based on the prospectively defined and agreed upon primary endpoint, statistical test, and NI margin. The primary endpoint, change in Hb value between baseline and mean of values during Weeks 28 to 52, represents the key measurement used to clinically define anemia, averaged over a time frame sufficient to capture steady state. In constructing the NI margin of -0.75 g/dL, we considered Hb changes that would result in a clinically meaningful difference to an individual patient, proportion of comparator (i.e., ESAs) efficacy preserved by the margin, measurement variability, and precedent from prior trials (i.e., darbepoetin, peginesatide, and methoxy polyethylene glycol-epoetin beta). Non-inferiority was defined as being achieved if the two-sided lower 95% confidence limit for the mean treatment difference (daprodustat minus comparator) was greater than the pre-specified NI margin, as is customary. Although some ASCEND studies were open label, bias due to knowledge of treatment assignment was minimized by evidence of appropriate titration in the two treatment arms and the central laboratory-Hb objective endpoint.

We found the conclusion of noninferiority to be robust to multiple sensitivity analyses which assessed the impact of important model assumptions, such as accounting for rescue therapy, endpoint missingness mechanisms (such as missing at random, or missing not at random), choice of analysis time window, and estimand strategy. We also generally found little heterogeneity between the subgroups studied, showing daprodustat was effective regardless of age, sex, race, body weight, dialysis type, CKD stage, prior ESA use, and Baseline Hb (Refer to Section 16).

Regarding ASCEND-D and ASCEND-ND

We placed particular emphasis on the efficacy results for ASCEND-D and ASCEND-ND, since it is well known hemoglobin can causally affect cardiovascular safety. In ASCEND-ND and ASCEND-D, the observed Hb versus time profile confirms that the titration algorithms resulted in similar Hb vs. time trajectories for daprodustat and the ESA comparator (Section 16). We are reassured that mean-level plots fell in the middle of the Hb target range for both arms during the EP. This provides assurance that the comparators were titrated and subsequently performed acceptably. In addition, the comparators performed reasonably similarly with other programs with regards to Hb vs. time. The within-subject variability in Hb data was also similar between arms. The proportion of participants who received transfusions during the evaluation period was generally similar between the daprodustat and ESA groups, as would be expected given the similarity of the achieved Hb levels (Section 6.3.1). Results from the key secondary analyses of the on-treatment monthly IV iron dose (mg/month) from Day 1 to Week 52 in ASCEND-D (not assessed in ASCEND-ND, due to infrequent indication for IV iron, but assessed in ASCEND-TD and ASCEND-ID) showed little or no difference between daprodustat and ESA in the mean IV iron dose. These supporting data further support the non-inferiority conclusion of the efficacy of daprodustat on Hb.

Disposition and conduct during ASCEND-D and ASCEND-ND was not of concern. For each study, the treatment arms were balanced in relation to baseline demographics and clinical characteristics, with appropriate representation across ages, genders, and races/ethnicities. As mentioned above, we did not identify a heterogeneous efficacy response in a subgroup that warranted further study or concern. Importantly, follow-up was remarkably complete (>95% of potential follow-up time was accounted for).

In ASCEND-D and ASCEND-ND, the randomized treatment titration algorithm was prespecified. We reviewed the titration algorithms and determined they reflected reasonable

clinical practice. Importantly, the target Hb range was 10 to 11 g/dL, which reflects current product labeling and best practice for ESA treatment. Instances where the algorithm was not explicitly followed were recorded as protocol violations. We reviewed the protocol violations, and they were balanced between arms. Additionally, rescue therapy and iron management criteria were prespecified and consistent across the ASCEND program.

These conclusions were robust among all subgroups studied (including race, age, gender, location, prior ESA use, dialysis status), and to a standard battery of sensitivity analyses, which included alternative estimand strategies, evaluation periods, and data missingness assumptions. Several supportive analyses (both prespecified and post-hoc) for Hb such as Hb vs. time, time-in-range, and visit-to-visit variability were similarly balanced between arms. The incidence of RBC transfusions and rescue therapies was generally similar between treatment arms as well. These analyses further strengthen the conclusion of non-inferiority.

Regarding ASCEND-ID, ASCEND-TD, and ASCEND-NHQ

ASCEND-ID established non-inferiority of daprodustat for efficacy (mean change from baseline in Hb over evaluation period of Weeks 28 to 52) to darbepoetin in the ID population. As the study was not designed to demonstrate general safety or cardiovascular outcomes, (b) (4) it is unclear if the risks in this population will be similar to the NDD population (b) (4) or the DD population. Further discussion regarding cardiovascular safety in the ID population is in Section 6.2.4 and Section 7.7.6.

In the ASCEND-TD, analysis of mean change in Hb between baseline over the evaluation period of Weeks 28 to 52 demonstrated that daprodustat TIW was non-inferior to epoetin alfa. The study was not designed to demonstrate general safety or cardiovascular outcomes. A detailed discussion of the CV safety and extrapolation of CV safety for the TIW dosing regimen in the DD population is in Section 7.7.5.

ASCEND-NHQ established superiority for the primary efficacy endpoint of mean change in Hb between baseline over the evaluation period of Weeks 24 to 28 compared to placebo. It is important to note that the evaluation period in this trial was shorter than the other trials and the comparator arm was placebo. In addition, the target hemoglobin level was higher (11 to 12 g/dL) compared to 10 to 11 g/dL for all other trials in the ASCEND program. The principal secondary endpoint was a patient reported outcome (PRO), the mean change in SF-26 Vitality Domain between baseline and Week 28. We did not agree with the Applicant's conclusions regarding demonstrated benefit on the PRO. This is a key efficacy review issue discussed under Section 6.3.2.

Lastly, for each ASCEND study, we reviewed the protocols, the statistical analysis plan, study report, and study data (Study Data Tabulation Model and Analysis Dataset Model). The quality of the original data submission was adequate. In general, the reviewers independently corroborated the Applicant's analyses using the submitted datasets.

In conclusion, daprodustat increased Hb to a similar extent as approved ESAs (darbepoetin alfa in the NDD population and darbepoetin alfa or epoetin alfa in the DD population) with similar continued need for rescue therapy. Most patients with CKD receiving hemodialysis require ESAs to correct anemia and reduce the need for RBC transfusion and its attendant risks, including the risks of alloreactivity and rejection after kidney transplantation. We expect the similarity in Hb

response and similarity in the need for rescue therapy between daprodustat and the ESA control arms to translate into similar reductions in the need for RBC transfusions, an accepted clinical benefit for treatments for anemia of CKD, because of avoiding the procedure and its attendant risks. There were no other benefits demonstrated on how patients feel, function, or survive.

7. Safety (Risk and Risk Management)

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

The safety of daprodustat and major human metabolites was evaluated in a comprehensive nonclinical program. Toxicological characterization was performed in good laboratory practice-compliant toxicology studies in mice, rats, dogs, and monkeys, in reproductive toxicology studies in rats and rabbits, and in a carcinogenicity assessment that included data from lifetime carcinogenicity studies in mice and rats. Monkeys were considered the most relevant nonclinical species for general toxicological evaluation based on the similarity of the daprodustat metabolite profile to humans and higher bioavailability compared to other species.

Daprodustat binding was shown to be specific for HIF-prolyl hydroxylases in primary pharmacology and secondary pharmacology (evaluations of potential targets not associated with the desired therapeutic effect) assessments. No significant risks were identified during in vitro and in vivo cardiovascular, respiratory, or neurologic safety pharmacology assessments.

Daprodustat is highly protein-bound in rats and rabbits (~100%), humans (>99%), monkeys and dogs (~98%), and mice (>99%). Because only the unbound fraction of drug is “active”, the high level of protein binding complicated the determination of appropriate exposure margins between nonclinical species and humans. Ultimately, exposure extrapolation based on body surface area (mg/m²) was deemed the most appropriate method to inform clinical risk.

The IND enabling nonclinical toxicology studies produced effects consistent with the exaggerated primary pharmacology of daprodustat (polycythemia) and secondary impacts on hemodynamics (systemic thrombosis, hemorrhage, congestion, and necrosis in lung, kidney, heart, and other organs). Thus, the clinical safety of daprodustat was predicted to be largely a function of its primary pharmacology and impacts on blood viscosity.

In a 6-month toxicology study in non-anemic rats, adverse findings associated with excess hematocrit (e.g., thrombosis, hemorrhage, congestion, and necrosis occurred in various organs, along with splenomegaly, extramedullary hematopoiesis, and platelet reductions) at the highest dose of 10 mg/kg/day (4 times the clinical dose based on body surface area) in both sexes. In males, these effects caused mortality and moribundity leading to early termination of all high-dose male animals, which occurred coincident with total blood hematocrit levels as high as 75% at Week 13 (versus total blood hematocrit levels of 52% in untreated controls), whereas females tolerated similar effects on hematocrit. Daprodustat-related gastric hemorrhage, erosions and/or ulcerations also occurred due to downstream complications related to increased blood viscosity at the highest dose. In the 6-month rat study, the no-observed-adverse-effect-level (NOAEL) was determined to be 4 mg/kg/day (1.6 times the clinical dose based on body surface area), based on the mortality and daprodustat-related toxicity related to excess red blood cell mass observed at

the highest dose of 10 mg/kg/day (4 times the clinical dose based on body surface area). The severity of findings increased with longer exposures, suggesting a potential for any negative impacts related to daprodustat to worsen over time. The narrow margin (2.5-fold dose-range) between the NOAEL and a severely toxic dose highlight a steep dose response curve for toxicity.

The toxicologic profile observed in studies in non-anemic monkeys up to 9-months in duration was similar to that observed in long-term rat studies, with systemic thrombosis/hemorrhage linked to excess hematocrit (total hematocrits as high as 74% at the highest dose compared to 42% in controls) and platelet reductions observed at ≥ 10 mg/kg/day (≥ 8 times the clinical dose based on body surface area). Findings also included dose-related gastric hemorrhage, erosions and/or ulcerations at ≥ 10 mg/kg/day. At 50 mg/kg/day (40 times the clinical dose based on body surface area), widespread and severe impacts of polycythemia occurred, which included subacute inflammation, edema, and hemorrhage in the superficial brainstem and other evidence of systemic congestion and bleeding, underscoring the importance of avoiding excess red blood cell mass in patients. All findings trended towards reversibility after the recovery period. In the 9-month monkey study, the NOAEL was determined to be 3 mg/kg/day (2.4 times the clinical dose based on body surface area), based on the daprodustat-related toxic effects coincident with excess red blood cell mass observed at ≥ 10 mg/kg/day (≥ 8 times the clinical dose based on body surface area). The narrow margin (3.3-fold dose-range) between the NOAEL and a significantly toxic dose corroborate the narrow therapeutic index observed in the rat.

Upwards of 150 genes known to be involved in angiogenesis, glucose metabolism, cell proliferation, and inhibition of apoptosis are regulated by HIFs in response to hypoxia (Selvaraju et al. 2014). However, no toxicity studies were conducted in animal models of anemia, and excess hematocrit in healthy animal models confounds identification of toxicity not related to excess erythrocytosis and high blood viscosity. Indeed, risks associated with daprodustat observed in long-term animal toxicology studies appear attributable to polycythemia and related sequelae. While data in non-anemic animals may somewhat overpredict risks of the use of daprodustat in patients with chronic anemia, the steepness of the dose-response curve for toxicity and narrow therapeutic index observed in animals require caution during clinical use.

Reproductive risks of daprodustat were assessed in a comprehensive battery of studies covering the entire reproductive cycle, including a fertility and early embryonic development study in female rats, embryo-fetal development studies in rats and rabbits, and a pre- and post-natal development study in rats. Adverse developmental effects in offspring observed in rats and rabbits in the fertility and early embryonic development, embryo-fetal development and pre- and post-natal development studies were considered primarily a consequence of maternal toxicity related to excess red blood cell mass. No adverse effects on fertility were observed in male rats. Impacts on fertility in female rats were consistent with the established consequences of maternal toxicity during early pregnancy. These data are presented in Section 8.4, and the reproductive and developmental toxicity findings are discussed in detail in Section 13.2.4. Labeling recommendations are consistent with erythropoietin stimulating agents, as the data suggest risk is related to daprodustat's primary pharmacology (i.e., risks with hematocrit overshoot).

Daprodustat and its major metabolites were determined not to be genotoxic using in vitro and in vivo assays, and daprodustat was deemed not to pose a significant carcinogenic risk based on a carcinogenicity assessment that included 2-year rat and mouse carcinogenicity studies.

A juvenile toxicity study was conducted in rats with dosing beginning on post-natal day 4 (generally approximates a full-term human neonate) to evaluate the pharmacological consequence of daprodustat increasing RBC mass during development. The study showed dose-related decreases in body weight/body weight gain in both sexes of young rats and delayed attainment of developmental landmarks in females (e.g., increased the mean age of vaginal patency and increased the estrous cycle length). Minimal decreases in femur length were also noted, which correlated with decreased body weight/body weight gain. Decreased body weight gain and developmental delay is reasonably explained by the impact of excess red blood cell mass in the young, developing non-anemic rat. While the toxicity profile in juvenile animals was qualitatively similar to that observed in adults, younger animals appeared more sensitive than adults (the NOAEL in juvenile rats was below the clinical dose based on body surface area). These data suggest that the use of daprodustat in pediatric patients warrants caution.

As discussed above, the plasma protein binding of daprodustat is extremely high (approaching 100%) in rat and rabbit, which precluded an accurate estimation of pharmacologically active (free) drug in rat and rabbit plasma for comparison to human free drug levels. Exposure margins (see [Table 68](#)) for labeling purposes were therefore derived using body surface area extrapolation. Supporting the use of body surface area extrapolation, this method predicts a 3-fold animal:human safety margin for gastric erosions/ulcers, an imbalance of which occurred in clinical trials. In contrast, the use of the maximum plasma concentration (C_{max}) or the area under the concentration-time curve (AUC) to determine the animal:human exposure multiple appears to grossly overpredict clinical safety (e.g., the use of unadjusted AUC produces a safety margin for gastric erosions/ulcers as high as 845-fold).

Overall, the nonclinical data support chronic clinical use of daprodustat for the treatment of anemia, but studies consistently demonstrate a narrow therapeutic index with the potential for severe consequences related to hematocrit overshoot. No other outstanding nonclinical safety issues of significant concern were observed at clinically relevant exposures in the nonclinical toxicology program. Therefore, the nonclinical pharmacology/toxicology data support approval of this NDA.

Table 68. Safety Margins Provided by the Nonclinical Studies Relative to the Maximum Recommended Clinical Dose of Daprodustat

Study	NOAEL (mg/kg/day)	C_{max} (μ g/kg)	AUC (μ g*hr/mL) ^b	Exposure Margins ^a		
				Safety Margins ^d		Exposure Multiple
				BSA	C_{max}	AUC
6-month rat	4 ^c	47	786	1.6	166	845
9-month monkey	3	0.7	3.5	2.4	2.3	3.8
Fertility-male rat	20	NA	NA	8.1	NA	NA
Rat embryofetal	7	52	128	2.8	184	138
Rabbit embryofetal	30	9	133	24.3	33	143
Pre/Postnatal	7	48	613	2.8	169	659
Juvenile	2/0.8	19	350	0.3	67	376

Source: Reviewer Analysis

^a Exposure margins based on body surface area (BSA) and C_{max} and area under the curve (AUC) of proposed maximum human dose of 24 mg/day.

^b Rat Fertility studies had no TK data, therefore the exposure margins are based on BSA alone.

^c Steep dose response in rats was observed with only a 2.5-fold difference between the dose of NOAEL and the dose which resulted in mortalities due to systemic thrombosis.

^d Safety margins are derived from dose multiple.

Abbreviations: AUC, area under the concentration-time curve; BSA, body surface area; C_{max} , maximum plasma concentration; NOAEL, no-observed adverse effect level; TK, toxicokinetic

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

Daprodustat is a small molecule inhibitor of HIF-PH enzymes. HIF-PHIs are a new class of drugs that increase hemoglobin by inducing endogenous EPO production (rather than exogenous administration as per the ESAs). Currently, the standard of care for the treatment of anemia due to CKD is ESA, which include Epogen/Procrit (epoetin alfa), Aranesp (darbepoetin alfa), Mircera (pegylated epoetin beta) and Retacrit (epoetin alfa-epbx). ESAs are approved for the treatment of anemia due to CKD, including patients on dialysis and not on dialysis. Given the similar end effect of increased EPO, as a common mechanism of action between daprodustat and ESAs, safety concerns observed with ESAs were considered potential safety concerns in our safety evaluation of daprodustat.

Briefly, current ESA labeling includes a boxed warning for increased risk of death, thrombosis, and thromboembolism (such as myocardial infarction stroke, venous thromboembolism, and thrombosis of vascular access) and congestive heart failure. Although these risks were observed in randomized controlled trials with randomized arms targeting higher hemoglobin levels using ESAs (i.e., >11 g/dL), a hemoglobin target level or dosing strategy that avoids the risks of ESAs has not been established. As a result, the ESA labeling recommends using the lowest ESA dose sufficient to reduce the need for red blood cell transfusions, and specifically warns against exceeding 11 g/dL of Hb. Additionally, the labeling for ESAs includes the following safety concerns in the warnings and precautions:

- Hypertension, worsening or new onset, with a contraindication in patients with uncontrolled hypertension
- Increased risk for seizures in patients with CKD
- Serious allergic reactions, including anaphylactic reactions, angioedema, bronchospasm, skin rash and urticaria
- Severe cutaneous reactions, including blistering and skin exfoliation

Vadadustat and roxadustat are also HIF-PH inhibitors under development and are approved in other countries.⁷ These molecules share a mechanism of action, targeted indication and patient population with daprodustat. The drug development programs have been broadly similar. In July 2021, the Cardiovascular and Renal Drugs Advisory Committee considered the roxadustat NDA for the treatment of anemia due to CKD in patients on dialysis (DD) and not on dialysis (NDD). The Committee did not support approval for either population and recommended that additional trials be conducted to determine a dose and dose strategy with a better benefit-risk profile. The FDA issued a complete response letter (CRL) for roxadustat for both NDD and DD populations, citing concerns for thrombosis and thromboembolism that were echoed by the members of the advisory committee. Vadadustat was also issued a CRL due similar safety concerns, as well as concerns for drug-induced liver injury. Generally, clinical trial results have disfavored HIF-PHIs versus active control or ESAs for MACE (which includes MI, CVA, and all-cause mortality). We summarized these results in [Table 69](#).

⁷ As of January 2023, Roxadustat has been approved in China, Japan, and the European Union. Vadadustat has been approved in Japan

Table 69. Results of MACE From Other HIF-PHI Development Programs:

	Roxadustat HR (95% CI)	Vadadustat HR (95% CI)
NDD		
On-Study	1.10* (0.96,1.27)	1.17 (1.01, 1.36)
On-Treatment	1.38* (1.11, 1.7)	1.50 (1.22,1.85)
On-Treatment window	Last dose given + 7 days	Last dose given +7 days
DD		
On-Study	1.14 (1.0, 1.3)	0.96 (0.83, 1.1)
On-Treatment	1.02 (0.88,1.2)	0.95 (0.8, 1.1)
On-Treatment window	Last dose given + 7 days	Last dose given + 28 days

Source: Clinical Reviewer's compilation from public data

* The comparator for roxadustat in the NDD studies was placebo, all other estimates are against ESA

Abbreviations: CI, confidence interval; DD, dialysis dependent; HIF-PHI, hypoxia-inducible factor prolyl-hydroxylase; MACE, major adverse cardiovascular events; NDD, non-dialysis dependent

7.3. Potential Risks or Safety Concerns Identified Through Postmarket Experience

Daprodustat was approved in Japan on June 29, 2020, for the treatment of anemia due to CKD. The Applicant estimates ~11000 PY of exposure has been obtained in Japan up until the cutoff date of June 2021. The Applicant claims there are no new safety concerns with daprodustat based on the available post-marketing data to date.

7.4. FDA Approach to the Safety Review

The safety review of daprodustat was divided into two populations of patients with CKD: those who were not dependent on chronic dialysis, and those who were dependent on chronic dialysis. We viewed the data this way because the patient populations are distinct with regards to cardiovascular physiology, severity of anemia, and patient management.

Of the five phase 3 clinical studies submitted to support this application (2 in NDD, 3 in DD), we focused our safety review on ASCEND-D and ASCEND-ND. ASCEND-D and ASCEND-ND were large, randomized, active controlled trials designed to assess noninferiority for MACE (as a coprimary endpoint). The sample size and study duration required to adequately power for MACE also fulfilled safety database size recommendations in ICH E1. The remaining phase 3 studies, ASCEND-NHQ, ASCEND-TD, and ASCEND-ID were designed to assess specific efficacy objectives (e.g., specific patient populations, dosing, or outcomes). These remaining studies had a limited size and duration, and importantly, unique design elements that limit their utility to formally integrate with the CVOT. Therefore, we concluded that pooling these smaller, disparate studies with the CVOTs was not useful.

ASCEND-D and ASCEND-ND were open-label (sponsor-blind) trials: patients and investigators were aware of treatment assignment, which may have influenced the reporting for adverse event occurrence – particularly for those who were randomized to a treatment that was similar to what they were treated with prior to the study. This reporting bias is likely attenuated for the serious adjudicated outcomes and objective laboratory evaluations. Although we did not identify any obvious signs of reporting bias, we placed particular emphasis on events adjudicated by a blinded committee (see below).

The safety population was defined as all subjects in the randomized population who received at least one dose of study drug and analysis was based on the actual treatment received. The ITT population was defined as all randomized subjects based on planned randomized treatment assignment. In ASCEND-D and ASCEND-ND, there was no meaningful difference between the ITT and safety populations since the number of subjects included in each arm differs by 2 to 5 patients. Nonetheless, we have attempted to maintain consistency throughout our analyses.

The general safety assessment considered subjects to be at risk for the total duration of follow-up regardless of being on or off treatment. FDA generally relied on the on-study (i.e., regardless of treatment discontinuation) analyses to ascertain estimates of treatment differences for all endpoints (e.g., AEs, vitals, laboratory assessments). Through the course of our review, we identified limitations in the prespecified on-treatment definitions⁸ related to disparate dosing frequency of assigned treatment (reference 7.6.1.1.2). We have generally found that the study design, and particularly the disposition of study patients, supports a robust and interpretable estimate of treatment difference from the ASCEND CVOTs.

AEs were reported by verbatim term and coded and categorized using Medical Dictionary for Regulatory Activities (MedDRA) version 24.0. To assess the reliability and quality of the data, the clinical reviewer compared the coding of verbatim reported adverse event terms, the MedDRA lowest coded level term, and preferred term in the safety datasets. No major data quality, translation (i.e., verbatim to preferred) or integrity issues were identified that would preclude performing a safety review for this NDA. In addition to adverse event analyses grouped by MedDRA preferred terms, we used FDA Medical Query (FMQ) groupings. FMQs are a standardized approach to group preferred terms of a similar concept; as such, FMQs consolidate a medical condition with scattered preferred terms and are more likely to identify any signal of safety issues. The review team used FMQ version 2.0. Extensive documentation is available publicly on the Federal Register.⁹

Cumulative incidence proportions estimate of within arm risk were based on incident outcomes. Treatment arm comparisons and confidence intervals were based on marginal, unadjusted, absolute risk differences. We acknowledge that exposure adjusted analyses would be more easily interpretable given the varying duration of follow up for participants in the event driven CVOTs. Nonetheless, the exploratory analyses were conducted to identify any potential safety signals which would then be subject to more rigorous analyses such as time-to-event analyses (e.g., Kaplan-Meier methods). Accordingly, safety signals identified in Section 7.6 which we felt merited potential concern were analyzed appropriately and discussed in detail in Section 7.7.

Key risks identified prior to enrollment were, at least in-part, informed by prior experience with ESAs and HIF-PHIs (Section 7.3). Briefly, major adverse cardiovascular events (MI, CVA), thromboembolism (deep vein thrombosis, pulmonary embolism, vascular access thrombosis [VAT]), and heart failure were key a priori concerns. Other adverse events associated with ESA treatment are of special interest, such as malignancy, hypertension, and seizures. Lastly, the

⁸ For adjudicated events the prespecified on-treatment window was Last Dose Given (LDG) + 28 days. For investigator-reported AEs, the prespecified on-treatment window was LDG + 1 day.

⁹ <https://www.regulations.gov/document/FDA-2022-N-1961-0001>

nonclinical program identified potential risks unique to daprodustat such as thrombosis and gastrointestinal erosions (see Section [7.6.1.8](#) for full listing and analyses).

The primary safety objective of ASCEND-D and ASCEND-ND was to assess NI of daprodustat to ESA in first occurrence of MACE. MACE was a composite endpoint consisting of all-cause mortality (ACM), nonfatal MI and nonfatal stroke. A pre-defined NI margin of 1.25 for the HR (daprodustat versus ESA) was adopted following discussions between the FDA and Applicant during the IND. All-cause mortality, rather than cardiovascular mortality, was included in the composite since ACM represents a clinically important and highly prevalent competing risk in the dialysis and non-dialysis dependent CKD populations. In addition, studies in anemia of CKD with similar designs and objectives (i.e., TREAT, CHOIR, EMERALD, PEARL) used all-cause mortality.

Analyses of individual CV events (ACM, MI, stroke, thromboembolic events, and hospitalization for HF) were conducted as additional secondary endpoints to provide a comprehensive evaluation of CV safety data obtained in the trials. The FDA considered additional exploratory time-to-event endpoints of CV MACE (composite of CV mortality, nonfatal MI, and nonfatal stroke) and VAT,¹⁰ a type of TEE, to be clinically meaningful; these results are presented below. The secondary endpoints and these additional safety endpoints were not controlled for multiple comparisons.

The Applicant used an external Clinical Events Committee (Duke Clinical Research Institute) group to conduct blinded adjudication of all investigator reported events suspected to meet the definition of MACE as well as other adjudicated CV events (including all-cause mortality, thromboembolic events, and hospitalization for HF), as well as select CKD progression events for the ASCEND-ND study. FDA did not review narratives for the adjudicated events or adjudication quality.

During our review, we identified investigator reported serious acute kidney injury (AKI) and gastrointestinal erosions/bleeding as potentially significant harms.

Analysis of MACE and CV Endpoints

The primary population for the analyses of MACE and CV endpoints is the ITT population, defined as all randomized subjects analyzed according to the treatment to which subjects were randomized.

The primary estimand strategy was the treatment policy strategy, referred to as the on-study analysis in the ASCEND-ND and ASCEND-D trials. The period for capturing CV safety endpoints began at randomization and ended at the date of study completion/subject withdrawal,¹¹ with the exception that if a death was reported in the clinical database after this

¹⁰ For VAT, either as a standalone endpoint or as a component of TEE, the event of interest is vascular access that becomes thrombosed.

¹¹ Withdrawal includes withdrawal of consent, lost to follow-up, and study site closure.

time, then the death was included in the analysis.¹² Intercurrent events¹³ such as treatment discontinuation, use of rescue therapies, or changes in other treatments were considered as part of the treatments (daprodustat versus control) being compared.

Within-treatment-group incidence rates, expressed as numbers of events per 100 PY, and CI for primary and secondary endpoints adjusted for follow-up time were calculated using the exact Poisson method.

The primary analysis of time to first MACE and other time-to-event endpoints was a Cox proportional hazards model controlling for treatment and adjusting for the following baseline variables used in stratified randomization: region (Asia Pacific, Eastern Europe/South Africa, Western Europe/Canada/Australia/New Zealand, Latin America, USA), current ESA use (ASCEND-ND only: User, Non-user), and dialysis type (ASCEND-D only: hemodialysis, peritoneal dialysis).

In addition to the hazard ratio estimate from the Cox model, the prespecified, unadjusted incidence rate difference (IRD) between treatments provided an interpretation (without hypothesis testing) on the absolute scale. IRD and CIs between treatments were calculated using Wald's method for normal approximation.

In a supportive analysis of MACE, the Applicant used another estimand strategy called the while-on-treatment strategy, referred to as the On-Treatment (OT) analysis, whereby response to treatment within a time window (ascertainment window) of the intercurrent event is of interest. We considered three variations of the OT analysis accounting for differing dosing intervals in the treatment arms (see Section [7.6.1.1.2](#)). The period for capturing MACE for each variation are the following:

- OT + 28 (prespecified): treatment start date to the earlier of the date of study completion/withdrawal or last non-zero dose date + 28 days.
- OT + Dosing Frequency (exploratory): treatment start date to the earlier of the date of study completion/withdrawal or last non-zero dose date + dosing interval, where the dosing interval was 1 day (daprodustat); 7, 14, or 28 days (darbepoetin alfa); 2 or 7 days (epoetin alfa).
- OT + Dosing Frequency + 28 (exploratory): treatment start date to the earlier of the date of study completion/withdrawal or last non-zero dose date + dosing interval + 28 days.

Sensitivity analyses were conducted using tipping point methods to assess departures from a non-informative censoring mechanism (i.e., missing at random) on the robustness of the primary MACE analysis conclusion. For each treatment arm, the assumed event rates ranged from all prematurely censored subjects who had a MACE at the time of censoring to all prematurely

¹² We note that analyses including deaths after study completion/withdrawal is not customary of survival analysis with a study completion date. Furthermore, including these deaths assumed that patients did not have nonfatal MACE events after the CV follow-up period and allowed for differing follow-up periods that depended on the occurrence of death. We requested the Applicant to conduct exploratory analyses of MACE for the entire population and for select subgroups excluding deaths after the date of study completion/withdrawal. Because these results did not materially change the interpretation of the MACE results that included deaths after study completion/withdrawal, we presented findings that included deaths after study completion/withdrawal.

¹³ Events occurring after treatment initiation that affect either the interpretation or the existence of the measurements associated with the clinical question of interest.

censored subjects who completed the study without experiencing the event. These event rates, including a censored-at-random scenario, were quantified using the log-hazard rate ratio comparing prematurely censored subjects to observed subjects. The multiply imputed time to first occurrence of MACE data were then compared between treatment groups using the primary analysis model of Cox proportional hazards (see Section [17.1](#)).

Subgroup Analysis of MACE

Prespecified subgroup analyses of MACE (without multiplicity control) included the following characteristics at randomization:

- Age category (<65, 65 to <75, ≥75 years)
- Sex (female, male)
- Race (American Indian or Alaskan Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, Mixed Race)¹⁴
- Regions combined (USA, non-USA)
- Current ESA use at randomization (ASCEND-ND only: ESA non-user, ESA user)
- Dialysis type (ASCEND-D only: hemodialysis, peritoneal dialysis)

Exploratory Analyses

Exploratory analyses conducted by FDA included:

- Time-to-event analysis of each adjudicated secondary CV endpoints by region (USA, non-USA)
- Time-to-event analysis of each adjudicated secondary CV endpoints by ESA use at randomization (ASCEND-ND only: ESA non-user, ESA user)
- Time-to-event analysis of HHF by history of heart failure (Yes, No), defined as a having any of the following medical history conditions: heart failure, left ventricular systolic dysfunction, left ventricular diastolic dysfunction, or pulmonary hypertension
- OT + 28 analysis of CV endpoints
- Competing risk analyses of time to first fatal/nonfatal (a) stroke, (b) MI, and (c) TEE with mortality from other causes as the competing event using Fine and Gray's subdistribution hazards regression adjusting for the same factors as the primary analysis for MACE
- Adjudicated CV endpoints in relation to average dose summaries¹⁵
- Adjudicated CV endpoints in relation to Hb summaries¹⁶

¹⁴ Because of model convergence issues for the small race subgroup of Native Hawaiian or Other Pacific Islander, we combined Native Hawaiian or Other Pacific Islander with Mixed Race into a category called "Other" for analysis.

¹⁵ Analysis description and results are discussed in Appendix Section [17.3](#).

¹⁶ Analysis description and results are discussed in Appendix Section [17.4](#).

7.5. Adequacy of the Clinical Safety Database

Each CVOT was adequately powered to rule out the risk of MACE according to the prespecified NI margin. Based on these calculations, the number of subjects and total exposure met ICH E1A recommendations and were sufficient to assess unexpected risks (i.e., signal detection) and general safety. For this reason, the safety database is adequate for the comprehensive safety assessment of daprodustat for the proposed indication, patient population, dosage regimen and duration.

Exposure within each of the 2 large CV outcomes studies was similar across the daprodustat and ESA comparators (Table 70). Greater than 50% of participants were on treatment for at least 18 months (ASCEND-ND) and 2 years (ASCEND-D). It is notable that the overall exposure and overall follow up are balanced between randomized treatment arms for both studies. Of note, a majority (77 to 85%) of the time on study was also on treatment (last row in Table 70).

Table 70. Duration of Exposure, ITT Population, Trials ASCEND-D, ASCEND-ND

Parameter	ASCEND-D		ASCEND-ND	
	Daprodustat N=1487 n (%)	rhEPO N=1477 n (%)	Daprodustat N=1937 n (%)	rhEPO N=1935 n (%)
Duration of treatment, months				
Mean (SD)	22 (11.6)	22.3 (11.5)	18.5 (12)	19 (12.1)
Median (Q1, Q3)	25.8 (10.8, 31.1)	25.8 (12, 31.3)	17.4 (7.4, 28.1)	17.5 (8.3, 28.6)
min, max	0, 45.1	0, 45.1	0.2, 49.7	0, 51.1
Total exposure (person years)	2712	2745	2982	3056
Patients treated, by duration, n (%)				
<6 months	218 (14.7)	204 (13.8)	398 (20.5)	356 (18.4)
≥6 to <12 months	178 (12.0)	167 (11.3)	314 (16.2)	337 (17.4)
≥12 to <24 months	254 (17.1)	271 (18.3)	566 (29.2)	543 (28.1)
≥24 to <36 months	714 (48.0)	696 (47.1)	460 (23.7)	486 (25.1)
≥36 to <48 months	118 (7.9)	136 (9.2)	197 (10.2)	206 (10.6)
≥48 months	0	0	2 (0.1)	5 (0.3)
Cumulative follow-up (patient-years)	3512	3483	3593	3592
Duration of overall follow up (months) (median (Q1, Q3))	29.9 [26.6, 34.6]	29.7 [26.0, 34.8]	22.3 [12.0, 32.2]	22.3 [12.0, 32.4]
Total exposure/follow-up (%)	77%	79%	83%	85%

Source: adex.xpt; Software: R; CDS STF V1

Abbreviations: Dapro, daprodustat; ITT, intent-to-treat; N, number of subjects in treatment arm; n, number of subjects with given treatment duration; Q1, first quartile; Q3, third quartile; rhEPO, recombinant human erythropoietin; SD, standard deviation

All the active-controlled global Phase 3 studies (including ASCEND-D and ASCEND-ND) used the same Hb target range (10 to 11 g/dL, Section 6) and the same protocol-specified dose levels and dosing frequencies in all territories to avoid the potential for difficulty in interpretation of safety data that might have arisen from varying target and actual-achieved levels of Hb.

Of the subjects assigned to ESA in ASCEND-D (n=1477), approximately 11% were on peritoneal dialysis, and thus randomized to darbepoetin alfa (n=169). Those randomized to darbepoetin outside of the United States (n=140) received drug product regulated under the European Union jurisdiction. The clinical reviewer confirmed that the primary safety conclusions (i.e., noninferiority for MACE) were unchanged if subjects assigned EU darbepoetin

were excluded. Additionally, the effect estimates for CV harms (i.e., hazard ratios for mortality, thrombosis, thromboembolism, heart failure using the same methodologies found in this review) were not different when omitting participants who received EU darbepoetin. The sample size (n=29) of US darbepoetin-treated subjects precluded a reliable estimate of risks comparing daprodustat to US darbepoetin in the peritoneal dialysis subgroup or EU-US darbepoetin comparisons. However, there were no other clinical observations of efficacy or safety in ASCEND-D or ASCEND-ND which suggest EU and US darbepoetin alfa have different clinical properties.

7.6. Safety Results

7.6.1. Safety Results, ASCEND-ND

7.6.1.1. MACE

7.6.1.1.1. Overview

Results for the pre-specified primary analysis of time to first MACE are shown in [Table 71](#). The HR (95% CI) from the primary analysis (on-study analysis) for time to first MACE was 1.03 (0.89, 1.19) comparing daprodustat to darbepoetin alfa. The upper bound of the 95% CI was lower than the prespecified risk margin of 1.25, i.e., the study ruled out a relative risk for MACE greater than 1.25. Kaplan-Meier survival curves for MACE overlapped throughout the follow-up period ([Figure 27](#)). The IRD was 0.2 events/100 PY (95% CI -1.3, 1.8) between daprodustat and darbepoetin alfa. The incident events of the MACE (daprodustat 378/1937 [10.9 events/100 PY], darbepoetin alfa 371/1935 [10.6 events/100 PY]) composite endpoint comprised mostly all-cause mortality (daprodustat 252/378, darbepoetin alfa 259/371), followed by nonfatal MI (daprodustat 96/378, darbepoetin alfa 91/371), and nonfatal stroke (daprodustat 30/378, darbepoetin alfa 21/371).

Because both co-primary endpoints (mean change in Hb and time to first MACE) ruled out their respective risk margins, the principal secondary endpoints were tested for superiority by comparing the one-sided superiority p-value against the rank-associated alpha derived from the Holm-Bonferroni method. None of the principal secondary endpoints (MACE, MACE or thromboembolic events, MACE or HHF, progression of CKD) were significant (results not shown).

Tipping point sensitivity analyses showed that the MACE analysis result (HR 1.09; 95% CI 0.4, 1.26) tipped, i.e., did not rule out the 1.25 risk margin, when censored subjects in the daprodustat arm were assumed to have a (worse) hazard rate of 1.7 times that observed among subjects in the daprodustat arm and censored subjects in the darbepoetin alfa arm were assumed to have a (better) hazard rate of 0.4 times that observed among subjects in the darbepoetin alfa arm. For more details (see [Section 17.1](#)). A less extreme assumption (daprodustat 1.7, darbepoetin alfa 0.6) resulted in a HR of 1.08 (95% CI 0.93, 1.25) and did not tip the MACE results. Note that assuming a hazard rate factor of 1.0 in each arm is similar to the non-informative censoring assumption. In summary, the primary analysis results for MACE, assuming non-informative censoring, was tipped only under fairly extreme assumptions of daprodustat having more MACE

events and the active control having fewer MACE events than the observed subjects. Therefore, the primary analysis assuming non-informative censoring could be relied upon for interpretation.

Competing risk analyses of time to first fatal/nonfatal (a) stroke, (b) MI, and (c) TEE with mortality from other causes as the competing event produced estimates that were similar to the primary analyses that censored subjects at death (results not shown). Competing risk analysis of these endpoints did not change the interpretation of their respective primary analysis.

Two other considerations related to the MACE findings are discussed in subsequent sections:

- The supportive OT+28 days analysis of MACE provided HR estimates (HR 1.40; 95% CI 1.17, 1.68) inconsistent with the primary analysis (on-study analysis). See the discussion in Section [7.6.1.1.2](#).
- A limitation to the use of MACE to assess CV risk was that MACE was a composite endpoint prespecified to contain all-cause mortality, therefore, including non-CV deaths. To better understand CV risk, secondary CV endpoints and CV MACE (a composite of CV mortality, nonfatal MI, and nonfatal stroke) were analyzed and are presented in Section [7.6.1.2.1](#).

Table 71. Summary of Results for MACE, ASCEND-ND (On-Study Analysis)

	Daprodustat N=1937 PY=3480	Darbepoetin alfa N=1935 PY=3489	Rate Diff Per 100 PY (95% CI)	Hazard Ratio (95% CI)
MACE, n [IR]	378 [10.9]	371 [10.6]	0.2 (-1.3, 1.8)	1.03 (0.89, 1.19)
All-cause mortality, n (%) ¹	252 (66.7)	259 (69.8)	-	-
Nonfatal myocardial infarction, n (%) ¹	96 (25.4)	91 (24.5)	-	-
Nonfatal stroke, n (%) ¹	30 (7.9)	21 (5.7)	-	-

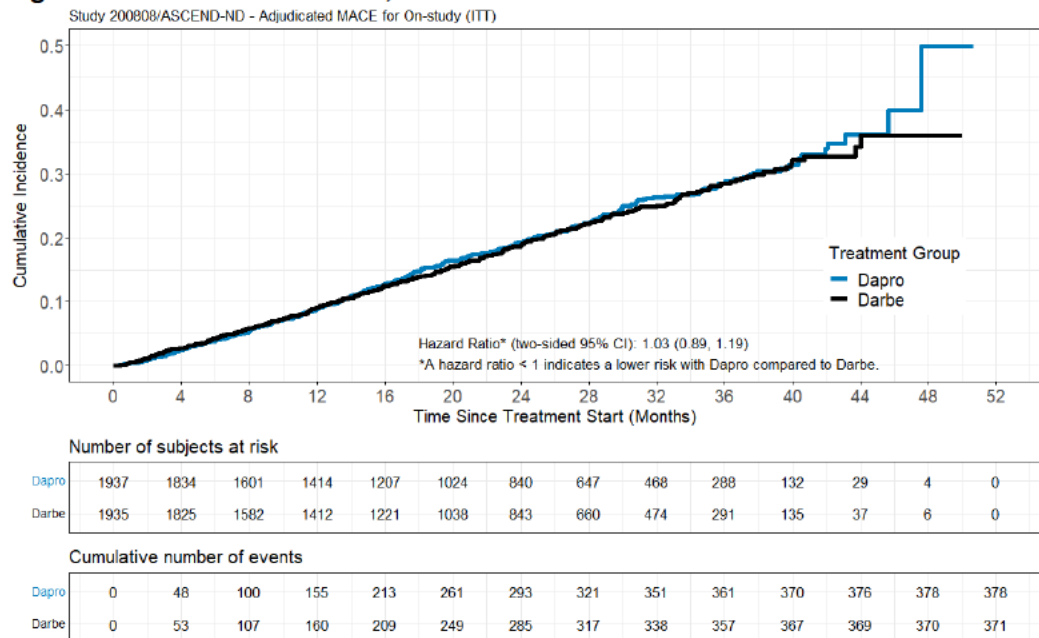
Source: FDA analysis.

Comparative estimates not calculated for MACE components.

¹ Percentage of MACE.

Abbreviations: CI, confidence interval; Diff, difference; IR, incidence rate per 100 PY; MACE, major adverse cardiovascular event; N, number of subjects; n, number of subjects with event; PY, person-year

Figure 27. Time to First MACE, ASCEND-ND



Source: FDA analysis.
Abbreviations: CI, confidence interval; Dapro, Daprodustat; Darbe, darbepoetin alfa; ITT, intention-to-treat; MACE, major adverse cardiovascular event

7.6.1.1.2. On-Study vs. On-Treatment Analyses

To assess risks, two approaches were considered: an on-study analysis and OT analysis. An on-study analysis considers a subject at risk of experiencing an event from the time of randomization until trial discontinuation, which includes events that occur while a subject is on treatment and off treatment (for those subjects who prematurely discontinued treatment but remained in the trial). An OT analysis considers a subject at risk of experiencing an event from the time of randomization to the time of treatment discontinuation plus a pre-defined fixed period of time thereafter (e.g., 28 days). Refer to Section 7.7.1 for discussion of the pros and cons of the two approaches in assessing risks.

In the development program for daprodustat, an on-study analysis approach was the primary analysis, and an OT analysis approach was a supportive analysis. Three variations of the OT analysis accounting for differing treatment dosing intervals, resulting in different ascertainment windows, were considered (see Section 7.7.1 and Table 72).

In ASCEND-ND, the prespecified primary on-study analysis approach captured events from randomization to study completion/withdrawal (mean follow-up duration 1.9 years). Treatment discontinuation was similar between treatment arms (daprodustat 38%, darbepoetin alfa 38%), as was study withdrawal (daprodustat 3%, darbepoetin alfa 3%) and use of rescue therapies (daprodustat 2%, darbepoetin alfa 3%).

The prespecified primary analysis of MACE was on-study (HR 1.03; 95% CI 0.89, 1.19; see Section 7.6.2.1.1). The prespecified supportive OT analysis (OT+28) resulted in a higher incidence of MACE in the daprodustat arm compared to the control arm and an HR estimate (HR 1.40; 95% CI 1.17, 1.68) that was greater than the on-study analysis estimate (Table 72).

Exploratory OT analyses adjusted by the dosing frequencies of the different treatments attenuated the effect estimate; the extent of attenuation depended on the ascertainment window chosen (OT+Dosing Frequency+28: HR 1.18 (95% CI 0.99, 1.40) and OT+Dosing Frequency: HR 1.09 (95% CI 0.89, 1.33)).

In summary, OT analyses produced HR estimates for MACE that were greater than the on-study analysis estimate, and the OT estimates differed depending on the ascertainment window used. However, comparisons based on the OT analyses are likely subject to bias, which cannot easily be corrected through analysis. The etiology and extent of this bias is thoroughly discussed in other work.¹⁷ The design and conduct of ASCEND-ND was sufficient to evaluate and estimate risks based on an on-study analysis approach.

Table 72. MACE On-Study and On-Treatment Analyses, ASCEND-ND

	Daprodustat N=1937 ^a PY=3480 ^a n [IR]	Darbepoetin alfa N=1935 ^a PY=3489 ^a n [IR]	Hazard Ratio (95% CI)
On-study (primary)	378 [10.9]	371 [10.6]	1.03 (0.89, 1.19)
On-treatment + 28 days (supportive)	274 [9.9]	202 [7.2]	1.40 (1.17, 1.68)
On-treatment + dosing frequency + 28 days (exploratory)	275 [10.0]	248 [8.6]	1.18 (0.99, 1.40)
On-treatment + dosing frequency (exploratory)	192 [7.3]	189 [6.8]	1.09 (0.89, 1.33)

Source: FDA analysis.

MACE: Composite of all-cause mortality, nonfatal MI, and nonfatal stroke.

Dosing frequency: Dapro: daily; Darbe: weekly, every 2 weeks, or every 4 weeks.

^a Number of subjects (N) and person-years (PY) correspond to the on-study (primary) analysis only.

Abbreviations: CI, confidence interval; IR, incidence rate per 100 PY; MACE, major adverse cardiovascular event; MI, myocardial infarction; N, number of subjects; n, number of subjects with event; PY, person-year

7.6.1.1.3. Subgroup Analyses

Results of the prespecified subgroup analyses of MACE are shown in [Figure 27](#). Estimated HRs for MACE were greater than 1.0 comparing daprodustat to darbepoetin alfa in the older age categories, among American Indian or Alaskan Native (HR 1.41; 95% CI 0.80, 2.49), among Black or African American (1.19; 95% CI 0.70, 2.02), among White (1.12; 95% CI 0.93, 1.34), in the USA region (HR 1.19; 95% CI 0.91, 1.54), and among ESA users at randomization (HR 1.12; 95% CI 0.91, 1.36). Key considerations with the subgroup analyses included:

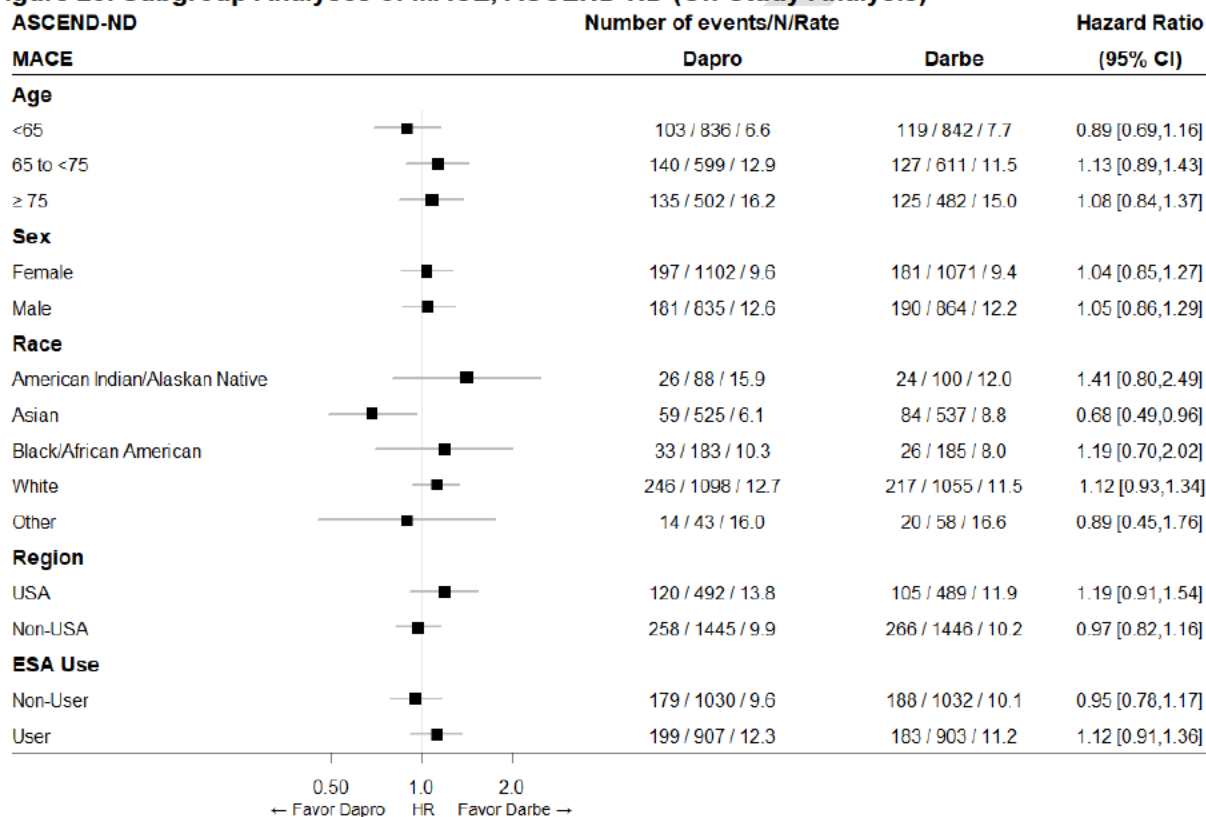
- In general, the chance of observing a signal that is incongruous with the overall study population is non-negligible. Therefore, the signal could be an overestimation of the truth.
- The low event rates and small samples sizes for the non-White race subgroups limited the interpretability of those analyses.
- The higher incidence of MACE in the daprodustat arm for the prespecified USA subgroup and ESA user subgroup were of particular interest because the USA is under FDA's jurisdiction and ESA is the standard of care. These subgroup sample sizes were moderate, approximately 500 (USA subgroup) and 900 (ESA-user subgroup) subjects,

¹⁷ <https://www.nejm.org/doi/full/10.1056/NEJMc2208182>

per treatment arm, and the treatment effect estimates were relatively precise compared to the other subgroups.

- Exploratory analyses across the secondary CV endpoints also showed higher risk estimates in the USA subgroup compared to the non-USA subgroup in the NDD population. This is discussed in Section 7.6.1.2.2. Exploratory analyses of ESA subgroups for the secondary CV endpoints did not indicate a consistently higher risk among ESA users compared to non-users (see Section 17.5).

Figure 28. Subgroup Analyses of MACE, ASCEND-ND (On-Study Analysis)



Source: FDA analysis.

MACE: Composite of all-cause mortality, nonfatal MI, and nonfatal stroke.

Rate: Incidence rate per 100 PY.

Abbreviations: CI, confidence interval; Dapro, daprodustat; Darbe, darbepoetin alfa; ESA, erythropoietin-stimulating agent; HR, hazard ratio; MACE, major adverse cardiovascular event; N, number of subjects; USA, United States of America

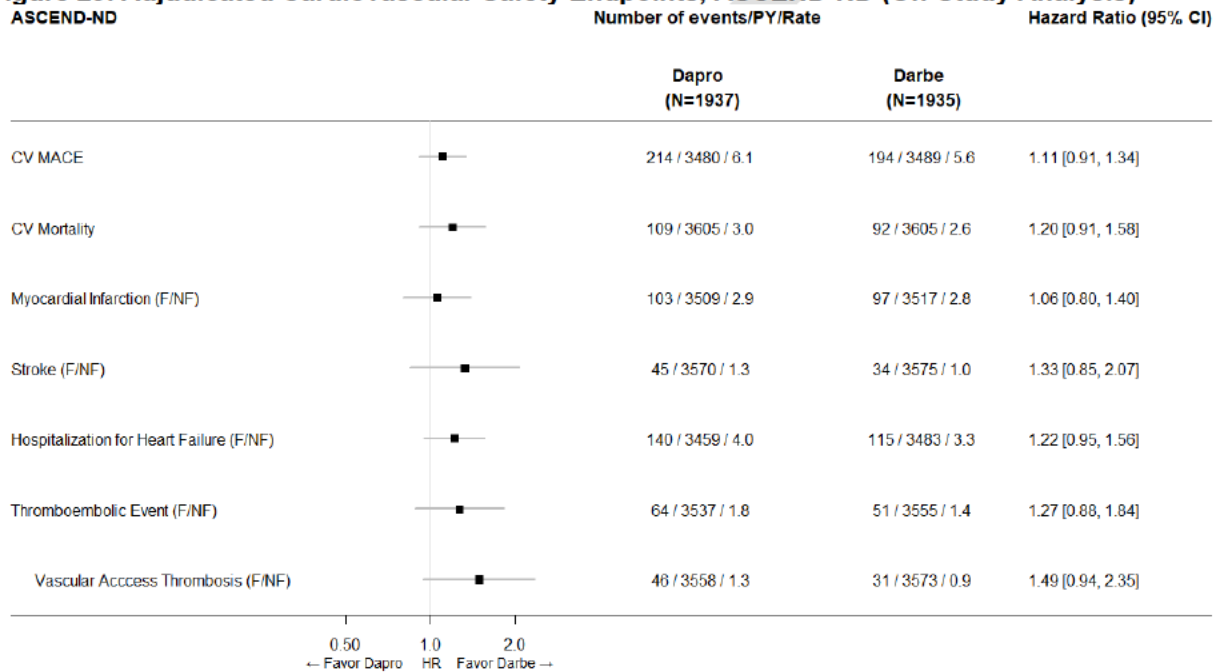
7.6.1.2. CV Endpoints

7.6.1.2.1. Overview

To better understand CV risk, adjudicated CV endpoints, not including all-cause mortality, were assessed. Results of the primary (on-study) time-to-event analysis of CV endpoints in ASCEND-ND are shown in Figure 29. As shown in the figure, the estimated HRs for CV MACE, CV mortality, MI, stroke, HHF, TEE, and VAT are all increased for the comparison of daprodustat to darbepoetin alfa, ranging from 1.06 to 1.49. The corresponding IRDs (per 100-person years) ranged from 0.2 to 0.7 (Appendix Section 17.6).

Limitations of these analyses included lower precision (compared to MACE) because of lower event rates (and hence wider 95% CIs) and no Type I error control for treatment arm comparisons of the CV endpoints. However, in ASCEND-ND, the consistently increased risk estimates across different CV endpoints measuring related aspects of CV risk in a CV outcomes trial raises concerns as to whether daprodustat is safe relative to darbepoetin alfa, which itself carries an increased CV risk. In ASCEND-D, there was not a consistent pattern of increased risk estimates in the CV endpoints (Section 7.6.2.2). The reasons for discrepant trials results for CV safety are not clear.

Figure 29. Adjudicated Cardiovascular Safety Endpoints, ASCEND-ND (On-Study Analysis)



Source: FDA analysis.

CV MACE: Composite of CV mortality, nonfatal MI, nonfatal stroke.

Rate: Number of events per 100 PY.

Abbreviations: CI, confidence interval; CV, cardiovascular; Dapro, daprodustat; Darbe, darbepoetin alfa; F/NF, fatal or nonfatal; HR, hazard ratio; MI, myocardial infarction; PY, person-year

7.6.1.2.2. Regional Subgroup Analyses

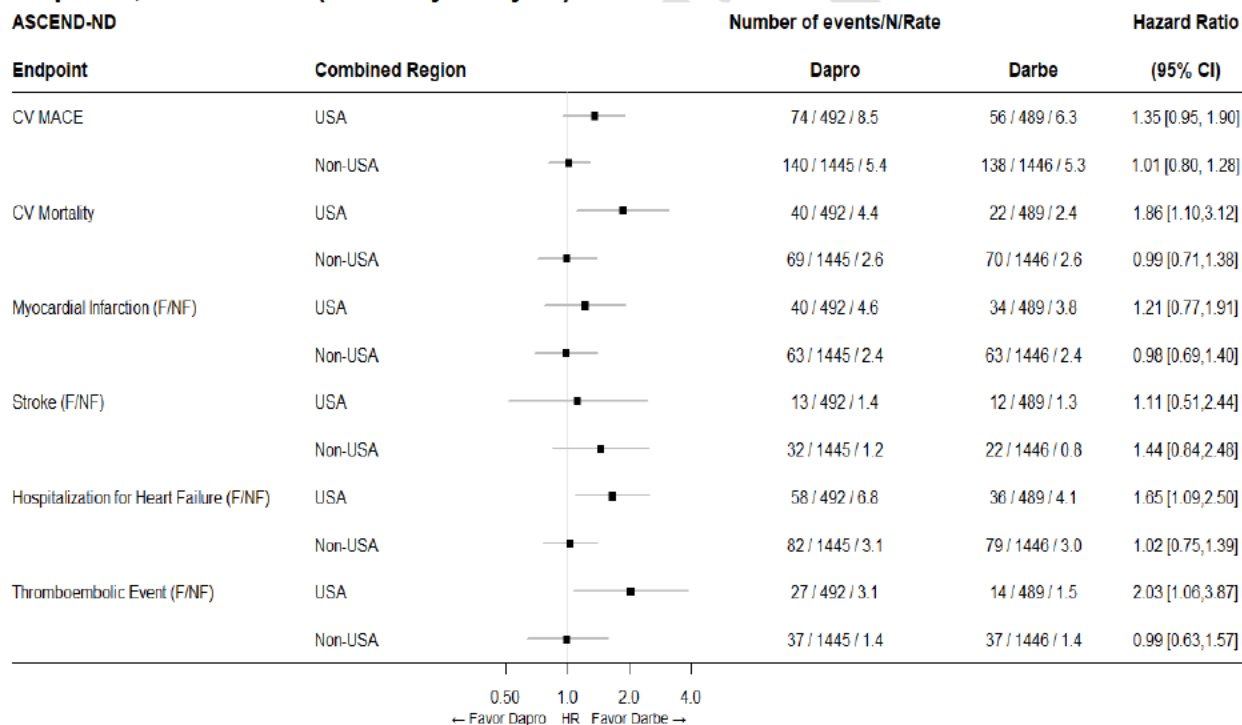
To further explore CV endpoints in the regional subgroups, exploratory analyses were conducted for each adjudicated CV endpoint in the USA and non-USA subgroups. A higher incidence of each CV endpoint was observed in the daprodustat arm compared to the darbepoetin alfa arm in the USA region (Figure 30). Except stroke, the non-USA subgroup HRs were approximately 1.0, and USA subgroup HRs were elevated (ranging from 1.21 to 2.03). The variability (95% CI widths) of HR estimates depended on the number of events and sample sizes, with lower precision for the USA subgroup compared to the non-USA subgroup and for endpoints like stroke and TEE.

There were differences in baseline characteristics of the USA compared to the non-USA subgroup. USA subjects used less ESAs at baseline, which was also reflected in baseline Hb category, and generally had more comorbid conditions at baseline (history of diabetes, heart

failure, cardiovascular disease). In addition, there were differences in enrollment by race between USA and non-USA subgroups (see Section 17.5). It is unclear if these differences contributed to the discrepant results between the USA and non-USA subgroups.

Although exploratory analyses could introduce bias and produce unreliable results, the higher risk estimates in the USA subgroup compared to the non-USA subgroup in the NDD population were seen across multiple CV endpoints and were consistent with the unfavorable MACE prespecified region subgroup results (Section 7.6.1.1.3). In summary, these higher incidences of CV endpoints in the daprodustat arm in the USA subgroup raised concerns as to whether daprodustat is safe relative to darbepoetin alfa, which itself carries an increased CV risk.

Figure 30. Combined Region (USA, non-USA) Subgroup Analyses for All Adjudicated CV Endpoints, ASCEND-ND (On-Study Analysis)



Source: FDA analysis.

CV MACE: Composite of CV mortality, nonfatal MI, and nonfatal stroke.

Rate: Incidence rate per 100 person-years.

Abbreviations: CI, confidence interval; CV, cardiovascular; Dapro, daprodustat; Darbe, darbepoetin alfa; F/NF, fatal or nonfatal; HR, hazard ratio; MACE, major adverse cardiovascular events; MI, myocardial infarction; USA, United States of America

7.6.1.2.3. Hospitalization for Heart Failure Subgroup Analyses

Exploratory subgroup analyses by history of heart failure (Yes, No) showed a higher incidence of HHF in the daprodustat arm compared to the control arm in the subgroup with a history of heart failure (Table 73). The HR for the subgroup with history of heart failure was 1.51 (95% CI 1.01, 2.25). In contrast, the HR for the subgroup without a history of heart failure was 0.99 (95% CI 0.72, 1.36).

Table 73. History of Heart Failure Subgroup Analysis¹ for Hospitalization for Heart Failure, ASCEND-ND (On-Study Analysis)

	Daprodustat n/N [IR]	Darbepoetin alfa n/N [IR]	Hazard Ratio (95% CI)
History of heart failure	65/348 [11.2]	38/339 [7.0]	1.51 (1.01, 2.25)
No history of heart failure	75/1586 [2.6]	76/1593 [2.6]	0.99 (0.72, 1.36)

Source: FDA analysis.

Incident Rate (IR): Number of events per 100 PY.

History of heart failure defined as having the medical history conditions: heart failure, left ventricular systolic dysfunction, left ventricular diastolic dysfunction, or pulmonary hypertension.

¹ Analyses did not include six subjects with missing history of heart failure.

Abbreviations: CI, confidence interval; PY, person-year

7.6.1.3. Overview of Treatment-Emergent Adverse Events Summary

Table 74 provides a frequency-based overview of treatment-emergent adverse events (TEAEs) reported in the safety population during the on-study period for ASCEND-ND. Based on the frequency-based analysis, daprodustat is associated with a higher rate of AEs leading to permanent discontinuation and AEs leading to dosage modification of study drug. However, the clinical relevance of these treatment differences cannot be interpreted without more granular data, which are presented and discussed in more detail in the next several sections. Otherwise, there were no remarkable differences between study arms.

Table 74. Overview of On-Study Adverse Events, ITT Population, ASCEND-ND

Event Category	ASCEND-ND Daprodustat N=1937 n (%)	ASCEND-ND rhEPO N=1935 n (%)	ASCEND-ND RD (%) (95% CI)
SAE	971 (50.1)	956 (49.4)	0.7 (-2.4, 3.9)
SAEs with fatal outcome	301 (15.5)	296 (15.3)	0.2 (-2.0, 2.5)
Life-threatening SAEs	54 (2.8)	59 (3.0)	-0.3 (-1.3, 0.8)
AE leading to permanent discontinuation of study drug	297 (15.3)	257 (13.3)	2.1 (-0.2, 4.3)
AE leading to dose modification of study drug	168 (8.7)	85 (4.4)	4.3 (2.7, 5.8)
AE leading to interruption of study drug	166 (8.6)	84 (4.3)	4.2 (2.7, 5.8)
AE leading to reduction of study drug	2 (0.1)	1 (0.05)	0.1 (-0.1, 0.2)
AE leading to dose delay of study drug	0	0	0 (0, 0)
AE	1627 (84.0)	1645 (85.0)	-1.0 (-3.3, 1.3)
Severe	377 (19.5)	360 (18.6)	0.9 (-1.6, 3.3)
Moderate	605 (31.2)	635 (32.8)	-1.6 (-4.5, 1.4)
Mild	290 (15.0)	295 (15.2)	-0.3 (-2.5, 2.0)

Source: adae.xpt; Software: R

Treatment-emergent adverse events defined as any AEs occurring or worsening between the first dose and the end of the follow-up period.

Risk difference and risk ratio (with 95% confidence interval) are shown between treatment and comparator. Severity as assessed by the investigator.

Abbreviations: AE, adverse event; CI, confidence interval; Dapro, daprodustat; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients with at least one event; RD, risk difference; rhEPO, recombinant human erythropoietin; SAE, serious adverse event

7.6.1.4. Deaths

Adjudicated causes of death in the On-Study analysis are summarized in [Table 75](#). In the ASCEND-ND study, all-cause mortality, a prespecified secondary endpoint, was similar between arms (HR 1.03; CI 0.87, 1.20) (see Section 17 for survival curves). There were 301/1937 deaths [8.3 deaths/100 PY] in the daprodustat arm and 298/1935 deaths [8.3 deaths/100 PY] in the darbepoetin alfa arm. The incidence of death from adjudicated cardiovascular causes was higher in subjects who received daprodustat. Death from undetermined cause was lower with daprodustat.

Importantly, undetermined deaths need to be interpreted with caution – as many of these are likely to be cardiovascular related. Thus, treatment effect estimates of adjudicated, cause specific mortalities was interpreted cautiously. Overall, we did not observe meaningful trends in mortality causes, and in aggregate, the rates of all-cause mortality are comparable between the two treatment arms

Table 75. Adjudicated Causes of Death—ASCEND-ND, (On-Study Analysis)

	Daprodustat N=1937	Darbepoetin alfa N=1935	Relative Risk	Risk Difference (%)
CV Death	109 (5.6%)	92 (4.8%)	1.2 [0.9, 1.6]	0.9 [-0.5, 2.3]
Heart failure	20 (1%)	13 (0.7%)	1.5 [0.8, 3.1]	0.4 [-0.2, 0.9]
Stroke	14 (0.7%)	11 (0.6%)	1.3 [0.6, 2.8]	0.2 [-0.4, 0.7]
Sudden cardiac death	34 (1.8%)	31 (1.6%)	1.1 [0.7, 1.8]	0.2 [-0.7, 1.0]
Acute myocardial infarction	9 (0.5%)	9 (0.5%)	1.0 [0.4, 2.5]	0.0 [-0.4, 0.4]
Presumed sudden death	11 (0.6%)	11 (0.6%)	1.0 [0.4, 2.3]	0.0 [-0.5, 0.5]
Presumed CV death	9 (0.5%)	11 (0.6%)	0.8 [0.3, 2.0]	-0.1 [-0.6, 0.4]
Non-CV Death	149 (7.7%)	148 (7.7%)	1.0 [0.8, 1.3]	0.0 [-1.6, 1.7]
Pulmonary	6 (0.3%)	5 (0.3%)	1.2 [0.4, 4.0]	0.0 [-0.3, 0.4]
Infection (includes sepsis)	90 (4.7%)	90 (4.7%)	1 [0.8, 1.3]	0.0 [-1.3, 1.3]
Renal	20 (1%)	22 (1.1%)	0.9 [0.5, 1.7]	-0.1 [-0.8, 0.6]
Malignancy	11 (0.6%)	14 (0.7%)	0.8 [0.4, 1.7]	-0.2 [-0.7, 0.4]
Undetermined	43 (2.2%)	58 (3%)	0.7 [0.5, 1.1]	-0.8 [-1.8, 0.2]

Source: Clinical Reviewer, R v. 4.2, ADSL.xpt; ADEVENT.xpt.

ITT population, groups correspond to planned treatment assignment. Events occurring 10 times or more are included. Ordered by risk difference.

Abbreviations: CI, confidence interval; CV, cardiovascular; ITT, intention-to-treat; N, number of patients in treatment arm; n, number of patients with adverse event

7.6.1.5. Serious Treatment-Emergent Adverse Events

[Table 76](#) provides a frequency-based comparison of SAE occurrence, by system organ class (SOC) and preferred term (PT). [Table 77](#) provides a frequency-based comparison of SAE occurrence, by narrow FMQ. Treatment emergent serious adverse events in the ITT population are reported below if they occurred in at least 1% of subjects in either arm. The intent for this threshold, while somewhat arbitrary, is to identify potential safety signals which would then undergo additional assessment to determine causality and estimate risk potential.

We noted the following potential safety issues:

- **Cardiovascular:** The treatment difference not favoring daprodustat in the proportion of subjects who were reported to have a SAE in the MedDRA Cardiac Disorders System

Organ Class is noteworthy (RD [95% CI]: 2.1 [0.0 - 4.2]). The MedDRA PTs contributing to most of the SOC treatment difference are ‘Cardiac failure, congestive’, ‘Cardiac failure’, ‘Myocardial infarction’, and ‘Cardiac arrest’. The first two PTs (‘Cardiac failure, congestive’, and ‘Cardiac failure’) and second two PTs (‘Myocardial infarction’, ‘Cardiac arrest’) may represent the same medical concept, but the clinical presentation may differ slightly or may represent heterogeneous reporting by the investigator. The treatment difference in FMQs, which counts these similar terms together, was 1.3% (-0.1 – 2.8) for heart failure, and 0.2 to 0.5% for myocardial infarction/ischemia/acute coronary syndrome.

- **Thrombosis:** There is a treatment difference not favoring daprodustat for the FMQ of thrombosis (RD of 0.9%). The treatment difference seems to be driven by arterial events, a nFMQ of arterial thrombosis (RD 1.0) is nested within the thrombosis nFMQ and includes MI and CVA (but excludes venous thromboembolism). Similar signals were seen for nFMQs of myocardial infarction (RD: 0.4) and myocardial ischemia (RD: 0.5) and acute coronary syndrome (RD: 0.2). Importantly these cover similar concepts in the continuum of acute coronary syndrome and are correlated (i.e., these queries include overlapping PTs) and thus not directly additive. There was a treatment difference not favoring daprodustat in the Stroke/TIA nFMQ (RD: 1.4) which seems notable, given the small proportion of ESA treated subjects with an event reported (3.3% in daprodustat and 1.9% in ESA).
- **Renal outcomes:** The treatment difference not favoring daprodustat in proportion of subjects who were reported to have a SAE in the MedDRA Renal System Organ Class is noteworthy (RD [95%CI]: 3.1 [0.8 - 5.4]). The PTs contributing mostly to this difference are AKI, CKD, and azotemia. Additionally, a treatment difference not favoring daprodustat in the proportion of subjects who were reported to have a SAE nFMQ for AKI (RD 1.5) emerged.
- **Gastrointestinal bleeding:** There was slight imbalance in the PT for serious gastrointestinal hemorrhage (RD 0.4%), and the Gastrointestinal SOC (0.9% RD) not favoring daprodustat. Additionally, the RD based on the FMQ for serious hemorrhage (RD 0.7) was consistent with the PT analysis. In isolation (i.e., only considering the readout from this analysis) one might reasonably suspect this treatment difference may be due to chance alone. However, gastrointestinal erosions were a prespecified adverse event of special interest (AESI) and were seen in the nonclinical program.

The safety signals for thrombosis and the cardiovascular organ class cover similar or overlapping concepts. Additionally, the safety issues covered by these medical concepts were prespecified and adjudicated as part of a primary composite endpoint (MACE) or as secondary endpoints (HHF, CVA, MI, TEE) and are major safety review issues (Sections [7.6.1.1](#), [7.6.1.2](#), and [7.6.1.8](#)). Nonetheless, the investigator-reported TESAEs provide further support for safety findings observed in primary safety and key secondary safety CV endpoints (see discussion in AESI section).

We considered the treatment differences not favoring daprodustat for gastrointestinal bleeding and AKI major safety review issues (see Section [7.7](#)). There were no other concerning safety signals for SAEs based on PTs, narrow FMQs and SOC occurring at an incidence of <1% (data not shown).

Table 76. Patients With On-Study Serious Adverse Events by System Organ Class and Preferred Term, Occurring in at Least 1% of Subjects in Any Arm, ITT Population, ASCEND-ND

	Daprodustat N=1937 n (%)	rhEPO N=1935 n (%)	RD (%) (95% CI)
Cardiac disorders (SOC)	256 (13.2)	215 (11.1)	2.1 (0.0, 4.2)
Cardiac failure congestive	39 (2.0)	34 (1.8)	0.3 (-0.6, 1.1)
Myocardial infarction	23 (1.2)	14 (0.7)	0.5 (-0.1, 1.1)
Cardiac arrest	25 (1.3)	19 (1.0)	0.3 (-0.4, 1.0)
Cardiac failure	39 (2.0)	30 (1.6)	0.5 (-0.4, 1.3)
Angina unstable	16 (0.8)	21 (1.1)	-0.3 (-0.9, 0.4)
Acute myocardial infarction	43 (2.2)	43 (2.2)	-0.0 (-0.9, 0.9)
Infections and infestations (SOC)	389 (20.1)	385 (19.9)	0.2 (-2.3, 2.7)
Peritonitis	28 (1.4)	18 (0.9)	0.5 (-0.2, 1.2)
Cellulitis	20 (1.0)	17 (0.9)	0.2 (-0.5, 0.8)
Pneumonia	99 (5.1)	112 (5.8)	-0.7 (-2.1, 0.8)
Sepsis	29 (1.5)	31 (1.6)	-0.1 (-0.9, 0.7)
Urinary tract infection	43 (2.2)	50 (2.6)	-0.4 (-1.3, 0.6)
Covid-19	60 (3.1)	56 (2.9)	0.2 (-0.9, 1.3)
Septic shock	20 (1.0)	24 (1.2)	-0.2 (-0.9, 0.5)
Metabolism and nutrition disorders (SOC)	141 (7.3)	131 (6.8)	0.5 (-1.1, 2.1)
Fluid overload	33 (1.7)	30 (1.6)	0.2 (-0.6, 1.0)
Hyperkalemia	33 (1.7)	33 (1.7)	-0.0 (-0.8, 0.8)
Renal and urinary disorders (SOC)	338 (17.4)	278 (14.4)	3.1 (0.8, 5.4)
Acute kidney injury	87 (4.5)	58 (3.0)	1.5 (0.3, 2.7)
Chronic kidney disease	92 (4.7)	69 (3.6)	1.2 (-0.1, 2.4)
Azotemia	61 (3.1)	49 (2.5)	0.6 (-0.4, 1.7)
End stage renal disease	55 (2.8)	52 (2.7)	0.2 (-0.9, 1.2)
Renal impairment	24 (1.2)	18 (0.9)	0.3 (-0.3, 1.0)
Renal failure	18 (0.9)	20 (1.0)	-0.1 (-0.7, 0.5)
Vascular disorders (SOC)	98 (5.1)	103 (5.3)	-0.3 (-1.7, 1.1)
Hypertension	19 (1.0)	15 (0.8)	0.2 (-0.4, 0.8)

Source: adae.xpt; Software: R

Treatment-emergent adverse events defined as any AEs occurring or worsening between the first dose and the end of the follow-up period.

Serious adverse events defined as any untoward medical occurrence that, at any dose results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.

Risk difference and risk ratio (with 95% confidence interval) are shown between treatment and comparator.

Abbreviations: AE, adverse event; CI, confidence interval; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients with adverse event; RD, risk difference; rhEPO, recombinant human erythropoietin; RR, risk ratio; SOC, system organ class

Table 77. Patients With On-Study Serious Adverse Events by System Organ Class and FDA Medical Query (Narrow), Occurring in at Least 1% of Subjects in Any Arm, ITT Population, ASCEND-ND

System Organ Class FMQ (Narrow)	ASCEND-ND		
	Daprodustat N=1937 n (%)	rhEPO N=1935 n (%)	RD (%) (95% CI)
Blood and lymphatic system disorders (SOC)			
Thrombosis	134 (6.9)	116 (6.0)	0.9 (-0.6, 2.5)
Anemia	48 (2.5)	62 (3.2)	-0.7 (-1.8, 0.3)
Thrombosis arterial	114 (5.9)	95 (4.9)	1.0 (-0.4, 2.4)
Thrombosis Venous	22 (1.1)	29 (1.5)	-0.4 (-1.1, 0.4)

System Organ Class FMQ (Narrow)	ASCEND-ND		
	Daprodustat N=1937 n (%)	rhEPO N=1935 n (%)	RD (%) (95% CI)
Cardiac disorders (SOC)			
Heart failure	123 (6.4)	97 (5.0)	1.3 (-0.1, 2.8)
Systemic hypertension	37 (1.9)	36 (1.9)	0.0 (-0.8, 0.9)
Myocardial infarction	65 (3.4)	57 (2.9)	0.4 (-0.7, 1.5)
Myocardial ischemia	100 (5.2)	90 (4.7)	0.5 (-0.8, 1.9)
Acute coronary syndrome	82 (4.2)	78 (4.0)	0.2 (-1.1, 1.5)
Arrhythmia	35 (1.8)	33 (1.7)	0.1 (-0.7, 0.9)
Endocrine disorders (SOC)			
Hyperglycemia	35 (1.8)	33 (1.7)	0.1 (-0.7, 0.9)
Hypoglycemia	20 (1.0)	17 (0.9)	0.2 (-0.5, 0.8)
General disorders and administration site conditions (SOC)			
Fall	11 (0.6)	11 (0.6)	-0.0 (-0.5, 0.5)
Infections and infestations (SOC)			
Bacterial infection	207 (10.7)	212 (11.0)	-0.3 (-2.2, 1.7)
Purulent material	12 (0.6)	14 (0.7)	-0.1 (-0.6, 0.4)
Viral infection	21 (1.1)	18 (0.9)	0.2 (-0.5, 0.8)
Pneumonia	112 (5.8)	129 (6.7)	-0.9 (-2.4, 0.6)
Musculoskeletal and connective tissue disorders (SOC)			
Osteoporosis	18 (0.9)	23 (1.2)	-0.3 (-0.9, 0.4)
Fracture	49 (2.5)	63 (3.3)	-0.7 (-1.8, 0.3)
Neoplasms benign, malignant and unspecified (incl cysts and polyps) (SOC)			
Malignancy	46 (2.4)	52 (2.7)	-0.3 (-1.3, 0.7)
Nervous system disorders (SOC)			
Stroke TIA	63 (3.3)	36 (1.9)	1.4 (0.4, 2.4)
Seizure	6 (0.3)	6 (0.3)	-0.0 (-0.4, 0.3)
Renal and urinary disorders (SOC)			
Acute kidney injury	94 (4.9)	64 (3.3)	1.5 (0.3, 2.8)
Renal and urinary tract infection	71 (3.7)	76 (3.9)	-0.3 (-1.5, 0.9)
Respiratory, thoracic, and mediastinal disorders (SOC)			
Dyspnea	17 (0.9)	16 (0.8)	0.1 (-0.5, 0.6)
Respiratory failure	42 (2.2)	40 (2.1)	0.1 (-0.8, 1.0)
Vascular disorders (SOC)			
Hemorrhage	86 (4.4)	72 (3.7)	0.7 (-0.5, 2.0)
Hypotension	19 (1.0)	18 (0.9)	0.1 (-0.6, 0.7)

Source: adae.xpt; Software: R

Treatment-emergent adverse events defined as any AEs occurring or worsening between the first dose and the end of the follow-up period.

Serious adverse events defined as any untoward medical occurrence that, at any dose results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.

Duration is 52 months.

Risk difference and risk ratio (with 95% confidence interval) are shown between treatment and comparator.

Each FMQ is aligned to a single SOC based on clinical judgment. However, please be aware that some FMQs may contain PTs from more than one SOC.

Abbreviations: AE, adverse event; CI, confidence interval; FMQ, FDA medical query; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients with adverse event; NA, not applicable; RD, risk difference; rhEPO, recombinant human erythropoietin; SOC, system organ class; TIA, transient ischemic attack

7.6.1.6. Adverse Events Leading to Treatment Discontinuation

In ASCEND-ND, daprodustat was associated with higher rate of AEs leading to permanent discontinuation, compared to darbepoetin alfa (15.3% versus 13.3%). As shown in [Table 78](#), most of this 2% treatment difference is attributable to the following AEs: thrombosis (Stroke/TIA and MI), bacterial infection, and hemorrhage. In aggregate, the treatment difference not favoring daprodustat is relatively small, and based on the 95% CI, could very likely be due to chance alone. When inspecting lower-level terms, such as the PT or FMQ, we did not find any revealing trends. Nonetheless, the isolation of the treatment difference to thrombosis and hemorrhage are addressed as major safety review issues given similar findings in other analyses (e.g., SAEs, MACE endpoint analyses). Bacterial infection, while imbalanced (22 vs. 11 subjects reported to have discontinued treatment due to this adverse event class), did not rise to the level of a review issue, because the imbalance was not isolated to a specific medical concept (e.g., fungal, bacterial, or viral, or infection loci), and supporting queries (such as all TEAEs, SAEs) did not establish a convincing signal.

Table 78. Patients With On-Study Adverse Events Leading to Treatment Discontinuation by System Organ Class and FDA Medical Query (Narrow), Occurring in at Least 1% of Subjects in Any Arm, ITT Population, Trials ASCEND-D, ASCEND-ND, and Pooled Trials

System Organ Class FMQ (Narrow)	ASCEND-ND		RD (%) (95% CI)
	Daprodustat N=1937 n (%)	rhEPO N=1935 n (%)	
Blood and lymphatic system disorders (SOC)			
Thrombosis	26 (1.3)	17 (0.9)	0.5 (-0.2, 1.1)
Cardiac disorders (SOC)			
Myocardial Infarction	21 (1.1)	13 (0.7)	0.4 (-0.2, 1.0)
Infections and infestations (SOC)			
Bacterial Infection	22 (1.1)	11 (0.6)	0.6 (-0.0, 1.1)
Neoplasms benign, malignant and unspecified (incl cysts and polyps) (SOC)			
Malignancy	35 (1.8)	34 (1.8)	0.0 (-0.8, 0.9)
Nervous system disorders (SOC)			
Stroke TIA	14 (0.7)	4 (0.2)	0.5 (0.1, 0.9)
Respiratory, thoracic and mediastinal disorders (SOC)			
Respiratory Failure	13 (0.7)	11 (0.6)	0.1 (-0.4, 0.6)
Vascular disorders (SOC)			
Hemorrhage	13 (0.7)	4 (0.2)	0.5 (0.0, 0.9)

Source: adae.xpt; Software: R

Treatment-emergent adverse events defined as any AEs occurring or worsening between the first dose and the end of the follow-up period.

Risk difference and risk ratio (with 95% confidence interval) are shown between treatment and comparator.

Each FMQ is aligned to a single SOC based on clinical judgment. However, some FMQs may contain PTs from more than one SOC.

Abbreviations: AE, adverse event; CI, confidence interval; FMQ, FDA medical query; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients with adverse event; NA, not applicable; RD, risk difference; rhEPO, recombinant human erythropoietin; SOC, system organ class; TIA, transient ischemic attack

7.6.1.7. Treatment-Emergent Adverse Events

[Table 79](#) provides a frequency-based comparison of common TEAEs reported in the safety population during the on-study period for the NDD population. This table includes AEs

occurring in greater than 5% in any arm, and with a nominal risk difference (in either direction, favoring or disfavoring daprodustat) of 1%. We cautiously interpreted some nonserious AEs treatment differences, as they are liable to ascertainment bias in an open label study (see Section 7.5). The frequency-based analysis demonstrated that:

- Events related to thrombosis and thromboembolism, AKI, and gastrointestinal bleeding/ulcerations had a treatment difference not favoring daprodustat, which were also detected in the SAE analysis (highlighted in blue). These AEs are discussed in detail in Section 7.6.1.8 and Section 7.7 as major safety review issues. Since the TEAEs also include serious AEs, we considered if any of these adverse events could be considered reinforcing to serious AEs. We did not identify treatment differences in potentially nonserious presentations of identified serious events (e.g., unspecified chest pain versus incident unstable angina).
- Small imbalances not favoring daprodustat for pruritis (1.4% RD) which occurred infrequently (5.5% vs. 4.1% with comparator), dyspepsia, nausea, and vomiting, which each occurred in ~1.3% more patients than the comparator, with 3.8% to 5.7% of comparator patients reporting such events.

The nonserious treatment differences not favoring daprodustat of pruritis, dyspepsia, nausea, and vomiting are reversible, showed small treatment differences (i.e., less than 2% difference in proportion of subjects reported), and were generally manageable. Importantly, these adverse events did not emerge as leading to treatment discontinuation. For these reasons, we felt that the level of concern for these common AEs did not raise to a safety review issue (i.e., contribute substantially to the benefit-risk assessment of the drug), but rather, could be labeled and considered in individual patient/physician decision making.

There were no clinically significant differences between study arms in the remainder of the TEAE groupings.

Table 79. Patients With Common Treatment-Emergent Adverse Events by System Organ Class by PT and FDA Medical Query (Narrow) Occurring at ≥5% Frequency in Either Arm, With at Least 1% Difference in Reporting Frequency Between Arms, On Study, ASCEND-ND

AE	Daprodustat N=1937 n (%)	rhEPO N=1935 n (%)	Relative Risk (95% CI)	Risk Difference (%) (95% CI)
Skin and subcutaneous tissue disorders SOC	300 (15.5) [412]	260 (13.5) [355]	1.15 [0.99 - 1.34]	2.04 [-0.18 - 4.25]
FMQ Pruritus N	106 (5.5) [123]	79 (4.1) [89]	1.34 [1.01 - 1.78]	1.39 [0.04 - 2.73]
Vascular disorders SOC	510 (26.3) [838]	532 (27.5) [877]	0.96 [0.86 - 1.06]	-1.19 [-3.99 - 1.6]
SMQ embolic and thrombotic events, arterial N	107 (5.5) [123]	84 (4.4) [97]	1.27 [0.96 - 1.68]	1.18 [-0.19 - 2.54]
SMQ embolic and thrombotic events N	198 (10.2) [271]	164 (8.5) [216]	1.2 [0.99 - 1.47]	1.74 [-0.1 - 3.57]
GI SOC	646 (33.4) [1235]	644 (33.3) [1299]	1 [0.92 - 1.09]	0.03 [-2.94 - 3]
SMQ gastrointestinal perforation, ulceration, hemorrhage or obstruction N	173 (8.9) [245]	152 (7.9) [198]	1.14 [0.92 - 1.4]	1.07 [-0.68 - 2.82]
Constipation PT	145 (7.5) [174]	111 (5.7) [124]	1.3 [1.03 - 1.66]	1.74 [0.18 - 3.31]
Nausea PT	121 (6.3) [141]	96 (5.0) [114]	1.26 [0.97 - 1.63]	1.28 [-0.17 - 2.73]
FMQ dyspepsia N (incl. abdominal pain, dyspepsia, esophageal pain, biliary dyspepsia)	102 (5.3) [112]	73 (3.8) [85]	1.39 [1.04 - 1.87]	1.49 [0.18 - 2.8]
Renal and urinary disorders SOC	547 (28.2) [728]	493 (25.5) [684]	1.11 [1 - 1.23]	2.74 [-0.06 - 5.53]
Acute kidney injury PT	126 (6.5) [152]	103 (5.3) [126]	1.22 [0.95 - 1.57]	1.18 [-0.31 - 2.66]
SMQ Acute renal failure N (includes AKI, renal impairment, azotemia, oliguria, renal failure, toxic nephropathy, anuria)	272 (14.0) [323]	234 (12.1) [278]	1.16 [0.99 - 1.37]	1.94 [-0.19 - 4.06]
Musculoskeletal and connective tissue disorders SOC	434 (22.4) [753]	454 (23.5) [753]	0.95 [0.85 - 1.07]	-1.08 [-3.73 - 1.57]
Back pain PT	100 (5.2) [118]	122 (6.3) [131]	0.82 [0.63 - 1.06]	-1.15 [-2.61 - 0.32]

Source: Clinical Reviewer, R ver. 4.2, ADAE.xpt and ADSL.xpt

Treatment-emergent adverse event defined as [definition]. MedDRA version X.

Abbreviations: AKI, acute kidney injury; CI, confidence interval; FMQ, FDA Medical Query; MedDRA, GI, gastrointestinal; Medical Dictionary for Regulatory Activities; N, number of patients in treatment arm; n, number of patients with adverse event in treatment arm; PT, preferred term; rhEPO, recombinant human erythropoietin; SOC, system organ class

7.6.1.8. Adverse Events of Special Interest (Serious)

FDA considers thrombosis (CVA, MI), thromboembolism (including VAT), and heart failure adverse events of special interest (see Section 7.2). Accordingly, these events were studied as a primary analysis (MACE) as well as post-hoc supportive analyses for individual components (see Sections 7.6.1.1 and 7.6.1.2).

The Agency also considered non-adjudicated endpoints including proliferative retinopathy, macular degeneration, pulmonary artery hypertension, exacerbation of rheumatoid arthritis, worsening hypertension, seizures, malignancy, and gastrointestinal erosions as AESI, since these are known risks for ESAs (see Section 7.2). or suspected risks based on nonclinical data (see Section 7.1).

The blinded adjudication process, while ensuring unbiasedness in the classification and counting of reported events, may ignore events which are clinically important in our assessment of risk, but not fulfilling prespecified criteria in the Clinical Events Committee charter. Therefore, we considered both investigator SAEs and adjudicated events in our assessment of harm. Additionally, qualitative aspects were collected in the adjudication process which provide additional insights into the severity and seriousness of counted events and associated treatment differences. We have displayed results for adjudicated events along with investigator reported SAE counts for related MedDRA terms and/or queries related to them in [Table 80](#).

This frequency-based analysis demonstrated that:

- Hospitalization for Heart Failure: adjudicated events and investigator reported SAEs (the seriousness implies hospitalization was substantially involved) have good concordance for measures of absolute and relative risks.
- Myocardial Infarction: adjudicated events and investigator reported SAEs have good concordance for measures of absolute and relative risks. Based on qualitative measures provided by blinded adjudicators, the severity of MI seems somewhat milder than epidemiological reports (i.e., a lower proportion of events in ASCEND-ND were classified as STEMI or fatal). However, there are limitations of cross study analyses (e.g., the populations or methods of ascertainment are different) limiting definitive conclusions on the degree of risk.
- Cerebrovascular Accident: adjudicated events and investigator reported SAEs have good concordance for measures of relative risks. There seems to be a slightly higher absolute risk of investigator reported stroke events in the daprodustat arm compared to ESA. This suggests that the absolute measure of harm for clinically important, but non-adjudicated stroke events may be higher than what adjudicated endpoints alone suggest.
- Thromboembolic Events: both SAEs and adjudicated events were dominated by VAT. Measures of relative risks were reasonably concordant. There was only one fatal adjudicated TEE event.
- Worsening Hypertension and Seizure: serious adverse events were reported in similar proportions of patients treated with daprodustat and ESA comparator.
- Gastrointestinal Erosions/Bleeding: imbalances not favoring daprodustat were seen in all nSMQs for hemorrhage, perforation, and ulcerations.

- **Retinopathies:** serious reports of retinopathies were reported in more subjects treated with daprodustat (n=9, 0.5%) than darbepoetin (n=5, 0.3%), however the frequency was very low, limiting conclusions.

Nonclinical findings relating to cyst progression in autosomal dominant polycystic kidney disease (ADPKD) for another HIF-PHI, roxadustat (FG-2216), were published in the literature during the conduct of the studies for daprodustat. There is a theoretical risk that the effects seen with another HIF-PHI, FG-2216 (Fibrogens HIF-PHI), in the mouse model of ADPKD may be relevant to humans. In Study ASCEND-ND, 122 patients were identified as having ADPKD (65 on daprodustat and 57 on darbepoetin alfa). There was no indication of an accelerated decline in eGFR in daprodustat treated patients compared with darbepoetin alfa in patients with ADPKD.

All the safety signals discussed here, besides seizure, hypertension, and retinopathies, were key safety review issues and are discussed in more detail in Section 7.7.

Table 80. Patients With Serious Investigator-Reported and Adjudicated Treatment-Emergent Adverse Events by System Organ Class, On Study, ASCEND-ND

AE	Daprodustat N=1937 n (%) [events]	rhEPO N=1935 n (%) [events]	RR (%) (95% CI)	RD (%) (95% CI)
Heart Failures				
Adjudicated	140 (7.2) [209]	116 (6.0) [146]	1.21 [0.95 - 1.53]	1.23 [-0.33 - 2.8]
Narrow FMQ	123 (6.4) [167]	97 (5.0) [121]	1.27 [0.98 - 1.64]	1.33 [-0.13 - 2.79]
MedDRA HLGT	108 (5.6) [151]	75 (3.9) [93]	1.44 [1.08 - 1.92]	1.7 [0.36 - 3.03]
Myocardial infarction				
Adjudicated MI	103 (5.3) [132]	97 (5.0) [123]	1.06 [0.81 - 1.39]	0.3 [-1.1 - 1.69]
Adjudicated type 1 (thrombotic) MI	52 (2.7) [62]	49 (2.5) [58]	1.06 [0.72 - 1.56]	0.15 [-0.85 - 1.15]
Adjudicated type 2 (O2 mismatch) MI	60 (3.1) [68]	54 (2.8) [63]	1.11 [0.77 - 1.59]	0.3 [-0.76 - 1.37]
Adjudicated NSTEMI	64 (3.3) [74]	61 (3.2) [76]	1.05 [0.74 - 1.48]	0.15 [-0.97 - 1.26]
Adjudicated STEMI	6 (0.3) [6]	8 (0.4) [8]	0.75 [0.26 - 2.15]	-0.1 [-0.48 - 0.27]
Adjudicated fatal MI	7 (0.4) [7]	7 (0.4) [7]	1 [0.35 - 2.84]	0 [-0.38 - 0.38]
SMQ MI N	85 (4.4) [107]	80 (4.1) [90]	1.06 [0.79 - 1.43]	0.25 [-1.02 - 1.52]
MedDRA HLT	98 (5.1) [119]	89 (4.6) [102]	1.1 [0.83 - 1.45]	0.46 [-0.9 - 1.81]
Stroke				
Adjudicated stroke	45 (2.3) [47]	34 (1.8) [37]	1.32 [0.85 - 2.05]	0.56 [-0.33 - 1.46]
Adjudicated ischemic stroke	37 (1.9) [37]	25 (1.3) [28]	1.48 [0.89 - 2.44]	0.62 [-0.17 - 1.41]
Adjudicated fatal stroke	9 (0.5) [9]	10 (0.5) [10]	0.9 [0.37 - 2.21]	-0.05 [-0.49 - 0.39]
FMQ stroke TIA N	63 (3.3) [65]	36 (1.9) [39]	1.75 [1.17 - 2.62]	1.39 [0.4 - 2.38]
TIA HLT	8 (0.4) [8]	3 (0.2) [3]	2.66 [0.71 - 10.0]	0.26 [-0.08 - 0.59]
Stroke HLT	55 (2.8) [58]	33 (1.7) [36]	1.66 [1.09 - 2.55]	1.13 [0.19 - 2.07]
Seizure				
FMQ seizure N	6 (0.3) [6]	6 (0.3) [7]	1 [0.32 - 3.09]	0 [-0.35 - 0.35]
Seizures (includes subtypes) HLGT	6 (0.3) [6]	6 (0.3) [7]	1 [0.32 - 3.09]	0 [-0.35 - 0.35]

AE	Daprodustat N=1937 n (%) [events]	rhEPO N=1935 n (%) [events]	RR (%) (95% CI)	RD (%) (95% CI)
Venous thrombosis/ Thromboembolism				
Adjudicated thromboembolic	64 (3.3) [98]	51 (2.6) [66]	1.25 [0.87 - 1.8]	0.67 [-0.4 - 1.74]
Adjudicated DVT	16 (0.8) [17]	20 (1.0) [22]	0.8 [0.41 - 1.54]	-0.21 [-0.81 - 0.4]
Adjudicated PE	12 (0.6) [12]	2 (0.1) [2]	6 [1.34 - 26.72]	0.52 [0.14 - 0.89]
Adjudicated VAT	46 (2.4) [69]	31 (1.6) [42]	1.48 [0.94 - 2.32]	0.77 [-0.11 - 1.65]
Adjudicated TEE - fatal	1 (0.1) [1]	1 (0.1) [1]	1 [0.06 - 15.94]	0 [-0.14 - 0.14]
Device malfunction events	16 (0.8) [17]	12 (0.6) [15]	1.33 [0.63 - 2.81]	0.21 [-0.33 - 0.74]
NEC HLT				
Cardiac and vascular procedural complications HLT	24 (1.2) [29]	17 (0.9) [23]	1.41 [0.76 - 2.61]	0.36 [-0.29 - 1]
Deep vein thrombosis PT	4 (0.2) [4]	10 (0.5) [10]	0.4 [0.13 - 1.27]	-0.31 [-0.69 - 0.07]
Pulmonary embolism PT	10 (0.5) [11]	5 (0.3) [5]	2 [0.68 - 5.83]	0.26 [-0.13 - 0.65]
Hypertension				
Accelerated and malignant hypertension HLT	24 (1.2) [33]	29 (1.5) [32]	0.83 [0.48 - 1.41]	-0.26 [-0.99 - 0.47]
Gastrointestinal erosions and/or bleeds				
SMQ gastrointestinal hemorrhage N	53 (2.7) [61]	40 (2.1) [42]	1.32 [0.88 - 1.98]	0.67 [-0.3 - 1.63]
SMQ gastrointestinal perforation N	42 (2.2) [52]	28 (1.5) [34]	1.5 [0.93 - 2.4]	0.72 [-0.12 - 1.56]
SMQ gastrointestinal ulceration N	19 (1.0) [20]	15 (0.8) [16]	1.26 [0.64 - 2.48]	0.2 [-0.38 - 0.79]
Prespecified AESI queries				
Cancer-related mortality and tumor progression and recurrence (GSK)	54 (2.8) [62]	61 (3.2) [69]	0.88 [0.62 - 1.27]	-0.4 [-1.4 - 0.7]
Cardiomyopathy (GSK)	4 (0.2) [4]	5 (0.3) [5]	0.8 [0.21 - 2.97]	-0.1 [-0.4 - 0.3]
Esophageal and gastric erosions (GSK)	55 (2.8) [58]	28 (1.4) [29]	1.96 [1.25 - 3.08]	1.4 [0.5 - 2.3]
Exacerbation of rheumatoid arthritis (GSK)	0 (0) [0]	1 (0.1) [1]	NE	0 [-0.1 - 0.1]
Proliferative retinopathy/macular edema/choroidal neovascularization (GSK)	9 (0.5) [9]	5 (0.3) [6]	1.8 [0.6 - 5.36]	0.2 [-0.2 - 0.6]
Pulmonary artery hypertension (GSK)	3 (0.2) [3]	2 (0.1) [3]	1.5 [0.25 - 8.96]	0.1 [-0.2 - 0.3]
Thrombosis and/or tissue ischemia (GSK)	153 (7.9) [185]	128 (6.6) [157]	1.19 [0.95 - 1.5]	1.3 [-0.3 - 2.9]
Worsening of hypertension (GSK)	46 (2.4) [57]	45 (2.3) [51]	1.02 [0.68 - 1.53]	0 [-0.9 - 1]

Source: Clinical Reviewer, R Ver. 4.3, adevent.xpt (Adevent.R, script on file)

On-study (primary) analysis only.

Abbreviations: AE, adverse event; AESI, adverse event of special interest; CI, confidence interval; DVT, deep venous thrombosis; FMQ, FDA medical query; GSK, GlaxoSmithKline; HLT, high level group term; HLT, high level term; IR, incidence rate per 100 PY; MACE, major adverse cardiovascular event; MedDRA, Medical Dictionary for Regulatory Activities; MI, myocardial infarction; N, narrow; N, number of subjects; n, number of subjects with event; NE, not estimable; NEC, not elsewhere classified; NSTEMI, non-ST-elevation myocardial infarction; PE, pulmonary embolism; PT, preferred term; PY, person-year; RD, risk difference; RR, relative risk; SMQ, standardized MedDRA query; STEMI, ST-elevation myocardial infarction; TEE, thromboembolic event; TIA, transient ischemic attack; VAT, venous arterial thromboembolism;

7.6.1.9. Laboratory Findings

Clinically relevant laboratory abnormalities in the NDD population are presented in sections pertaining to the key safety review issues of the respective organ system. Specifically, liver biochemistries are presented in Section 7.7. [Table 81](#) shows other pertinent laboratory abnormalities for laboratory values sufficiently collected over the clinical trial. There is a high baseline rate of electrolyte abnormalities in CKD patients, which makes it challenging to resolve treatment-emergent treatment differences.

There were no clinically significant differences between trial arms in relation to the change values of laboratory parameters from baseline to end of treatment values. We did not find any credible treatment differences.

Table 81. Patients With One or More Chemistry Analyte Values With Elevated or Low Values Meeting Specified Levels, ITT Population, Trials ASCEND-D, ASCEND-ND, and Pooled Trials

Level	ASCEND-ND		
	Daprodustat N=1937 n/N _w (%)	rhEPO N=1935 n/N _w (%)	RD (%) (95% CI)
Sodium, low (mEq/L)			
Level 1 (<132)	238/1937 (12.3)	259/1935 (13.4)	-1.1 (-3.2, 1.0)
Level 2 (<130)	126/1937 (6.5)	145/1935 (7.5)	-1.0 (-2.6, 0.6)
Level 3 (<125)	39/1937 (2.0)	52/1935 (2.7)	-0.7 (-1.6, 0.3)
Sodium, high (mEq/L)			
Level 1 (>150)	11/1937 (0.6)	7/1935 (0.4)	0.2 (-0.2, 0.6)
Level 2 (>155)	3/1937 (0.2)	2/1935 (0.1)	0.1 (-0.2, 0.3)
Level 3 (>160)	1/1937 (0.05)	0/1935 (0)	0.1 (-0.0, 0.2)
Potassium, low (mEq/L)			
Level 1 (<3.6)	274/1937 (14.1)	264/1935 (13.6)	0.5 (-1.7, 2.7)
Level 2 (<3.4)	144/1937 (7.4)	152/1935 (7.9)	-0.4 (-2.1, 1.3)
Level 3 (<3)	29/1937 (1.5)	28/1935 (1.4)	0.1 (-0.7, 0.8)
Potassium, high (mEq/L)			
Level 1 (>5.5)	707/1937 (36.5)	722/1935 (37.3)	-0.8 (-3.9, 2.2)
Level 2 (>6)	266/1937 (13.7)	309/1935 (16.0)	-2.2 (-4.5, 0.0)
Level 3 (>6.5)	97/1937 (5.0)	105/1935 (5.4)	-0.4 (-1.8, 1.0)
Chloride, low (mEq/L)			
Level 1 (<95)	263/1937 (13.6)	268/1935 (13.9)	-0.3 (-2.4, 1.9)
Level 2 (<88)	28/1937 (1.4)	47/1935 (2.4)	-1.0 (-1.9, -0.1)
Level 3 (<80)	2/1937 (0.1)	1/1935 (0.05)	0.1 (-0.1, 0.2)
Chloride, high (mEq/L)			
Level 1 (>108)	1053/1937 (54.4)	1080/1935 (55.8)	-1.5 (-4.6, 1.7)
Level 2 (>112)	382/1937 (19.7)	396/1935 (20.5)	-0.7 (-3.3, 1.8)
Level 3 (>115)	110/1937 (5.7)	111/1935 (5.7)	-0.1 (-1.5, 1.4)
Calcium, low (mg/dL)			
Level 1 (<8.4)	831/1937 (42.9)	809/1935 (41.8)	1.1 (-2.0, 4.2)
Level 2 (<8)	451/1937 (23.3)	444/1935 (22.9)	0.3 (-2.3, 3.0)
Level 3 (<7.5)	268/1937 (13.8)	270/1935 (14.0)	-0.1 (-2.3, 2.1)
Calcium, high (mg/dL)			
Level 1 (>10.5)	118/1937 (6.1)	107/1935 (5.5)	0.6 (-0.9, 2.0)
Level 2 (>11)	42/1937 (2.2)	37/1935 (1.9)	0.3 (-0.6, 1.1)
Level 3 (>12)	11/1937 (0.6)	12/1935 (0.6)	-0.1 (-0.5, 0.4)

Level	ASCEND-ND		
	Daprodustat N=1937 n/N _w (%)	rhEPO N=1935 n/N _w (%)	RD (%) (95% CI)
Phosphate, low (mg/dL)			
Level 1 (<2.5)	178/1937 (9.2)	168/1935 (8.7)	0.5 (-1.3, 2.3)
Level 2 (<2)	41/1937 (2.1)	39/1935 (2.0)	0.1 (-0.8, 1.0)
Level 3 (<1.4)	14/1937 (0.7)	10/1935 (0.5)	0.2 (-0.3, 0.7)
Albumin, low (g/dL)			
Level 1 (<3.1)	319/1937 (16.5)	276/1935 (14.3)	2.2 (-0.1, 4.5)
Level 2 (<2.5)	63/1937 (3.3)	60/1935 (3.1)	0.2 (-1.0, 1.3)
Level 3 (<2)	16/1937 (0.8)	13/1935 (0.7)	0.2 (-0.4, 0.7)
Blood urea nitrogen, high (mg/dL)			
Level 1 (>23)	1921/1937 (99.2)	1926/1935 (99.5)	-0.4 (-0.9, 0.1)
Level 2 (>27)	1902/1937 (98.2)	1906/1935 (98.5)	-0.3 (-1.1, 0.5)
Level 3 (>31)	1875/1937 (96.8)	1874/1935 (96.8)	-0.0 (-1.2, 1.1)
Cholesterol, total, high (mg/dL)			
Level 1 (>200)	528/1929 (27.4)	543/1927 (28.2)	-0.8 (-3.6, 2.0)
Level 2 (>210)	439/1929 (22.8)	455/1927 (23.6)	-0.9 (-3.5, 1.8)
Level 3 (>225)	307/1929 (15.9)	319/1927 (16.6)	-0.6 (-3.0, 1.7)
HDL, males, low (mg/dL)			
Level 1 (<40)	471/829 (56.8)	433/859 (50.4)	6.4 (1.7, 11.2)
Level 2 (<30)	161/829 (19.4)	133/859 (15.5)	3.9 (0.3, 7.6)
Level 3 (<20)	15/829 (1.8)	14/859 (1.6)	0.2 (-1.1, 1.4)
HDL, females, low (mg/dL)			
Level 1 (<50)	704/1100 (64.0)	648/1068 (60.7)	3.3 (-0.8, 7.4)
Level 2 (<40)	398/1100 (36.2)	346/1068 (32.4)	3.8 (-0.2, 7.8)
Level 3 (<20)	6/1100 (0.5)	5/1068 (0.5)	0.1 (-0.5, 0.7)
LDL, high (mg/dL)			
Level 1 (>130)	362/1927 (18.8)	390/1926 (20.2)	-1.5 (-4.0, 1.0)
Level 2 (>160)	129/1927 (6.7)	149/1926 (7.7)	-1.0 (-2.7, 0.6)
Level 3 (>190)	35/1927 (1.8)	43/1926 (2.2)	-0.4 (-1.3, 0.5)

Source: ad b.xpt; Software: R

Note that glucose values for hyperglycemia do not follow a nested format like the other labs. Level 1 corresponds to the diagnosis of prediabetes and is not inclusive of Level 2 and 3. Level 2 corresponds to the diagnosis of diabetes. Level 3 represents significant hyperglycemia that may indicate need for insulin or increased risk for diabetic ketoacidosis or other complications.

Threshold levels 1, 2, and 3 as defined by the [Standard Safety Tables & Figures Integrated Guide](#).

Risk difference and risk ratio (with 95% confidence interval) are shown between treatment and comparator.

Abbreviations: CI, confidence interval; ITT, intent-to-treat; LDL, low-density lipoprotein; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data; RD, risk difference; rhEPO, recombinant human erythropoietin

7.6.1.10. Vital Signs' Analyses

Hypertension is a known risk for ESAs, and thus are of special interest for daprodustat. Vitals for SBP, DBP, and HR were systematically collected during the study. One measurement of SBP, DBP, and HR was taken with the subject in a semi-supine or seated position after a 5-minute rest period. Triplicate measurements were taken at baseline and the end of study visit (or early treatment discontinuation visit, as applicable).

We graphically inspected aggregate plots vs. time and maximum on-treatment vs. baseline values for each and did not identify trends. One limitation of the SBP and DBP data is that they were collected using cuffs, which are not as definitive as ambulatory blood pressure monitoring.

For brevity, we only report shift tables below. It is important to note that these tables were not controlled for baseline hypertension status. Nonetheless, the objective measurement of hypertension appears similar between daprodustat and ESA comparator. The use of antihypertensive agents was stable throughout the treatment period for both treatment arms, and was balanced between arms, consistent with trends seen in investigator adverse event reports.

Table 82. Percentage of Patients With Maximum Systolic and Diastolic Blood Pressure by Category of Blood Pressure Post-Baseline, ITT Population, ASCEND-ND

Blood Pressure	ASCEND-ND		
	Daprodustat N=1937 n/N _w (%)	rhEPO N=1935 n/N _w (%)	RD (%) (95% CI)
Systolic blood pressure (mm Hg)			
<90	0/1937 (0)	0/1935 (0)	0 (0, 0)
≥90	1937/1937 (100)	1935/1935 (100)	0 (0, 0)
≥120	1918/1937 (99.0)	1925/1935 (99.5)	-0.5 (-1.0, 0.1)
≥140	1665/1937 (86.0)	1672/1935 (86.4)	-0.5 (-2.6, 1.7)
≥160	891/1937 (46.0)	923/1935 (47.7)	-1.7 (-4.8, 1.4)
≥180	273/1937 (14.1)	338/1935 (17.5)	-3.4 (-5.7, -1.1)
Diastolic blood pressure (mm Hg)			
<60	4/1936 (0.2)	13/1935 (0.7)	-0.5 (-0.9, -0.0)
≥60	1932/1936 (99.8)	1922/1935 (99.3)	0.5 (0.0, 0.9)
≥90	865/1936 (44.7)	879/1935 (45.4)	-0.7 (-3.9, 2.4)
≥110	67/1936 (3.5)	75/1935 (3.9)	-0.4 (-1.6, 0.8)
≥120	10/1936 (0.5)	23/1935 (1.2)	-0.7 (-1.3, -0.1)

Source: advs.xpt; Software: R

Risk difference and risk ratio (with 95% confidence interval) are shown between treatment and comparator.

Abbreviations: CI, confidence interval; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data; RD, risk difference; rhEPO, recombinant human erythropoietin

7.6.2. Safety Results, ASCEND-D

7.6.2.1. MACE

7.6.2.1.1. Overview

Results for the prespecified primary analysis of time to first MACE are shown in [Table 83](#). The HR (95% CI) for the primary analysis (on-study analysis) for time to first MACE was 0.93 (0.81, 1.07) comparing daprodustat to ESA. The upper bound of the 95% CI was lower than the prespecified risk margin of 1.25, therefore the study ruled out the risk margin for MACE. Kaplan-Meier survival curves for MACE overlapped during the follow-up period ([Figure 31](#)). The IRD was -0.8 events/100 PY (95% CI -2.4, 0.8) between daprodustat and ESA. Incident events of the MACE composite endpoint (daprodustat 374/1487 [11.1 events/100 PY], ESA 394/1477 [11.9 events/100 PY]) comprised mostly all-cause mortality (daprodustat 244/374, ESA 233/394), followed by nonfatal MI (daprodustat 101/374, ESA 126/394) and nonfatal stroke (daprodustat 29/374, ESA 35/394).

Because both co-primary endpoints (mean change in Hb and time to first MACE) ruled out their respective risk margins, the principal secondary endpoints were tested for superiority by

comparing the one-sided superiority p-value against the rank-associated alpha derived from the Holm-Bonferroni method. None of the principal secondary endpoints (MACE, MACE or thromboembolic events, MACE or HHF, average monthly IV iron dose) were significant.

Tipping point sensitivity analyses to the MACE primary analysis showed that the results (HR 1.08; 95% CI 0.94, 1.25) tipped, i.e., did not rule out the 1.25 risk margin, when censored subjects in daprodustat arm were assumed to have a (worse) hazard rate of 4.5 times the observed rate in the daprodustat arm and censored subjects in the ESA arm were assumed to have a (better) hazard rate of 0.4 times the observed rate in the ESA arm (see Section 17.1). In summary, the primary analysis results for MACE, assuming non-informative censoring, was tipped only under extreme assumptions of daprodustat having more MACE and the active control having less MACE than the observed subjects. Therefore, the primary analysis assuming non-informative censoring could be relied upon for interpretation.

Competing risk analyses of time to first fatal/nonfatal (a) stroke, (b) MI, and (c) TEE with all-cause mortality as the competing event produced estimates that were similar to the primary analyses that censored subjects at death (results not shown). Competing risk analysis of these endpoints did not change the interpretation of their respective primary analysis.

Table 83. Summary of Results for MACE, ASCEND-D (On-Study Analysis)

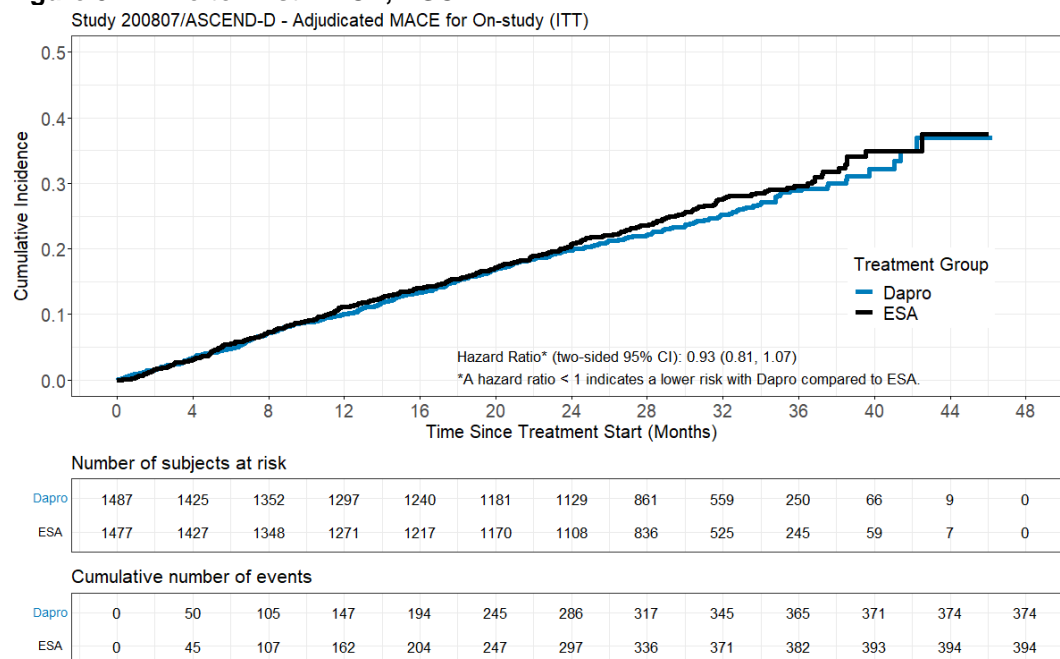
	Daprodustat N=1487 PY=3377	ESA N=1477 PY=3323	Rate Diff per 100 PY (95% CI)	Hazard Ratio (95% CI)
MACE, n [IR]	374 [11.1]	394 [11.9]	-0.8 (-2.4, 0.8)	0.93 (0.81, 1.07)
All-cause mortality, n (%) ¹	244 (65.2)	233 (59.1)	-	-
Nonfatal myocardial infarction, n (%) ¹	101 (27.0)	126 (32.0)	-	-
Nonfatal stroke, n (%) ¹	29 (7.8)	35 (8.9)	-	-

Source: FDA analysis.

¹ Percentage of MACE.

Abbreviations: CI, confidence interval; Diff, difference; ESA, erythropoietin-stimulating agents; IR, incidence rate per 100 PY; MACE, major adverse cardiovascular event; N, number of subjects; n, number of subjects with event; PY, person-year

Figure 31. Time to First MACE, ASCEND-D



Source: FDA analysis.
MACE: Composite of all-cause mortality, nonfatal MI, and nonfatal stroke.
Abbreviations: CI, confidence interval; Dapro, daprodustat; ESA, erythropoietin-stimulating agents; ITT, intention-to-treat; MACE, major adverse cardiovascular event; MI, myocardial infarction

7.6.2.1.2. On-Study vs. On-Treatment Analyses

The pre-specified primary analysis of MACE was on-study (HR 0.93; CI 0.81, 1.07). The pre-specified supportive OT analysis (OT + 28) of MACE (HR 0.96; CI 0.81, 1.14) was consistent with the primary on-study analysis (Table 84). Exploratory OT analyses adjusted by the dosing frequencies of the different treatments were also consistent with the primary on-study analysis.

Table 84. MACE On-Study and On-Treatment Analyses, ASCEND-D

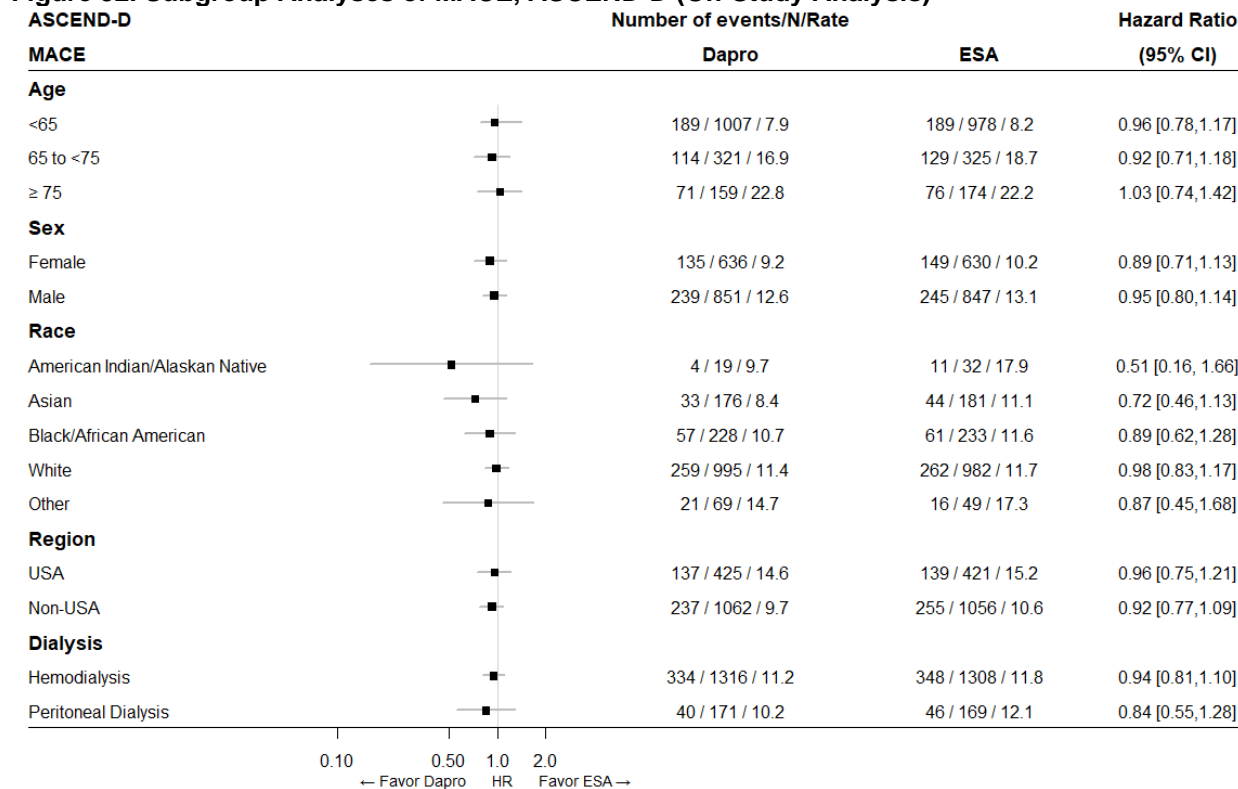
	Daprodustat N=1487 ^a PY=3377 ^a n [IR]	ESA N=1477 ^a PY=3323 ^a n [IR]	Hazard Ratio (95% CI)
On-study (primary)	374 [11.1]	394 [11.9]	0.93 (0.81, 1.07)
On-treatment + 28 days (supportive)	255 [9.9]	271 [10.4]	0.96 (0.81, 1.14)
On-treatment + dosing frequency + 28 days (exploratory)	255 [9.9]	278 [10.6]	0.94 (0.79, 1.11)
On-treatment + dosing frequency (exploratory)	177 [7.1]	207 [8.1]	0.88 (0.72, 1.07)

Source: FDA analysis.
MACE: Composite of all-cause mortality, nonfatal MI, and nonfatal stroke
Dosing frequency: Daprodustat: daily; ESA: TIW, weekly, every 2 weeks, or every 4 weeks
^a Number of subjects (N) and person-years (PY) correspond to the on-study (primary) analysis only.
Abbreviations: CI, confidence interval; ESA, erythropoietin stimulating agents; IR, incidence rate per 100 PY; MACE, major adverse cardiovascular event; MI, myocardial infarction; N, number of subjects; n, number of subjects with event; PY, person-year; TIW, three times a week

7.6.2.1.3. Subgroup Analyses

Results for prespecified subgroup analyses of MACE are shown in [Figure 32](#). Subgroup HR estimates were less than or equal to 1.03 and generally consistent with the overall study population MACE estimate (HR 0.93; 95% CI 0.81, 1.07).

Figure 32. Subgroup Analyses of MACE, ASCEND-D (On-Study Analysis)



Source: FDA analysis.

MACE: Composite of all-cause mortality, nonfatal MI, and nonfatal stroke.

Rate: incidence rate per 100 PY

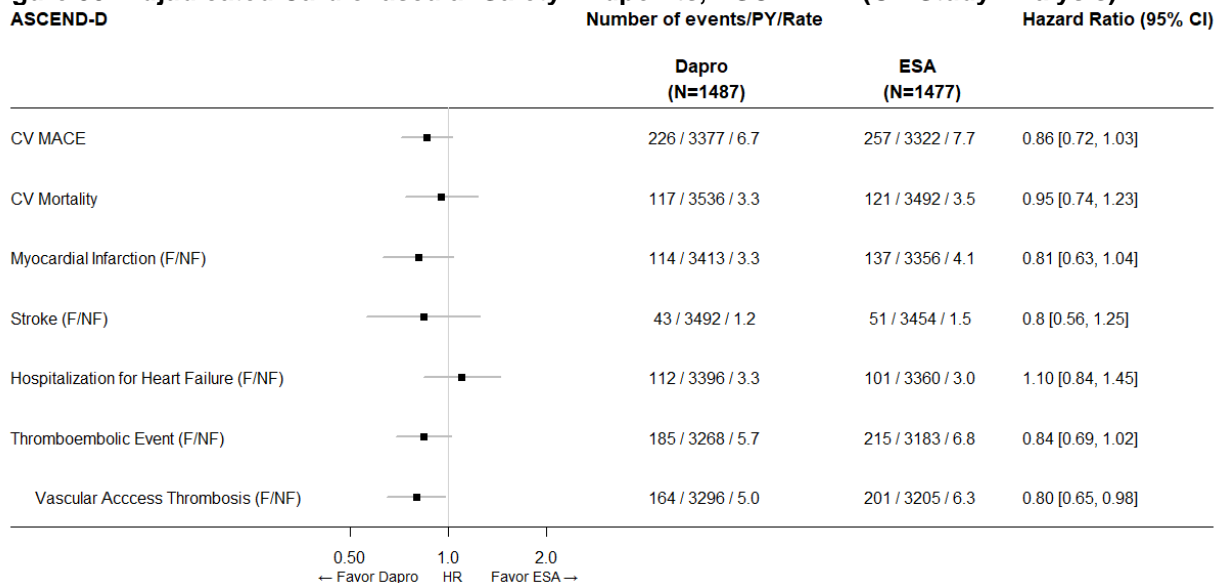
Abbreviations: CI, confidence interval; Dapro, daprodustat; ESA, erythropoiesis-stimulating agent; HR, hazard ratio; MACE, major adverse cardiovascular event; MI, myocardial infarction; N, number of subjects; USA, United States of America

7.6.2.2. CV Endpoints

7.6.2.2.1. Overview

To better understand CV risk, adjudicated CV endpoints, not including all-cause mortality, were assessed. Results of the primary (on-study) time-to-event analysis of CV endpoints in ASCEND-D are shown in [Figure 33](#). A slightly higher incidence of HHF was observed in the daprodustat arm (112/1487 [3.3 events/100 PY]) compared to the ESA arm (101/1477 [3.0 events/100 PY]) corresponding to a HR of 1.10 (95% CI 0.84, 1.45). A higher incidence of HHF was also observed in the daprodustat arm in ASCEND-ND (Section [7.6.1.2](#)). A higher incidence was not observed for other adjudicated CV endpoints in the daprodustat arm. The corresponding IRDs (per 100-person years) ranged from -1.1 to 0.3 (Appendix Section [17.6](#)).

Figure 33. Adjudicated Cardiovascular Safety Endpoints, ASCEND-D (On-Study Analysis)



Source: FDA analysis.

CV MACE: Composite of CV mortality, nonfatal MI, and nonfatal stroke.

Rate: number of events per 100 PY.

Abbreviations: CI, confidence interval; CV, cardiovascular; Dapro, daprodustat; ESA, erythropoietin-stimulating agents; F/NF, fatal or nonfatal; HR, hazard ratio; PY, person-year

7.6.2.2.2. Hospitalization for Heart Failure Subgroup Analyses

Exploratory subgroup analyses by history of heart failure (Yes, No) showed a higher incidence of HHF in the daprodustat arm compared to the control arm in the subgroup with a history of heart failure ([Table 85](#)). The HR for the subgroup with history of heart failure was 1.44 (95% CI 0.97, 2.14). In contrast, the HR for the subgroup without a history of heart failure was 0.91 (95% CI 0.63, 1.32).

Table 85. History of Heart Failure Subgroup Analysis¹ for Hospitalization for Heart Failure, ASCEND-D (On-Study Analysis)

	Daprodustat n/N [IR]	rhEPO n/N [IR]	Hazard Ratio (95% CI)
History of heart failure	58/399 [6.8]	44/389 [5.1]	1.44 (0.97, 2.14)
No history of heart failure	54/1088 [2.1]	57/1086 [2.3]	0.91 (0.63, 1.32)

Source: FDA analysis.

History of heart failure defined as having the medical history conditions: heart failure, left ventricular systolic dysfunction, left ventricular diastolic dysfunction, or pulmonary hypertension.

¹ Analyses did not include two subjects with missing history of hemofiltration.

Abbreviations: CI, confidence interval; IR, incidence rate per 100 PY; PY, person-year

7.6.2.3. Overview of Treatment-Emergent Adverse Events Summary

[Table 86](#) provides a frequency-based overview of TEAEs reported in the safety population during the on-study period for ASCEND-D. Based on the frequency-based analysis, daprodustat

has similar rates of SAEs, AEs leading to discontinuation or interruption of study drug, and adverse events. There were no remarkable differences between study arms. However, the clinical relevance of these findings cannot be interpreted without more granular data which are presented and discussed in more detail in the next several sections).

Table 86. Overview of On-Study Adverse Events, ITT Population, Trials ASCEND-D

Event Category	ASCEND-D		
	Daprodustat N=1487 n (%)	rhEPO N=1477 n (%)	RD (%) (95% CI)
SAE	945 (63.6)	954 (64.6)	-1.0 (-4.5, 2.4)
SAEs with fatal outcome	289 (19.4)	299 (20.2)	-0.8 (-3.7, 2.1)
Life-threatening SAEs	60 (4.0)	77 (5.2)	-1.2 (-2.7, 0.3)
AE leading to permanent discontinuation of study drug	275 (18.5)	266 (18.0)	0.5 (-2.3, 3.3)
AE leading to dose modification of study drug	123 (8.3)	124 (8.4)	-0.1 (-2.1, 1.9)
AE leading to interruption of study drug	121 (8.1)	123 (8.3)	-0.2 (-2.2, 1.8)
AE leading to reduction of study drug	2 (0.1)	1 (0.07)	0.1 (-0.2, 0.3)
AE leading to dose delay of study drug	0	0	0 (0, 0)
AE	1378 (92.7)	1357 (91.9)	0.8 (-1.1, 2.7)
Severe	332 (22.3)	334 (22.6)	-0.3 (-3.3, 2.7)
Moderate	525 (35.3)	449 (30.4)	4.9 (1.5, 8.3)
Mild	171 (11.5)	198 (13.4)	-1.9 (-4.3, 0.5)

Source: adae.xpt; Software: R

Abbreviations: AE, adverse event; CI, confidence interval; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients with at least one event; RD, risk difference; rhEPO, recombinant human erythropoietin; RR, risk ratio; SAE, serious adverse event

7.6.2.4. Deaths

In the ASCEND-D study, all-cause mortality was similar between arms (HR 0.96; CI 0.82, 1.13) (see [Figure 71](#) for survival curves). There were 294/1487 deaths [8.3 deaths/100 PY] in the daprodustat arm and 300/1477 deaths [8.6 deaths/100 PY] in the ESA arm ([Table 87](#)). The incidence of death from cardiovascular causes was lower in subjects who received daprodustat. Death from undetermined cause was higher with daprodustat. This is an opposite trend to what was seen in the non-dialysis population.

Table 87. Adjudicated Causes of Death—ASCEND-D, (On-Study Analysis)

	Daprodustat N=1487	ESA N=1477	Relative Risk	Risk Difference
CV death	117 (7.9%)	121 (8.2%)	1.0 [0.8, 1.2]	-0.3 [-2.3, 1.6]
Sudden cardiac death	47 (3.2%)	39 (2.6%)	1.2 [0.8, 1.8]	0.5 [-0.7, 1.7]
Acute MI	12 (0.8%)	9 (0.6%)	1.3 [0.6, 3.1]	0.2 [-0.4, 0.8]
Heart failure	14 (0.9%)	11 (0.7%)	1.3 [0.6, 2.8]	0.2 [-0.5, 0.9]
Other cardiovascular causes	6 (0.4%)	4 (0.3%)	1.5 [0.4, 5.4]	0.1 [-0.3, 0.6]
Stroke	13 (0.9%)	15 (1%)	0.7 [0.4, 1.8]	-0.1 [-0.8, 0.6]
Presumed sudden death	11 (0.7%)	15 (1%)	0.7 [0.3, 1.6]	-0.3 [-1.0, 0.4]
Presumed cardiovascular death	10 (0.7%)	15 (1%)	0.7 [0.3, 1.5]	-0.3 [-1.0, 0.3]

	Daprodustat N=1487	ESA N=1477	Relative Risk	Risk Difference
Non-CV death	132 (8.9%)	155 (10.5%)	0.9 [0.7, 1.1]	-1.6 [-3.8, 0.5]
Accidental	5 (0.3%)	5 (0.3%)	1.0 [0.3, 3.4]	0.0 [-0.4, 0.4]
Gastrointestinal	7 (0.5%)	7 (0.5%)	1.0 [0.4, 2.8]	0.0 [-0.5, 0.5]
Renal	15 (1%)	15 (1%)	1.0 [0.5, 2.0]	-0.0 [-0.7, 0.7]
Hemorrhage (ex. stroke)	5 (0.3%)	6 (0.4%)	0.8 [0.3, 2.7]	-0.1 [-0.5, 0.4]
Malignancy	15 (1%)	19 (1.3%)	0.8 [0.4, 1.5]	-0.3 [-1.0, 0.5]
Infection (includes sepsis)	77 (5.2%)	90 (6.1%)	0.9 [0.6, 1.1]	-1.0 [-2.6, 0.8]
Undetermined	45 (3%)	24 (1.6%)	1.9 [1.1, 3.0]	1.4 [0.3, 2.5]

Source: Clinical Reviewer, R v. 4.2, ADSL.xpt, ADEVENT.xpt.

ITT population, groups correspond to planned treatment assignment. Events occurring 10 times or more are included. Ordered by risk difference.

Abbreviations: CV, cardiovascular; ESA, erythropoiesis-stimulating agents; ITT, intent-to-treat; MI, myocardial infarction

7.6.2.5. Serious Treatment-Emergent Adverse Events

[Table 88](#) provides a frequency-based comparison of investigator-reported SAE occurrence, by SOC and preferred term. [Table 89](#) provides a frequency-based comparison of SAE occurrence by narrow FMQ. Treatment emergent serious adverse events in the ITT population are reported if they occurred in at least 1% of subjects in either arm.

The nFMQs for hyperglycemia and bacterial infection showed a treatment difference not favoring daprodustat. The hyperglycemia query was driven by diverse sequelae of diabetes (e.g., foot infections, neuropathies), and was not corroborated by reports of new onset diabetes.

The SOC for infections and infestations were balanced overall, however, there were several PTs which showed treatment differences not favoring daprodustat, including peritonitis, dialysis access infections (including "Device related infection" and "Arteriovenous fistula site infection"), osteomyelitis. When considering nFMQs related to organism or location (e.g., viral, bacterial, respiratory, integumentary), no common themes in treatment differences was observed. Importantly, reports of serious sepsis and shock were balanced between treatment arms.

The GI hemorrhage PT was somewhat more notable because the signal was also seen in ASCEND-ND and was a prespecified AESI. This is discussed in [Section 7.7](#).

We did not identify any other safety signals not favoring daprodustat. In contrast to ASCEND-ND, cardiovascular endpoints, such as thrombosis and thromboembolisms were favoring daprodustat in this population.

Table 88. Patients With On-Study Serious Adverse Events by System Organ Class and Preferred Term, Occurring in at Least 1% of Subjects in Any Arm, ITT Population, ASCEND-D

	Daprodustat N=1487 n (%)	rhEPO N=1477 n (%)	RD (%) (95% CI)
Blood and lymphatic system disorders (SOC)	60 (4.0)	64 (4.3)	-0.3 (-1.7, 1.1)
Anemia	38 (2.6)	50 (3.4)	-0.8 (-2.1, 0.4)
Cardiac disorders (SOC)	254 (17.1)	282 (19.1)	-2.0 (-4.8, 0.8)
Cardiac failure congestive	24 (1.6)	19 (1.3)	0.3 (-0.5, 1.2)
Myocardial infarction	23 (1.5)	23 (1.6)	-0.0 (-0.9, 0.9)
Cardiac arrest	42 (2.8)	42 (2.8)	-0.0 (-1.2, 1.2)
Cardiac failure	21 (1.4)	24 (1.6)	-0.2 (-1.1, 0.7)
Angina pectoris	21 (1.4)	20 (1.4)	0.1 (-0.8, 0.9)
Angina unstable	15 (1.0)	13 (0.9)	0.1 (-0.6, 0.8)
Acute myocardial infarction	36 (2.4)	43 (2.9)	-0.5 (-1.7, 0.7)
Cardio-respiratory arrest	11 (0.7)	17 (1.2)	-0.4 (-1.1, 0.3)
Coronary artery disease	12 (0.8)	20 (1.4)	-0.5 (-1.3, 0.2)
Atrial fibrillation	31 (2.1)	43 (2.9)	-0.8 (-2.0, 0.3)
Gastrointestinal disorders (SOC)	161 (10.8)	151 (10.2)	0.6 (-1.6, 2.8)
Gastrointestinal hemorrhage	21 (1.4)	15 (1.0)	0.4 (-0.4, 1.2)
General disorders and administration site conditions (SOC)	106 (7.1)	106 (7.2)	-0.0 (-1.9, 1.8)
Non-cardiac chest pain	13 (0.9)	22 (1.5)	-0.6 (-1.4, 0.2)
Infections and infestations (SOC)	475 (31.9)	480 (32.5)	-0.6 (-3.9, 2.8)
Peritonitis	39 (2.6)	34 (2.3)	0.3 (-0.8, 1.4)
Arteriovenous fistula site infection	17 (1.1)	7 (0.5)	0.7 (0.0, 1.3)
Device related infection	15 (1.0)	12 (0.8)	0.2 (-0.5, 0.9)
Osteomyelitis	20 (1.3)	14 (0.9)	0.4 (-0.4, 1.2)
Cellulitis	22 (1.5)	29 (2.0)	-0.5 (-1.4, 0.5)
Gastroenteritis	13 (0.9)	15 (1.0)	-0.1 (-0.8, 0.6)
Pneumonia	119 (8.0)	108 (7.3)	0.7 (-1.2, 2.6)
Sepsis	48 (3.2)	49 (3.3)	-0.1 (-1.4, 1.2)
Gangrene	15 (1.0)	20 (1.4)	-0.3 (-1.1, 0.4)
Urinary tract infection	25 (1.7)	25 (1.7)	-0.0 (-0.9, 0.9)
Covid-19	29 (2.0)	44 (3.0)	-1.0 (-2.1, 0.1)
Septic shock	21 (1.4)	31 (2.1)	-0.7 (-1.6, 0.3)
Injury, poisoning and procedural complications (SOC)	234 (15.7)	252 (17.1)	-1.3 (-4.0, 1.3)
Fall	19 (1.3)	7 (0.5)	0.8 (0.1, 1.5)
Arteriovenous fistula site hemorrhage	17 (1.1)	16 (1.1)	0.1 (-0.7, 0.8)
Arteriovenous graft thrombosis	8 (0.5)	17 (1.2)	-0.6 (-1.3, 0.0)
Arteriovenous fistula thrombosis	43 (2.9)	71 (4.8)	-1.9 (-3.3, -0.5)
Metabolism and nutrition disorders (SOC)	125 (8.4)	131 (8.9)	-0.5 (-2.5, 1.6)
Fluid overload	57 (3.8)	53 (3.6)	0.2 (-1.1, 1.6)
Hypoglycemia	16 (1.1)	13 (0.9)	0.2 (-0.5, 0.9)
Hyperkalemia	26 (1.7)	39 (2.6)	-0.9 (-1.9, 0.2)
Nervous system disorders (SOC)	103 (6.9)	134 (9.1)	-2.1 (-4.1, -0.2)
Cerebrovascular accident	15 (1.0)	17 (1.2)	-0.1 (-0.9, 0.6)
Product issues (SOC)	11 (0.7)	23 (1.6)	-0.8 (-1.6, -0.1)
Device malfunction	7 (0.5)	15 (1.0)	-0.5 (-1.2, 0.1)

	Daprodustat N=1487 n (%)	rhEPO N=1477 n (%)	RD (%) (95% CI)
Respiratory, thoracic, and mediastinal disorders (SOC)	131 (8.8)	154 (10.4)	-1.6 (-3.7, 0.5)
Dyspnea	21 (1.4)	16 (1.1)	0.3 (-0.5, 1.1)
Respiratory failure	21 (1.4)	18 (1.2)	0.2 (-0.6, 1.0)
Pleural effusion	16 (1.1)	13 (0.9)	0.2 (-0.5, 0.9)
Acute respiratory failure	24 (1.6)	27 (1.8)	-0.2 (-1.2, 0.7)
Acute pulmonary edema	12 (0.8)	16 (1.1)	-0.3 (-1.0, 0.4)
Pulmonary edema	17 (1.1)	22 (1.5)	-0.3 (-1.2, 0.5)
Vascular disorders (SOC)	132 (8.9)	153 (10.4)	-1.5 (-3.6, 0.6)
Hypertension PT	20 (1.3)	14 (0.9)	0.4 (-0.4, 1.2)
Hypotension	14 (0.9)	24 (1.6)	-0.7 (-1.5, 0.1)

Source: adae.xpt; Software: R

Abbreviations: CI, confidence interval; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients with adverse event; PT, preferred term; RD, risk difference; rhEPO, recombinant human erythropoietin; SOC, system organ class

Table 89. Patients With On-Study Serious Adverse Events by System Organ Class and FDA Medical Query (Narrow), Occurring in at Least 1% of Subjects in Any Arm, ITT Population, ASCEND-D

System Organ Class FMQ (Narrow)	ASCEND-D		RD (%) (95% CI)
	Daprodustat N=1487 n (%)	rhEPO N=1477 n (%)	
Blood and lymphatic system disorders (SOC)			
Thrombosis	180 (12.1)	219 (14.8)	-2.7 (-5.2, -0.3)
Anemia	47 (3.2)	57 (3.9)	-0.7 (-2.0, 0.6)
Thrombosis arterial	148 (10.0)	190 (12.9)	-2.9 (-5.2, -0.6)
Thrombosis venous	69 (4.6)	110 (7.4)	-2.8 (-4.5, -1.1)
Cardiac disorders (SOC)			
Heart Failure	87 (5.9)	91 (6.2)	-0.3 (-2.0, 1.4)
Systemic Hypertension	38 (2.6)	36 (2.4)	0.1 (-1.0, 1.2)
Myocardial Infarction	57 (3.8)	64 (4.3)	-0.5 (-1.9, 0.9)
Myocardial Ischemia	90 (6.1)	100 (6.8)	-0.7 (-2.5, 1.0)
Acute Coronary Syndrome	73 (4.9)	83 (5.6)	-0.7 (-2.3, 0.9)
Arrhythmia	54 (3.6)	81 (5.5)	-1.9 (-3.4, -0.4)
Endocrine disorders (SOC)			
Hyperglycemia	41 (2.8)	26 (1.8)	1.0 (-0.1, 2.1)
Hypoglycemia	16 (1.1)	13 (0.9)	0.2 (-0.5, 0.9)
Infections and infestations (SOC)			
Bacterial Infection	299 (20.1)	287 (19.4)	0.7 (-2.2, 3.5)
Purulent Material	20 (1.3)	19 (1.3)	0.1 (-0.8, 0.9)
Viral Infection	39 (2.6)	45 (3.0)	-0.4 (-1.6, 0.8)
Pneumonia	137 (9.2)	134 (9.1)	0.1 (-1.9, 2.2)
Musculoskeletal and connective tissue disorders (SOC)			
Osteoporosis	16 (1.1)	14 (0.9)	0.1 (-0.6, 0.8)
Fracture	60 (4.0)	59 (4.0)	0.0 (-1.4, 1.5)
Neoplasms benign, malignant and unspecified (incl cysts and polyps) (SOC)			
Malignancy	42 (2.8)	41 (2.8)	0.0 (-1.1, 1.2)
Nervous system disorders (SOC)			
Stroke TIA	50 (3.4)	63 (4.3)	-0.9 (-2.3, 0.5)
Seizure	10 (0.7)	15 (1.0)	-0.3 (-1.0, 0.3)

System Organ Class FMQ (Narrow)	ASCEND-D		
	Daprodustat N=1487 n (%)	rhEPO N=1477 n (%)	RD (%) (95% CI)
Psychiatric disorders (SOC)			
Arthritis	17 (1.1)	20 (1.4)	-0.2 (-1.0, 0.6)
Renal and urinary disorders (SOC)			
Renal and Urinary Tract Infection	40 (2.7)	48 (3.2)	-0.6 (-1.8, 0.7)
Respiratory, thoracic and mediastinal disorders (SOC)			
Dyspnea	23 (1.5)	19 (1.3)	0.3 (-0.6, 1.1)
Respiratory Failure	59 (4.0)	71 (4.8)	-0.8 (-2.3, 0.6)
Vascular disorders (SOC)			
Hemorrhage	115 (7.7)	129 (8.7)	-1.0 (-3.0, 1.0)
Hypotension	20 (1.3)	27 (1.8)	-0.5 (-1.4, 0.4)

Source: adae.xpt; Software: R

Risk difference and risk ratio (with 95% confidence interval) are shown between treatment and comparator.

Abbreviations: CI, confidence interval; FMQ, FDA medical query; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients with adverse event; RD, risk difference; rhEPO, recombinant human erythropoietin; SOC, system organ class; TIA, transient ischemic attack

7.6.2.6. Adverse Events Leading to Treatment Discontinuation

In ASCEND-D, daprodustat is not associated with higher rate of AEs leading to permanent discontinuation, compared to epoetin alfa (18.5% versus 18.0%).

Table 90. Patients With On-Study Adverse Events Leading to Treatment Discontinuation by System Organ Class, MedDRA Preferred Term, and FDA Medical Query (Narrow), Occurring in at Least 1% of Subjects in Any Arm, ITT Population, ASCEND-D

Adverse Event	Daprodustat N=1487 n (%)	rhEPO N=1477 n (%)	RD (%) (95% CI)
Blood and lymphatic system disorders (SOC)	58 (3.9)	58 (3.9)	-0.0 (-1.4, 1.4)
Cardiac arrest (PT)	24 (1.6)	25 (1.7)	-0.1 (-1.0, 0.8)
Thrombosis arterial FMQ	12 (0.8)	15 (1.0)	-0.2 (-0.9, 0.5)
Thrombosis FMQ	14 (0.9)	18 (1.2)	-0.3 (-1.0, 0.5)
Cardiac disorders (SOC)			
Myocardial ischemia FMQ	14 (0.9)	14 (0.9)	-0.0 (-0.7, 0.7)
Acute coronary syndrome FMQ	11 (0.7)	14 (0.9)	-0.2 (-0.9, 0.5)
Myocardial infarction FMQ	11 (0.7)	14 (0.9)	-0.2 (-0.9, 0.5)
Infections and infestations (SOC)	56 (3.8)	49 (3.3)	0.4 (-0.9, 1.8)
Bacterial infection FMQ	29 (2.0)	18 (1.2)	0.7 (-0.2, 1.6)
Sepsis (PT)	15 (1.0)	7 (0.5)	0.5 (-0.1, 1.2)
Neoplasms benign, malignant and unspecified (incl cysts and polyps) (SOC)			
Malignancy FMQ	32 (2.2)	35 (2.4)	-0.2 (-1.3, 0.9)
Nervous system disorders (SOC)			
Stroke TIA FMQ	13 (0.9)	17 (1.2)	-0.3 (-1.0, 0.4)

Adverse Event	Daprodustat N=1487 n (%)	rhEPO N=1477 n (%)	RD (%) (95% CI)
Respiratory, thoracic and mediastinal disorders (SOC)			
Respiratory failure FMQ	14 (0.9)	19 (1.3)	-0.3 (-1.1, 0.4)
Vascular disorders (SOC)			
Hemorrhage FMQ	10 (0.7)	16 (1.1)	-0.4 (-1.1, 0.3)

Source: adae.xpt; Software: R

Treatment-emergent adverse events defined as any AEs occurring or worsening between the first dose and the end of the follow-up period.

Risk difference and risk ratio (with 95% confidence interval) are shown between treatment and comparator.

Abbreviations: AE, adverse event; CI, confidence interval; FMQ, FDA medical query; ITT, intent-to-treat; MedDRA, Medical Dictionary for Regulatory Activities; N, number of patients in treatment arm; n, number of patients with adverse event; PT, preferred term; RD, risk difference; rhEPO, recombinant human erythropoietin; SOC, system organ class; TIA, transient ischemic attack

7.6.2.7. Treatment-Emergent Adverse Events

[Table 91](#) provides a frequency-based comparison of common TEAEs reported in the safety population during the on-study period for the DD population. This table includes AEs occurring in greater than 5% in any arm, and with a nominal risk difference (in either direction, favoring or disfavoring daprodustat) of 1%. You may note that there are more PTs and FMQs that are included in this table than that of the ND study: this is because these patients are more comorbid and as a result there is a higher number of patients reporting at least one event and events reported overall (see Sections [7.6.1.3](#) and [7.6.2.3](#)) rather than an incongruous reporting method.

The frequency-based analysis demonstrated that:

- Thrombotic and thromboembolic events (including MI, CVA) had a nominal treatment difference favoring daprodustat, which is consistent with the statistical analyses of adjudicated endpoints discussed in Section [7.4](#). These AEs are discussed in detail in Section [7.6.1.8](#) and Section [7.7](#) as major review issues.
- Nonspecific gastrointestinal related AEs (abdominal pain and dyspepsia nFMQs) were reported in more subjects treated with daprodustat. These will be included in the label.
- Dizziness, falls, and hypotension (FMQ) were more frequently reported in daprodustat subjects compared to rhEPO, with the risk difference ranging from 1.2 to 2.8% (over a median on-study duration of approximately 26 months). Hypotension can occur during dialysis and whether the dizziness, falls and hypotension are interrelated is difficult to determine. The adverse event of dizziness will be included in the adverse reaction table of the label.

We did not note any clinically significant differences between study arms in other nonserious adverse events.

Table 91. Patients With Common Treatment-Emergent Adverse Events by System Organ Class by PT and FDA Medical Query (Narrow) Occurring at ≥5% Frequency, With a Treatment Difference >1%, ASCEND-D

Adverse Event	Daprodustat		RR (%) (95% CI)	RD (95% CI)
	N=1487 n (%)	at rhEPO N=1477 n (%)		
Gastrointestinal disorders SOC	638 (43%) [1509]	618 (41.9%) [1494]	1.03 [0.9 - 1.12]	-2.4 - 4.7
FMQ abdominal Pain N	157 (11%) [197]	117 (7.9%) [163]	1.33 [1.1 - 1.7]	0.6 - 4.7
FMQ dyspepsia N	101 (7%) [115]	73 (5%) [92]	1.38 [1 - 1.8]	0.2 - 3.6
SMQ gastrointestinal nonspecific symptoms	465 (31%) [905]	429 (29.1%) [870]	1.08 [1 - 1.2]	-1 - 5.6
Skin and subcutaneous tissue disorders SOC	258 (17%) [389]	228 (15.5%) [370]	1.13 [1 - 1.3]	-0.7 - 4.6
Pyrexia PT	77 (5%) [95]	93 (6.3%) [106]	0.82 [0.6 - 1.1]	-2.8 - 0.6
Cardiac disorders SOC	391 (26%) [698]	430 (29.2%) [812]	0.9 [0.8 - 1]	-6 - 0.4
SMQ ischemic heart disease N	140 (9%) [185]	158 (10.7%) [227]	0.88 [0.7 - 1.1]	-3.4 - 0.9
SMQ supraventricular tachyarrhythmias N	100 (7%) [124]	131 (8.9%) [186]	0.76 [0.6 - 1]	-4.1 - -0.2
Atrial fibrillation PT	71 (5%) [86]	96 (6.5%) [128]	0.74 [0.5 - 1]	-3.4 - -0.1
FMQ peripheral edema N	87 (6%) [97]	66 (4.5%) [77]	1.31 [1 - 1.8]	-0.2 - 3

Adverse Event	Daprodust at N=1487 n (%)	rhEPO N=1477 n (%)	RR (%) (95% CI)	RD (95% CI)
Thrombosis/Thromboembolism				
FMQ thrombosis arterial N	223 (15%) [327]	261 (17.7%) [430]	0.85 [0.7 - 1]	-2.66 [-5.3 - 0]
FMQ thrombosis venous N	158 (11%) [244]	186 (12.6%) [314]	0.84 [0.7 - 1]	-1.96 [-4.3 - 0.4]
Dialysis related complication PT	46 (3%) [71]	77 (5.2%) [113]	0.59 [0.4 - 0.9]	-2.12 [-3.6 - -0.7]
Arteriovenous fistula site complication PT	91 (6%) [136]	108 (7.3%) [155]	0.84 [0.6 - 1.1]	-1.19 [-3 - 0.6]
Arteriovenous fistula thrombosis PT	98 (7%) [123]	117 (7.9%) [161]	0.83 [0.6 - 1.1]	-1.32 [-3.2 - 0.5]
SMQ central nervous system hemorrhages and cerebrovascular conditions N	76 (5%) [92]	101 (6.9%) [124]	0.75 [0.6 - 1]	-1.72 [-3.4 - 0]
Fluid status				
Dialysis hypotension PT	159 (11%) [288]	133 (9%) [255]	1.19 [1 - 1.5]	-1.71 [-0.4 - 3.9]
FMQ hypotension N	158 (11%) [198]	139 (9.4%) [192]	1.13 [0.9 - 1.4]	-1.23 [-0.9 - 3.4]
Fluid overload PT	115 (8%) [169]	135 (9.2%) [188]	0.85 [0.7 - 1.1]	-1.4 [-3.4 - 0.6]
SMQ shock N	108 (7%) [125]	130 (8.8%) [156]	0.83 [0.6 - 1.1]	-1.53 [-3.5 - 0.4]
Hypotension PT	146 (10%) [180]	127 (8.6%) [176]	1.14 [0.9 - 1.4]	-1.24 [-0.9 - 3.3]
Infections/Infestations				
FMQ viral infection N	244 (16%) [299]	227 (15.4%) [309]	1.07 [0.9 - 1.3]	-1.06 [-1.6 - 3.7]
FMQ bacterial infection N	544 (37%) [1169]	516 (35%) [1118]	1.05 [1 - 1.2]	-1.7 [-1.8 - 5.2]
SMQ sepsis N	112 (8%) [153]	131 (8.9%) [166]	0.85 [0.7 - 1.1]	-1.33 [-3.3 - 0.7]

Adverse Event	Daprodustat		RR (%) (95% CI)	RD (95% CI)	RD (95% CI)
	N=1487 n (%)	rhEPO N=1477 n (%)			
Musculoskeletal					
SMQ arthritis N	85 (6%) [101]	69 (4.7%) [89]	1.23 [0.9 - 1.05 1.7]	-0.5 - 2.7	
Back pain PT	90 (6%) [122]	108 (7.3%) [132]	0.83 [0.6 - 1.1]	-1.25 [-3.1 - 0.5]	
Pain in extremity PT	100 (7%) [124]	83 (5.6%) [110]	1.2 [0.9 - 1.6]	-0.6 - 2.9	
Respiratory, thoracic and mediastinal disorders SOC	412 (28%) [744]	450 (30.5%) [811]	0.91 [0.8 - 1]	-2.73 [-6 - 0.5]	
FMQ respiratory failure N	72 (5%) [91]	95 (6.4%) [118]	0.75 [0.6 - 1]	-1.59 [-3.3 - 0.1]	
Miscellaneous					
FMQ dizziness N	108 (7%) [129]	83 (5.6%) [102]	1.29 [1 - 1.7]	-0.1 - 3.4	
FMQ fall B	341 (23%) [513]	298 (20.2%) [480]	1.14 [1 - 1.3]	-0.2 - 5.8	
FMQ hyperglycemia N	106 (7%) [144]	89 (6%) [111]	1.18 [0.9 - 1.6]	-0.7 - 2.9	
Headache PT	143 (10%) [236]	157 (10.7%) [277]	0.91 [0.7 - 1.1]	-1 [-3.2 - 1.2]	
SMQ hemorrhages N	334 (23%) [532]	353 (23.9%) [582]	0.94 [0.8 - 1.1]	-4.5 - 1.6	
Hypertension			330 (22%) [563]	325 (22%) [530]	1.01 [0.9 - 1.2] 0.22 [-2.8 - 3.2]

Source: Clinical Reviewer, R Ver. 4.2; Medical Dictionary for Regulatory Activities, version 24.
Abbreviations: CI, confidence interval; FMQ, FDA Medical Query; MedDRA, Medical Dictionary for Regulatory Activities; N, number of patients in treatment arm; n, number of patients with at least one event; PT, preferred term; RD, risk difference; rhEPO, recombinant human erythropoietin; RR, relative risk; SMQ, standardized MedDRA query; SOC, system organ class

7.6.2.8. Adverse Events of Special Interest

We took a similar approach to the DD population as was done for the NDD population for AESI. (7.6.1.8 for a discussion of the approach). [Table 92](#) shows a frequency-based listing of adjudicated events along with related investigator-reported SAEs as MedDRA groupings, preferred terms, or queries. This frequency-based analysis demonstrated that:

- **Adjudicated events (MI, CVA, TEE, and HHF):** adjudicated events and investigator reported SAEs have reasonable concordance for measures of absolute and relative risks.
- **Worsening Hypertension and Seizure:** adverse events were reported in similar proportions of patients treated with daprodustat and ESA comparator.
- **Gastrointestinal erosions/bleeding:** in contrast to ASCEND-ND, harms were not evident in narrow SMQs for perforation, and ulcerations.

- **Retinopathies:** serious reports of retinopathies were reported in more subjects treated with daprodustat (n=12, 0.8%) than darbepoetin (n=3, 0.2%), however the frequency was low.

ADPKD was reported in 5% of patients overall. As this population is already on dialysis, progression of renal disease due to ADPKD was not assessed.

Worsening of hypertension is an important risk of ESAs, which includes a section in warnings and precautions, and a contraindication in patients with uncontrolled hypertension. The Applicant prespecified an AESI query (narrow SMQ for Hypertension). Serious worsening hypertension was reported in 3.1% of patients in either arm. The nonserious events were sufficiently balanced between arms to not be included in table 91 (i.e., there was less than a 1% risk difference). However, for labeling purposes, the TEAESI (narrow SMQ, serious and nonserious) was reported in 351 (24%) of patients treated with daprodustat, and 348 (23.6%) of patients treated with ESA comparator, with event rates of 12 per 100 PY for both (HR: 0.98, 95% CI [0.87 – 1.09]). The PT “Hypertension” dominated the query results (~85%). The estimates were similar for broad and narrow FMQs and SMQs. Worsening hypertension is an adverse event of special interest and similar results were observed between daprodustat and ESA comparator. We recommend including hypertension in the warnings and precautions of the prescribing information.

All the safety signals discussed here, besides seizure, hypertension, and retinopathy were major review issues and are discussed in more detail in Section [7.7](#)

Table 92. Patients With Serious Investigator-Reported and Adjudicated Treatment-Emergent Adverse Events by System Organ Class, On Study, ASCEND-D

Adverse Event	Daprodustat	rhEPO	Relative Risk	Risk Difference
	N=1487 n (%) [events]	N=1477 n (%) [events]		
Heart failure				
Adjudicated heart failure	115 (7.8) [162]	107 (7.2) [159]	1.07 [0.83 - 1.38]	0.54 [-1.36 - 2.44]
Heart failure (nFMQ)	87 (5.9) [113]	91 (6.2) [120]	0.95 [0.72 - 1.26]	-0.3 [-2.02 - 1.41]
Heart failures HLGT	58 (3.9) [72]	58 (3.9) [73]	0.99 [0.7 - 1.42]	-0.02 [-1.42 - 1.38]
Myocardial infarction				
SMQ myocardial infarction	77 (5.2) [88]	86 (5.83) [108]	0.89 [0.66 - 1.2]	-0.64 [-2.28 - 1.01]
N				
Ischemic coronary artery disorders HLT	89 (6.0) [109]	98 (6.7) [130]	0.9 [0.68 - 1.19]	-0.64 [-2.4 - 1.11]

Adverse Event	Daprodustat N=1487 n (%) [events]	rhEPO N=1477 n (%) [events]	Relative Risk	Risk Difference
AMI (adjudicated)				
Adjudicated MI	114 (7.7) [137]	136 (9.2) [177]	0.83 [0.66 - 1.06]	-1.53 [-3.54 - 0.47]
Adjudicated type 1 (thrombotic) MI	60 (4.1) [67]	73 (5.0) [85]	0.82 [0.59 - 1.14]	-0.9 [-2.4 - 0.59]
Adjudicated type 2 (O2 mismatch) MI	59 (4.0) [65]	68 (4.6) [85]	0.86 [0.61 - 1.21]	-0.63 [-2.09 - 0.83]
ECG not available	27 (1.8) [31]	37 (2.5) [46]	0.73 [0.44 - 1.19]	-0.69 [-1.74 - 0.36]
Adjudicated NSTEMI	70 (4.7) [83]	90 (6.1) [114]	0.77 [0.57 - 1.05]	-1.38 [-3.01 - 0.25]
Adjudicated STEMI	11 (0.7) [11]	6 (0.4) [6]	1.82 [0.68 - 4.92]	0.34 [-0.21 - 0.88]
Adjudicated fatal MI	11 (0.7) [11]	8 (0.5) [8]	1.37 [0.55 - 3.39]	0.2 [-0.38 - 0.78]
Stroke				
FMQ stroke TIA N	50 (3.4) [55]	63 (4.3) [70]	0.79 [0.55 - 1.14]	-0.9 [-2.28 - 0.48]
TIA HLT	9 (0.6) [9]	11 (0.8) [13]	0.81 [0.34 - 1.96]	-0.14 [-0.73 - 0.45]
Stroke HLT	41 (2.8) [45]	51 (3.5) [56]	0.8 [0.53 - 1.2]	-0.69 [-1.95 - 0.56]
Adjudicated stroke	43 (2.9) [45]	51 (3.5) [56]	0.84 [0.56 - 1.25]	-0.56 [-1.82 - 0.71]
Adjudicated ischemic stroke	33 (2.2) [35]	36 (2.4) [41]	0.91 [0.57 - 1.45]	-0.22 [-1.3 - 0.87]
Adjudicated fatal stroke	10 (0.7) [10]	12 (0.8) [12]	0.83 [0.36 - 1.91]	-0.14 [-0.76 - 0.48]
FMQ seizure N	10 (0.7) [14]	15 (1.0) [17]	0.66 [0.3 - 1.47]	-0.34 [-1 - 0.32]
Seizures (includes subtypes) HLT	10 (0.7) [14]	15 (1.0) [17]	0.66 [0.3 - 1.47]	-0.34 [-1 - 0.32]
Thromboembolic events				
Device malfunction events	9 (0.6) [10]	18 (1.2) [20]	0.5 [0.22 - 1.1]	-0.61 [-1.3 - 0.07]
NEC HLT				
Vascular complications associated with device	3 (0.2) [4]	2 (0.1) [2]	1.49 [0.25 - 8.92]	0.07 [-0.23 - 0.36]
HLT				
Cardiac and vascular procedural complications	126 (8.5) [169]	143 (9.7) [203]	0.88 [0.7 - 1.1]	-1.2 [-3.27 - 0.87]
HLT				
Deep vein thrombosis PT	8 (0.5) [8]	4 (0.3) [4]	1.99 [0.6 - 6.59]	0.27 [-0.19 - 0.73]
Pulmonary embolism PT	5 (0.3) [5]	14 (1.0) [15]	0.36 [0.13 - 0.98]	-0.61 [-1.19 - -0.04]
Adjudicated thromboembolic	185 (12.5) [294]	215 (14.6) [365]	0.86 [0.71 - 1.03]	-2.1 [-4.57 - 0.36]
Adjudicated DVT	23 (1.6) [24]	19 (1.3) [20]	1.2 [0.66 - 2.2]	0.26 [-0.59 - 1.12]
Adjudicated PE	9 (0.6) [9]	14 (1.0) [15]	0.64 [0.28 - 1.47]	-0.34 [-0.98 - 0.29]
Adjudicated VAT	164 (11.1) [261]	201 (13.6) [330]	0.81 [0.67 - 0.98]	-2.57 [-4.94 - -0.2]
Adjudicated TEE - fatal	2 (0.1) [2]	2 (0.1) [2]	0.99 [0.14 - 7.05]	0 [-0.27 - 0.26]

Adverse Event	Daprodustat N=1487 n (%) [events]	rhEPO N=1477 n (%) [events]	Relative Risk	Risk Difference
Accelerated and malignant hypertension HLT	23 (1.6) [31]	29 (2.0) [36]	0.79 [0.46 - 1.36]	-0.42 [-1.36 - 0.53]
Gastrointestinal bleeding				
SMQ gastrointestinal hemorrhage N	50 (3.4) [58]	46 (3.1) [53]	1.08 [0.73 - 1.6]	0.25 [-1.02 - 1.53]
SMQ gastrointestinal perforation N	55 (3.7) [69]	57 (3.9) [77]	0.96 [0.67 - 1.38]	-0.16 [-1.53 - 1.22]
SMQ gastrointestinal ulceration N	26 (1.8) [28]	28 (1.9) [31]	0.92 [0.54 - 1.57]	-0.15 [-1.11 - 0.82]
Prespecified AESI queries				
Cancer-related mortality and tumor progression and recurrence	54 (3.6) [62]	51 (3.5) [58]	1.05 [0.72 - 1.53]	0.2 [-1.2 - 1.5]
Cardiomyopathy	9 (0.6) [9]	8 (0.5) [8]	1.12 [0.43 - 2.89]	0.1 [-0.5 - 0.6]
Esophageal and gastric erosions	53 (3.6) [60]	45 (3.1) [53]	1.17 [0.79 - 1.73]	0.5 [-0.8 - 1.8]
Proliferative retinopathy/macular edema/choroidal neovascularization	12 (0.8) [14]	3 (0.2) [3]	3.98 [1.12 - 14.07]	0.6 [0.1 - 1.1]
Pulmonary artery hypertension	2 (0.1) [2]	9 (0.6) [11]	0.22 [0.05 - 1.02]	-0.5 [-0.9 - 0]
Thrombosis and/or tissue ischemia	210 (14.2) [274]	255 (17.3) [364]	0.82 [0.69 - 0.97]	-3.1 [-5.8 - -0.5]
Worsening of hypertension	46 (3.1) [64]	46 (3.1) [57]	0.99 [0.67 - 1.49]	0 [-1.3 - 1.2]
Exacerbation of rheumatoid arthritis	1 (0.1) [1]	0 (0) [0]	NE	0 [-0.1 - 0.2]

Source: Clinical Reviewer, R Ver. 4.3, adevent.xpt (Adevent.R, AESI.R, script on file)

On-study analysis

Abbreviations: AESI, adverse event of special interest; AMI, acute myocardial infarction; DVT, deep venous thrombosis; ECG, electrocardiogram; FMQ, FDA medical query; HLT, high level term; HLT, high level term; MI, myocardial infarction; nFMQ, FDA medical query (narrow); NSTEMI, non-ST-elevation myocardial infarction; rhEPO, recombinant human erythropoietin; SMQ, standardized MedDRA query; NEC, not elsewhere classified; PE, pulmonary embolism; PT, preferred term; STEMI, ST-elevation myocardial infarction; TEE, thromboembolic event; TIA, transient ischemic attack; VAT, venous arterial thromboembolism

7.6.2.9. Laboratory Findings

[Table 93](#) shows other laboratory abnormalities for laboratory values routinely collected in ASCEND-D. There is a high baseline rate of electrolyte abnormalities in CKD patients, which makes it challenging to resolve treatment-emergent treatment differences.

There were no clinically significant differences between trial arms in relation to the change values of laboratory parameters from baseline to end of treatment values. We did not find any credible treatment differences.

Table 93. Patients With One or More Chemistry Analyte Values With Elevated or Low Values Meeting Specified Levels, ITT Population, ASCEND-D

Level	ASCEND-D		
	Daprodustat N=1487 n/N _w (%)	rhEPO N=1477 n/N _w (%)	RD (%) (95% CI)
Potassium, low (mEq/L)			
Level 1 (<3.6)	265/1486 (17.8)	278/1476 (18.8)	-1.0 (-3.8, 1.8)
Level 2 (<3.4)	154/1486 (10.4)	176/1476 (11.9)	-1.6 (-3.8, 0.7)
Level 3 (<3)	34/1486 (2.3)	41/1476 (2.8)	-0.5 (-1.6, 0.6)
Potassium, high (mEq/L)			
Level 1 (>5.5)	797/1486 (53.6)	815/1476 (55.2)	-1.6 (-5.2, 2.0)
Level 2 (>6)	465/1486 (31.3)	433/1476 (29.3)	2.0 (-1.4, 5.3)
Level 3 (>6.5)	225/1486 (15.1)	206/1476 (14.0)	1.2 (-1.4, 3.7)
Calcium, low (mg/dL)			
Level 1 (<8.4)	933/1486 (62.8)	901/1476 (61.0)	1.7 (-1.8, 5.2)
Level 2 (<8)	607/1486 (40.8)	581/1476 (39.4)	1.5 (-2.0, 5.0)
Level 3 (<7.5)	381/1486 (25.6)	333/1476 (22.6)	3.1 (0.0, 6.2)
Calcium, high (mg/dL)			
Level 1 (>10.5)	157/1486 (10.6)	161/1476 (10.9)	-0.3 (-2.6, 1.9)
Level 2 (>11)	54/1486 (3.6)	45/1476 (3.0)	0.6 (-0.7, 1.9)
Level 3 (>12)	16/1486 (1.1)	9/1476 (0.6)	0.5 (-0.2, 1.1)
Phosphate, low (mg/dL)			
Level 1 (<2.5)	326/1486 (21.9)	345/1476 (23.4)	-1.4 (-4.5, 1.6)
Level 2 (<2)	133/1486 (9.0)	137/1476 (9.3)	-0.3 (-2.4, 1.7)
Level 3 (<1.4)	47/1486 (3.2)	43/1476 (2.9)	0.2 (-1.0, 1.5)
Albumin, low (g/dL)			
Level 1 (<3.1)	259/1486 (17.4)	204/1476 (13.8)	3.6 (1.0, 6.2)
Level 2 (<2.5)	49/1486 (3.3)	48/1476 (3.3)	0.0 (-1.2, 1.3)
Level 3 (<2)	14/1486 (0.9)	14/1476 (0.9)	-0.0 (-0.7, 0.7)

Source: ad b.xpt; Software: R

Abbreviations: CI, confidence interval; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data; RD, risk difference; rhEPO, recombinant human erythropoietin

Table 94. Patients With One or More Hematology Analyte Values Exceeding Specified Levels, ITT Population, Trials ASCEND-D

Level	ASCEND-D		
	Daprodustat N=1487 n/N _w (%)	rhEPO N=1477 n/N _w (%)	RD (%) (95% CI)
Complete blood count			
WBC, low (cells/uL)			
Level 1 (<3500)	248/1484 (16.7)	229/1474 (15.5)	1.2 (-1.5, 3.8)
Level 2 (<3000)	119/1484 (8.0)	116/1474 (7.9)	0.1 (-1.8, 2.1)
Level 3 (<1000)	1/1484 (0.07)	1/1474 (0.07)	-0.0 (-0.2, 0.2)
WBC, high (cells/uL)			
Level 1 (>10800)	262/1484 (17.7)	227/1474 (15.4)	2.3 (-0.4, 4.9)
Level 2 (>13000)	100/1484 (6.7)	91/1474 (6.2)	0.6 (-1.2, 2.3)
Level 3 (>15000)	36/1484 (2.4)	45/1474 (3.1)	-0.6 (-1.8, 0.5)
Platelets, low (cells/uL)			
Level 1 (<140000)	521/1485 (35.1)	476/1474 (32.3)	2.8 (-0.6, 6.2)
Level 2 (<125000)	355/1485 (23.9)	329/1474 (22.3)	1.6 (-1.5, 4.6)
Level 3 (<1e+05)	137/1485 (9.2)	142/1474 (9.6)	-0.4 (-2.5, 1.7)

Level	ASCEND-D		
	Daprodustat N=1487 n/N _w (%)	rhEPO N=1477 n/N _w (%)	RD (%) (95% CI)
WBC differential			
Lymphocytes, low (cells/uL)			
Level 1 (<1000)	857/1483 (57.8)	841/1474 (57.1)	0.7 (-2.8, 4.3)
Level 2 (<750)	493/1483 (33.2)	438/1474 (29.7)	3.5 (0.2, 6.9)
Level 3 (<500)	155/1483 (10.5)	135/1474 (9.2)	1.3 (-0.9, 3.4)
Lymphocytes, high (cells/uL)			
Level 1 (>4000)	12/1483 (0.8)	12/1474 (0.8)	-0.0 (-0.7, 0.6)
Level 2 (>10000)	0/1483 (0)	2/1474 (0.1)	-0.1 (-0.3, 0.1)
Level 3 (>20000)	0/1483 (0)	0/1474 (0)	0 (0, 0)
Neutrophils, low (cells/uL)			
Level 1 (<2000)	289/1483 (19.5)	281/1474 (19.1)	0.4 (-2.4, 3.3)
Level 2 (<1000)	24/1483 (1.6)	20/1474 (1.4)	0.3 (-0.6, 1.1)
Level 3 (<500)	4/1483 (0.3)	7/1474 (0.5)	-0.2 (-0.6, 0.2)
Eosinophils, high (cells/uL)			
Level 1 (>650)	281/1483 (18.9)	292/1474 (19.8)	-0.9 (-3.7, 2.0)
Level 2 (>1500)	42/1483 (2.8)	38/1474 (2.6)	0.3 (-0.9, 1.4)
Level 3 (>5000)	1/1483 (0.07)	1/1474 (0.07)	-0.0 (-0.2, 0.2)
Cholesterol, total, high (mg/dL)			
Level 1 (>200)	334/1484 (22.5)	352/1472 (23.9)	-1.4 (-4.4, 1.6)
Level 2 (>210)	265/1484 (17.9)	263/1472 (17.9)	-0.0 (-2.8, 2.8)
Level 3 (>225)	169/1484 (11.4)	175/1472 (11.9)	-0.5 (-2.8, 1.8)
HDL, males, low (mg/dL)			
Level 1 (<40)	594/848 (70.0)	553/844 (65.5)	4.5 (0.1, 9.0)
Level 2 (<30)	258/848 (30.4)	240/844 (28.4)	2.0 (-2.4, 6.3)
Level 3 (<20)	39/848 (4.6)	32/844 (3.8)	0.8 (-1.1, 2.7)
HDL, females, low (mg/dL)			
Level 1 (<50)	474/636 (74.5)	431/628 (68.6)	5.9 (0.9, 10.9)
Level 2 (<40)	295/636 (46.4)	274/628 (43.6)	2.8 (-2.7, 8.2)
Level 3 (<20)	18/636 (2.8)	9/628 (1.4)	1.4 (-0.2, 3.0)
LDL, high (mg/dL)			
Level 1 (>130)	213/1484 (14.4)	256/1473 (17.4)	-3.0 (-5.7, -0.4)
Level 2 (>160)	56/1484 (3.8)	70/1473 (4.8)	-1.0 (-2.4, 0.5)
Level 3 (>190)	16/1484 (1.1)	16/1473 (1.1)	-0.0 (-0.8, 0.7)

Source: ad b.xpt; Software: R

Risk difference and risk ratio (with 95% confidence interval) are shown between treatment and comparator.

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; ITT, intent-to-treat; LDL, low-density lipoprotein; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data; RD, risk difference; rhEPO, recombinant human erythropoietin; RR, risk ratio; WBC, white blood cells

7.6.2.10. Vital Signs' Analyses

Hypertension is a known risk for ESAs, and thus are of special interest for daprodustat. Vitals for SBP, DBP, and HR were systematically collected during the study. One measurement of SBP, DBP, and HR was taken with the subject in a semi-supine or seated position after a 5-minute rest period. Triplicate measurements were taken at baseline and the end of study visit (or early treatment discontinuation visit, as applicable).

We graphically inspected aggregate plots vs. time and maximum on-treatment vs. baseline values for each and did not identify trends. One limitation of the SBP and DBP data is that they were collected using cuffs, which are not as definitive as ambulatory blood pressure monitoring.

For brevity, we only report shift tables below. It is important to note that these tables were not controlled for baseline hypertension status. Nonetheless, the objective measurement of hypertension appears similar between daprodustat and ESA comparator. The use of antihypertensive agents was stable throughout the treatment period for both treatment arms, and was balanced between arms, consistent with trends seen in investigator adverse event reports.

Table 95. Percentage of Patients With Maximum Systolic Blood Pressure by Category of Blood Pressure Post-Baseline, ITT Population, ASCEND-D

	ASCEND-D		
	Daprodustat N=1487 n/N _w (%)	rhEPO N=1477 n/N _w (%)	RD (%) (95% CI)
Systolic blood pressure (mm Hg)			
<90	1/1482 (0.07)	1/1467 (0.07)	-0.0 (-0.2, 0.2)
≥90	1481/1482 (99.9)	1466/1467 (99.9)	0.0 (-0.2, 0.2)
≥120	1457/1482 (98.3)	1445/1467 (98.5)	-0.2 (-1.1, 0.7)
≥140	1335/1482 (90.1)	1325/1467 (90.3)	-0.2 (-2.4, 1.9)
≥160	901/1482 (60.8)	902/1467 (61.5)	-0.7 (-4.2, 2.8)
≥180	368/1482 (24.8)	400/1467 (27.3)	-2.4 (-5.6, 0.7)
Diastolic blood pressure (mm Hg)			
<60	13/1482 (0.9)	10/1467 (0.7)	0.2 (-0.4, 0.8)
≥60	1469/1482 (99.1)	1457/1467 (99.3)	-0.2 (-0.8, 0.4)
≥90	840/1482 (56.7)	841/1467 (57.3)	-0.6 (-4.2, 2.9)
≥110	124/1482 (8.4)	131/1467 (8.9)	-0.6 (-2.6, 1.5)
≥120	46/1482 (3.1)	38/1467 (2.6)	0.5 (-0.7, 1.7)

Source: advs.xpt; Software: R

Risk difference and risk ratio (with 95% confidence interval) are shown between treatment and comparator.

Post-dialysis data used for patients undergoing dialysis.

Abbreviations: CI, confidence interval; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data; RD, risk difference; rhEPO, recombinant human erythropoietin

7.7. Key Safety Review Issues

7.7.1. Difference Between On-Study Versus On-Treatment Analyses Results – ASCEND-ND

Issue

In ASCEND-ND, the prespecified supportive OT analysis (OT+28) resulted in a higher incidence of MACE in the daprodustat arm compared to the control arm and an HR estimate (HR 1.40; 95% CI 1.17, 1.68) that was greater than the on-study analysis estimate (HR 1.03; 95% CI 0.89, 1.19).

Background

To assess risks, two approaches were considered: an on-study analysis and OT analysis. An on-study analysis considers a subject at risk of experiencing an event from the time of randomization until trial discontinuation, which includes events that occur while a subject is on treatment and off treatment (for those subjects who prematurely discontinued treatment but remained in the trial). An OT analysis considers a subject at risk of experiencing an event from the time of randomization to the time of treatment discontinuation plus a fixed pre-specified period of time thereafter (e.g., 28 days).

An on-study analysis approach is valuable in the assessment of risks that have a long latency period. In addition, such analyses preserve the integrity of randomization. A limitation of an on-study analysis approach is that it may be less sensitive than the OT analysis to detecting true adverse effects of the study drug, especially in cases of high rates or between-group imbalances of premature treatment discontinuation (these subjects who remain in the trial are still considered at risk for adverse reactions in the analysis but are no longer taking study drug) or use of rescue medication (that carry their own risks).

An OT analysis approach is sensitive to risks thought to be pharmacodynamic responses to the drug, and as such, the risk would be expected to occur while exposed to treatment or shortly thereafter. However, the OT analysis approach has the potential to break the integrity of randomization and introduce bias as there may be differences between treatment arms in the types of subjects who adhere to treatment. Such differences cannot easily be adjusted by statistical analysis.

Assessment

In the development program for daprodustat, an on-study analysis approach was the prespecified primary analysis and an OT analysis approach was a supportive analysis. Three variations of the OT analysis accounting for differing treatment dosing intervals, resulting in different ascertainment windows, were considered (see Section [7.6.1.1.2](#) and [Table 72](#)).

In ASCEND-ND, the prespecified primary on-study analysis approach captured events from randomization to study completion/withdrawal (mean follow-up duration 1.9 years). Treatment discontinuation was similar between treatment arms (daprodustat 38%, darbepoetin alfa 38%), as was study withdrawal (daprodustat 3%, darbepoetin alfa 3%) and use of rescue therapies (daprodustat 2%, darbepoetin alfa 3%).

The prespecified primary analysis of MACE was on-study (HR 1.03; 95% CI 0.89, 1.19; see Section [7.6.1.1.1](#)). The prespecified supportive OT analysis (OT+28) resulted in a higher incidence of MACE in the daprodustat arm compared to the control arm and an HR estimate (HR 1.40; 95% CI 1.17, 1.68) that was greater than the on-study analysis estimate ([Table 72](#)). Exploratory OT analyses adjusted by the dosing frequencies of the different treatments attenuated the effect estimate; the extent of attenuation depended on the ascertainment window chosen (OT+Dosing Frequency+28: HR 1.18 (95% CI 0.99, 1.40) and OT+Dosing Frequency: HR 1.09 (95% CI 0.89, 1.33)).

Conclusion

In summary, OT analyses produced HR estimates for MACE that were greater than the on-study analysis estimate, and the OT estimates differed depending on the ascertainment window used. However, as noted above, comparisons based on the OT analyses are likely subject to bias, which cannot easily be corrected through analysis. The design and conduct of ASCEND-ND appeared sufficient to evaluate and estimate risks based on an on-study analysis approach.

7.7.2. Higher Incidence of Cardiovascular Endpoints – ASCEND-ND

Issue

In ASCEND-ND, the estimated HRs for CV MACE, CV mortality, MI, stroke, HHF, TEE, and VAT are all increased for the comparison of daprodustat to darbepoetin alfa, ranging from 1.06 to 1.49.

Background

A limitation to the use of MACE to assess CV risk was that MACE was a composite endpoint prespecified to contain all-cause mortality, therefore non-CV deaths were included. To better understand CV risk, secondary CV endpoints and CV MACE (a composite of CV mortality, nonfatal MI, and nonfatal stroke) were analyzed.

Assessment

Table 96. Summary of Results for Adjudicated Cardiovascular Safety Endpoints, ASCEND-ND

	Daprodustat N=1937 n [IR]	Darbepoetin alfa N=1935 n [IR]	Rate Diff Per 100 PY (95% CI)	Hazard Ratio (95% CI)
CV MACE	214 [6.1]	194 [5.6]	0.6 (-0.5, 1.7)	1.11 (0.91, 1.34)
CV mortality	109 [3.0]	92 [2.6]	0.5 (-0.3, 1.2)	1.20 (0.91, 1.58)
Fatal or nonfatal myocardial infarction	103 [2.9]	97 [2.8]	0.2 (-0.6, 1.0)	1.06 (0.80, 1.40)
Fatal or nonfatal stroke	45 [1.3]	34 [1.0]	0.3 (-0.2, 0.8)	1.33 (0.85, 2.07)
Hospitalization for heart failure	140 [4.0]	115 [3.3]	0.7 (-0.2, 1.6)	1.22 (0.95, 1.56)
Thromboembolic event	64 [1.8]	51 [1.4]	0.4 (-0.2, 1.0)	1.27 (0.88, 1.84)
Vascular access thrombosis	46 [1.3]	31 [0.9]	0.4 (-0.1, 0.9)	1.49 (0.94, 2.35)

Source: FDA analysis.

CV MACE: Composite of CV mortality, nonfatal MI, and nonfatal stroke.

Abbreviations: CI, confidence interval; CV, cardiovascular; Diff, difference; IR, incidence rate per 100 PY; MACE, major adverse cardiovascular event; MI, myocardial infarction; N, number of subjects; n, number of subjects with event; PY, person-year

Results of the primary (on-study) time-to-event analysis of CV endpoints in ASCEND-ND are shown in [Figure 29](#) and in [Table 96](#). As shown in the figure and table, the estimated HRs for CV MACE, CV mortality, MI, stroke, HHF, TEE, and VAT are all increased for the comparison of daprodustat to darbepoetin alfa, ranging from 1.06 to 1.49. The corresponding IRDs (per 100-person years) ranged from 0.2 to 0.7.

The review team also carefully considered if the thrombotic and thromboembolic risks observed in ASCEND-ND could be mitigated using a population selection approach. Population selection is the most feasible mitigation measure, given thrombosis/thromboembolism does not typically

present with premonitory/prodromal symptoms and often occurs unpredictably or sporadically. The Agency conducted exploratory subgroup analyses excluding patients with preexisting CVD,¹⁸ which is a straightforward population with lower baseline absolute risk for MI, CVA, TEE. The rate with daprodustat vs. darbepoetin alfa was not appreciably lower in the no CVD group for a simple aggregate of MI, CVA, or TEE (3.6/100 PY versus 5.1/100 PY), nor was the absolute harm appreciably lower (0.70/100 PY versus 0.86/100 PY).

Following the Cardiovascular and Renal Drugs Advisory Committee meeting on October 26, 2022, the Applicant proposed (b) (4)

(b) (4)

This proposal was based on (b) (4)

However, we do not consider (b) (4)

these (b) (4) reliable enough (b) (4)

Conclusion

Limitations of the CV analyses included lower precision (compared to MACE) because of lower event rates (and hence wider 95% CIs) and no Type I error control for treatment arm comparisons of the CV endpoints. However, in ASCEND-ND, the consistently increased risk estimates across different CV endpoints measuring related aspects of CV risk in a CV outcomes trial raised concerns as to whether daprodustat is safe relative to darbepoetin alfa, which itself carries an increased CV risk. In ASCEND-D, there was not a consistent pattern of increased risk estimates in the CV endpoints (Section 7.6.2.2.1). The reasons for discrepant trials results for CV safety are not clear.

7.7.3. Higher Incidence of Cardiovascular Endpoints in the USA Subgroup – ASCEND-ND

Issue

A higher incidence of CV endpoints was observed in the daprodustat arm compared to the darbepoetin alfa arm in the USA region in the ASCEND-ND study. Except stroke, the HRs of the non-USA subgroup analyses were approximately 1.0, and the HRs of the USA subgroup analyses were elevated (HRs ranging from 1.21 to 2.03).

Background

The estimated HR for MACE was greater than 1.0 comparing daprodustat to darbepoetin alfa in the USA subgroup (HR 1.19; 95% CI 0.91, 1.54), which was of particular interest because the USA is under FDA's jurisdiction. To further explore CV endpoints in the regional subgroups,

¹⁸ Defined by the Applicant as any subject having one or more of the following: angina pectoris, myocardial infarction, stroke, coronary artery disease, transient ischemic attack, heart failure, atrial fibrillation, cardiac arrest, valvular heart disease.

exploratory analyses were conducted for each adjudicated CV endpoint in the USA and non-USA subgroups.

Assessment

A higher incidence of each CV endpoint was observed in the daprodustat arm compared to the darbepoetin alfa arm in the USA subgroup ([Figure 30](#)). Except stroke, the HRs of the non-USA subgroup analyses were approximately 1.0, and the HRs of the USA subgroup analyses were elevated (ranging from 1.21 to 2.03). The variability (95% CI width) of HR estimates depended on the number of events and sample sizes, with lower precision for the USA subgroup compared to the non-USA subgroup and for endpoints like stroke and TEE.

There were differences in baseline characteristics of the USA compared to the non-USA subgroup. USA subjects used less ESAs at baseline, which was also reflected in baseline Hb category, and generally had more comorbid conditions at baseline (history of diabetes, heart failure, cardiovascular disease). In addition, there were differences in enrollment by race between USA and non-USA subgroups ([Table 97](#)). It is unclear if these differences contributed to the discrepant results between the USA and non-USA subgroups.

Table 97. Selected Baseline Demographics for the US and Non-US Populations of ASCEND-ND, ITT Population

	Non-US N=2891	US N=981
Demographics		
Age (median [IQR])	66 [56, 74]	69 [60, 77]
Male (%)	1258 (43.5)	441 (45.0)
Race (%)		
American Indian or Alaskan Native	184 (6.4)	4 (0.4)
Asian	1034 (36)	28 (3)
Black or African American	47 (1.6)	321 (33)
White	1530 (53)	623 (64)
Bodyweight (kg) (median [IQR])	68 [58, 80]	81 [68, 97]
Medical History		
Baseline ESA user (%)	1515 (52)	295 (30)
Diabetes (%)	1542 (53)	676 (69)
History of stroke (%)	176 (6.1)	80 (8.2)
History of MI (%)	183 (6.3)	86 (8.8)
History of HF (%)	467 (16)	220 (22)
History of thrombosis (%)	92 (3.2)	58 (5.9)
History of CVD (%)	964 (33)	468 (48)
Hospitalization within 6 months	387 (13)	76 (7.7)
Concomitant Drugs		
Vitamin K antagonist use (%)	85 (2.9)	41 (4.2)
Aspirin use (%)	705 (24)	454 (46)
Clopidogrel use (%)	232 (8.0)	114 (12)

	Non-US N=2891	US N=981
CKD/Anemia Biomarkers		
eGFR (median [IQR])	17 [11, 25]	21 [15, 29]
Urine albumin/creatinine (median [IQR])	90 [18, 231]	51 [8.7, 175]
Systolic BP (mmHg) (median [IQR])	135 [125, 147]	135 [124, 147]
CRP (median [IQR])	1.9 [0.7, 5.1]	2.40 [1.0, 6.1]
Hemoglobin at baseline		
<9 g/dL	440 (15.2)	203 (21)
9-<10 g/dL	1029 (36)	430 (44)
10-11 g/dL	1088 (38)	305 (31)
>11 g/dL	334 (12)	43 (4.4)

Source: Clinical Reviewer; ADSL.xpt; R v. 3.4.

Abbreviations: BP, blood pressure; CKD, chronic kidney disease; CRP, C-reactive protein; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; ESA, erythropoiesis-stimulating agent; HF, heart failure; IQR, interquartile range; ITT, intention-to-treat; MI, myocardial infarction; US, United States

Conclusion

Although exploratory analyses could introduce bias and produce unreliable results, the higher risk estimates in the USA subgroup compared to the non-USA subgroup in the NDD population were seen across multiple CV endpoints and were consistent with the unfavorable MACE prespecified region subgroup results (Section 7.6.1.1.3). In summary, these higher incidences of CV endpoints in the daprodustat arm in the USA subgroup raised concerns as to whether daprodustat is safe relative to darbepoetin alfa, which itself carries an increased CV risk.

7.7.4. Higher Incidence of Hospitalization for Heart Failure - ASCEND-ND, ASCEND-D

Issue

A higher incidence of HHF with daprodustat compared to active control was observed in both ASCEND-ND and ASCEND-D studies. In ASCEND-D, only HHF (among CV endpoints) showed an increased risk estimate in the daprodustat arm compared to active control. In ASCEND-ND, HHF with other CV endpoints showed an increased risk estimate in the daprodustat arm compared to active control.

Background

To explore contributing factors that might have resulted in the HHF imbalance between treatments, subgroup analyses by history of heart failure were conducted.

Assessment

In ASCEND-ND (Section 7.6.1.2.1), a higher incidence of HHF was observed in the daprodustat arm (140/1937 [4.0 events/100 PY]) compared to the darbepoetin alfa arm (115/1935 [3.3 events/100 PY]), corresponding to a HR of 1.22 (95% CI 0.95, 1.56). A higher incidence was also observed for other adjudicated CV endpoints in the daprodustat arm in ASCEND-ND.

In ASCEND-D (Section 7.6.2.2.1), a higher incidence of HHF was observed in the daprodustat arm (112/1487 [3.3 events/100 PY]) compared to the ESA arm (101/1477 [3.0 events/100 PY])

corresponding to a HR of 1.10 (CI 0.84, 1.45). A higher incidence was not observed for other adjudicated CV endpoints in the daprodustat arm.

In both ASCEND-ND and ASCEND-D, exploratory subgroup analyses by history of heart failure (Yes, No) showed a higher incidence of HHF in the daprodustat arm compared to the control arm in the subgroup with a history of heart failure ([Table 73](#), [Table 85](#)). The HR for the subgroup with history of heart failure was 1.51 (95% CI 1.01, 2.25) in ASCEND-ND and 1.44 (95% CI 0.97, 2.14) in ASCEND-D. In contrast, the HR for the subgroup without a history of heart failure was 0.99 (95% CI 0.72, 1.36) in ASCEND-ND and 0.91 (95% CI 0.63, 1.32) in ASCEND-D.

The observed increased risk for HHF was attenuated in the DD population. Potential explanations include more frequent fluid management (e.g., dialysis), and more intense monitoring (e.g., more frequent health care interactions, where eminent heart failure exacerbations are identified and mitigated).

Conclusion

Limitations of the HHF analyses, including the subgroup analyses by history of HF, include lower precision (compared to MACE) because of lower event rates (and hence wider 95% CIs) and no Type I error control for multiple comparisons between treatment arms. However, the increased risks for HHF were observed in both the NDD and DD populations and in the subgroup with history of heart failure of both populations. While the precision and post-hoc nature of this analysis has limitations, there is a plausible mechanism for the subgroup interaction and an appreciable effect modification observed in both studies. For these reasons, we concluded that the HHF risk could be reliably mitigated by including a warning for patients with a history of heart failure in the DD population.

7.7.5. Three Times a Week (TIW) Dosing in ASCEND-TD

Issue

ASCEND-TD established non-inferiority of daprodustat TIW to IV epoetin alfa on Hb in the DD (hemodialysis only) population. However, the study was not designed to demonstrate general or cardiovascular safety. Is the safety established from ASCEND-D sufficient to establish safety for a TIW dosing regimen?

Background

Patients on hemodialysis typically have in-center hemodialysis sessions three times a week, where hemoglobin is routinely monitored and IV epoetin alfa is administered during the hemodialysis session. A daprodustat TIW dosing regimen may offer patient convenience. Additionally, three times a week dosing would enable institutionalization of treatment (i.e., daprodustat treatment could be managed by the hemodialysis center for patients on hemodialysis), which may confer enhanced adherence over outpatient-based anemia treatment. ASCEND-TD was designed to demonstrate efficacy, but not cardiovascular or general safety in the DD population (n=407, 2:1 randomization to daprodustat: epoetin alfa). Briefly, ASCEND-

TD was a randomized, double-blind, active-controlled, double dummy, parallel-group, multi-center study in hemodialysis-dependent patients (refer to Section 6 for additional study design details). This study demonstrated NI for hemoglobin using a dosing scheme with two times higher starting dose and titration.

Assessment

The clinical trial data in ASCEND-TD were insufficient to make an independent determination of safety, particularly CV safety. The results of the adjudicated endpoints are presented in Table 98. ASCEND-TD was a fixed-duration study, and these results represent the proportion of incident AEs over the 52-week duration. There are small imbalances not favoring daprodustat in MI (4.1% vs. 3.6%) and stroke (3.0% vs. 0%), and other small imbalances favoring daprodustat in TEE (10.0% vs. 12.4%) and HHF (1.9% vs. 2.9%). However, given the small sample size and low event rates with unstable estimates (e.g., only 1 additional MI with rhEPO would eradicate the MI imbalance), the precision of treatment effect estimates precludes definitive inference. Therefore, the cardiovascular safety of the TIW regimen in hemodialysis is indeterminate based on the existing AE data.

Table 98. Patients With Adjudicated Cardiovascular Safety Events, ITT Population, ASCEND-TD

	Daprodustat N=270 n (%)	rhEPO N=137 n (%)	RD (%) (95% CI)
All-cause mortality	18 (6.7)	10 (7.3)	-0.6 (-5.9, 4.6)
CV mortality	7 (2.6)	5 (3.6)	-1.1 (-4.7, 2.6)
All MI	11 (4.1)	5 (3.6)	0.4 (-3.5, 4.4)
Fatal	1 (0.4)	0 (0)	0.4 (-0.4, 1.1)
Nonfatal	10 (3.7)	5 (3.6)	0.1 (-3.8, 3.9)
All stroke	8 (3.0)	0 (0)	3.0 (0.9, 5.0)
Fatal	1 (0.4)	0 (0)	0.4 (-0.4, 1.1)
Nonfatal	7 (2.6)	0 (0)	2.6 (0.7, 4.5)
All thromboembolic events	27 (10.0)	17 (12.4)	-2.4 (-9.0, 4.2)
Fatal	0 (0)	0 (0)	0 (0, 0)
Nonfatal	27 (10.0)	17 (12.4)	-2.4 (-9.0, 4.2)
All heart failure	5 (1.9)	4 (2.9)	-1.1 (-4.3, 2.2)
Fatal	1 (0.4)	1 (0.7)	-0.4 (-2.0, 1.2)
Nonfatal	4 (1.5)	3 (2.2)	-0.7 (-3.6, 2.1)
Hospitalized	3 (1.1)	3 (2.2)	-1.1 (-3.8, 1.7)

Source: adevent.xpt; Software: R

Risk difference (with 95% confidence interval) is shown between treatment and comparator.

Abbreviations: CI, confidence interval; CV, cardiovascular; ITT, intent-to-treat; MI, myocardial infarction; N, number of patients in treatment arm; n, number of patients with adverse event; RD, risk difference; rhEPO, recombinant human erythropoietin

Cardiovascular safety is related to hemoglobin levels – as was shown in CHOIR, CREATE, TREAT and the Normal Hematocrit study. Additionally, it is postulated that Hb rate of rise may contribute to the pro-thrombotic effects of ESAs, and by extension to HIF-PHIs. The Office of Clinical Pharmacology reviewed a PK/PD model describing the relationship of daprodustat PK to hemoglobin concentrations. This model demonstrates that the hemoglobin trajectory versus time is nearly identical for TIW and once daily (QD) dosing regimens of equivalent pharmacodynamic potency (2-fold higher doses administered TIW had similar pharmacodynamic effect to once daily regimens). The treatment differences between QD and

TIW dosing in Hb fluctuations (i.e., the peak to trough ratio) were minimal, and not clinically important (see Section 14.5.3.1). Additionally, there are lower systemic erythropoietin levels observed with HIF-PHIs than with exogenous administration.

However, there may be other mechanisms by which daprodustat can contribute to cardiovascular harm. There is a worrisome trend in cardiovascular endpoints for daprodustat in the NDD population that does not have a clear explanation despite similar Hb responses to daprodustat and darbepoetin alfa in that trial. Therefore, the similar Hb levels with TIW vs. once daily dosing of equivalent potency do not provide adequate assurance that the TIW dosing regimen will be safe.

TIW dosing results in a C_{max} approximately two-fold higher than a once daily regimen of approximately equivalent pharmacodynamic potency. To explore the effects of C_{max} on cardiovascular safety, we conducted multivariate exposure-response analyses using a logistic regression model and Cox proportional hazards regression model to quantify the relationship between daprodustat PK exposures (e.g., average C_{max} prior to the occurrence of the first event, or average on-treatment C_{max} for patients without an event) and safety measures (thromboembolic event, nonfatal MI, and nonfatal hospitalization for HF) by Week 52 based on data from ASCEND-D and ASCEND-TD. The post-hoc analyses from these Cox and logistical regression models show C_{max} is not a significant factor in any of those safety measures and no differences between the average C_{max} up to the event (in those with the event) vs. the average C_{max} for 52 weeks (in those without the event) were detected (see Section 14.5.3.2). However, these data were derived from a small subset of patients and events. Additionally, because the effect of C_{max} on safety was based on a post-randomization analysis, the analysis may be subject to confounding.

We also considered whether safety for TIW could be extrapolated from the once daily safety database from ASCEND-D (Table 99). For example, a patient who received 24 mg TIW in ASCEND-TD would be expected to have C_{max} similar to those in ASCEND-D receiving 24 mg daily (the highest studied once daily dose), hence providing support for a 24 mg TIW regimen.

Table 99. Summary of Maximum Daprodustat Dose Attained On-Treatment for ASCEND-D and ASCEND-TD, Vertically Aligned By Equal Potency

QD regimen	1 mg	2 mg	4 mg	6 mg	8 mg	10 mg	12 mg	16 mg	24 mg
Max QD dose used in ASCEND-D, n (%)	Not used	Not used	73 (4.9%)	455 (30.7%)	265 (17.9%)	165 (11.1%)	159 (10.7%)	119 (8%)	246 (16.6%)
N=1482									
Equivalent maintenance doses									
TIW regimen	2 mg	4 mg	8 mg	12 mg	16 mg	20 mg	24 mg	32 mg	48 mg
Max TIW dose used in ASCEND-TD, n(%)	Not used	Not used	11 (4.1%)	91 (33.7%)	48 (17.8%)	32 (11.9%)	35 (13%)	22 (8.1%)	31 (11.5%)
N=270									

Source: Clinical Reviewer's Analysis. R ver 3.4. adex.xpt (Studies 200807 and 204837)
Abbreviations: QD, once a day (daily); TIW, three times a week

However, (b) (4) TIW dosing has important limitations. For example, the patients needing 24 mg QD (high potency regimen) are likely different to those who were titrated to a 24 mg TIW regimen (moderate potency regimen; equivalent to ~10 mg daily). These patients may differ by disease severity, on-treatment hemoglobin levels, drug absorption, distribution, metabolism, and excretion (ADME), or other observed or unobserved

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characteristics. Differences in some patient characteristics for those on higher vs. lower daprodustat doses were observed in ASCEND-D ([Table 100](#)). (b) (4)

Table 100. Summary of Clinical Characteristics by Highest On-Treatment Dose, ASCEND-D

	4 mg Max	6 mg Max	8 mg Max	10 mg Max	12 mg Max	16 mg Max	24 mg Max
n	73	455	265	165	159	119	246
Age, years (mean (SD))	60.6 (13.5)	59.6 (13.9)	57.6 (14.8)	55.7 (14.7)	54.9 (13.5)	54.7 (14.3)	54.8 (14.1)
Race (%)							
Asian	6 (8.2)	49 (10.8)	34 (12.8)	22 (13.3)	23 (14.5)	19 (16.0)	23 (9.3)
Black or African American	4 (5.5)	45 (9.9)	30 (11.3)	23 (13.9)	36 (22.6)	15 (12.6)	75 (30.5)
White	61 (83.6)	341 (74.9)	187 (70.6)	110 (66.7)	85 (53.5)	78 (65.5)	130 (52.8)
Location (%)							
Asia	4 (5.5)	38 (8.4)	29 (10.9)	20 (12.1)	18 (11.3)	14 (11.8)	19 (7.7)
East EU	31 (42.5)	154 (33.8)	66 (24.9)	56 (33.9)	22 (13.8)	35 (29.4)	53 (21.5)
West (NZ, CA, West EU)	28 (38.4)	116 (25.5)	57 (21.5)	22 (13.3)	30 (18.9)	16 (13.4)	17 (6.9)
Latinx regions	4 (5.5)	59 (13.0)	62 (23.4)	18 (10.9)	23 (14.5)	16 (13.4)	31 (12.6)
USA	6 (8.2)	88 (19.3)	51 (19.2)	49 (29.7)	66 (41.5)	38 (31.9)	126 (51.2)
Standardized BL ESA dose	2.7 (1.7)	5.1 (3.0)	8.7 (6.3)	6.8 (4.5)	11.7 (10.5)	9.2 (6.3)	12.1 (10.5)
ESA hyporesponder (%)	0 (0.0)	5 (1.1)	35 (13.2)	9 (5.5)	48 (30.2)	23 (19.3)	63 (25.6)
History of T2D (%)	23 (31.5)	161 (35.4)	101 (38.1)	75 (45.5)	76 (47.8)	50 (42.0)	127 (51.6)
History of CVA (%)	2 (2.7)	30 (6.6)	15 (5.7)	10 (6.1)	11 (6.9)	11 (9.2)	17 (6.9)
History of MI (%)	10 (13.7)	42 (9.2)	20 (7.5)	7 (4.2)	20 (12.6)	11 (9.2)	23 (9.3)
History of HF (%)	26 (35.6)	114 (25.1)	76 (28.7)	50 (30.3)	43 (27.0)	23 (19.3)	66 (26.8)
History of TEE (%)	18 (24.7)	82 (18.0)	48 (18.1)	33 (20.0)	27 (17.0)	21 (17.6)	42 (17.1)
History of CVD (%)	35 (47.9)	215 (47.3)	117 (44.2)	67 (40.6)	68 (42.8)	52 (43.7)	111 (45.1)
Body weight (kg)	75.1 (19.4)	79.4 (21.2)	77.6 (20.8)	80.6 (21.6)	79.7 (22.8)	78.6 (21.8)	85.9 (25.8)
Ferritin (mean (SD))	627.5 (482.7)	641.8 (503.3)	671.4 (482.4)	745.2 (630.9)	783.7 (526.6)	791.9 (688.8)	863.2 (513.8)
Absolute reticulocyte count (mean (SD))	47.5 (28.1)	46.8 (21.6)	48.8 (24.8)	48.4 (23.1)	52.8 (27.3)	54.7 (29.3)	58.3 (29.2)

Source: Clinical Reviewer's Analysis. R ver 3.4. adsl.xpt, adex.xpt

Abbreviations: BL, baseline; CA, Canada; HF, heart failure; CVA, cerebral vascular accident; CVD, cardiovascular disease; ESA, erythropoiesis-stimulating agents; EU, European Union; MI, myocardial infarction; NZ, New Zealand; SD, standard deviation; T2D, type 2 diabetes; TEE, thromboembolic event; USA, United States of America

7.7.6. CV Safety in Incident Dialysis

Issue

ASCEND-ID established non-inferiority of daprodustat for efficacy in the ID population. However, the study was not designed to demonstrate general safety or cardiovascular outcomes. Is the safety established from ASCEND-D and ASCEND-ND sufficient to establish safety for ID patients?

Background

The incident dialysis ID population is defined broadly as patients who have recently transitioned to chronic dialysis, or transition to dialysis is eminent. The ASCEND-ID study defined the ID population as those who are either planning to start chronic dialysis within the next 6 weeks from the date of the screening visit (which was 6 weeks prior to randomization) or those who had started chronic dialysis (hemodialysis or peritoneal dialysis) for no more than 90 days prior to randomization. Importantly, the inclusion criteria for ASCEND-ND and ASCEND-D ensure virtually no overlap with the ASCEND-ID population: ASCEND-ND enrolled subjects with stage 3, 4, or 5 CKD, who did not have clinical evidence of impending need to initiate dialysis within 90 days after study start, whereas ASCEND-D required participants to have been on dialysis for greater than 90 days prior to screening (which was at least 4 weeks from randomization)

ID patients are at an acutely elevated risk for cardiovascular morbidity and mortality.

Additionally, the cardiovascular safety profile in the DD and NDD populations are disparate (ASCEND-ND being broadly unfavorable to daprodustat, whereas ASCEND-D being broadly favorable to daprodustat), and the ID population is a transient state between being dialysis dependent and not dialysis dependent. These disparate results, as well as lack in understanding the root cause for the cardiovascular differences seen with daprodustat in the NDD vs. DD populations make reliance (i.e., extrapolation) of the safety profile from one or both populations to the ID population challenging.

Assessment

[Table 101](#) provides a comparison between daprodustat and rhEPO of the proportion of subjects experiencing an adjudicated cardiovascular event during the 52-week study period in ASCEND-ID. Daprodustat had a slightly higher incidence of ACM and HF events, a slightly lower

incidence of TEE, and comparable rates for MI and CVA compared to rhEPO. While being specific to the ID population, this trial was too small (n=312) and short term (52 weeks) and the number of events of interest were too low to provide precise estimates of risk. Therefore, the cardiovascular safety of daprodustat in the ID population is indeterminate based on the existing AE data.

Table 101. Patients With Adjudicated Cardiovascular Safety Events, ITT Population, Trials ASCEND-ID

	Daprodustat N=157 n (%)	rhEPO N=155 n (%)	RD (%) (95% CI)
All-cause mortality	17 (10.8)	12 (7.7)	3.1 (-3.3, 9.5)
CV mortality	5 (3.2)	3 (1.9)	1.2 (-2.3, 4.7)
All MI	5 (3.2)	5 (3.2)	-0.0 (-4.0, 3.9)
Fatal	0 (0)	0 (0)	0 (0, 0)
Nonfatal	5 (3.2)	5 (3.2)	-0.0 (-4.0, 3.9)
All stroke	1 (0.6)	1 (0.6)	-0.0 (-1.8, 1.8)
Fatal	1 (0.6)	0 (0)	0.6 (-0.6, 1.9)
Nonfatal	0 (0)	1 (0.6)	-0.6 (-1.9, 0.6)
All thromboembolic events	10 (6.4)	12 (7.7)	-1.4 (-7.1, 4.3)
Fatal	0 (0)	0 (0)	0 (0, 0)
Nonfatal	10 (6.4)	12 (7.7)	-1.4 (-7.1, 4.3)
All heart failure	9 (5.7)	5 (3.2)	2.5 (-2.1, 7.1)
Fatal	0 (0)	0 (0)	0 (0, 0)
Nonfatal	9 (5.7)	5 (3.2)	2.5 (-2.1, 7.1)
Hospitalized	8 (5.1)	5 (3.2)	1.9 (-2.6, 6.3)

Source: adevent.xpt; Software: R

Risk difference (with 95% confidence interval) is shown between treatment and comparator.

Abbreviations: CI, confidence interval; CV, cardiovascular; ITT, intent-to-treat; MI, myocardial infarction; N, number of patients in treatment arm; n, number of patients with adverse event; RD, risk difference; rhEPO, recombinant human erythropoietin

Given this considerable uncertainty regarding the safety of daprodustat in the ID population, we conducted exploratory analyses of ASCEND-ND in an attempt to provide some insight on the safety in the ID population. Approximately 35% (1345/3872) of subjects in ASCEND-ND transitioned to dialysis while on study (i.e., were incident dialysis).

We considered TEAE around the time of dialysis initiation (i.e., the TEAE and dialysis initiation must occur together, within an arbitrary, post-hoc window) as an endpoint to explore the safety of ND subjects who transitioned to ID during the course of ASCEND-ND. We chose several windows for defining a co-occurring event. [Table 102](#) below shows the incidence of SAEs by treatment arm and by SOC and nFMQ within 30 days before or after dialysis initiation in those subjects who transitioned to dialysis in ASCEND-ND. There are slightly higher incidences of thrombosis, myocardial ischemic-type events, and acute kidney injury with daprodustat compared to rhEPO during this timeframe.

Table 102. Patients With On-Study Serious Adverse Events Within 30 Days Before or After Dialysis Initiation, by System Organ Class and FDA Medical Query (Narrow), Occurring in at Least 0.5% of Subjects in Any Arm, ITT Population, ASCEND-ND

System Organ Class FMQ (Narrow)	ASCEND-ND			
	Daprodustat N=1937 n (%)	rhEPO N=1935 n (%)	RD (%) (95% CI)	RR (95% CI)
Blood and lymphatic system disorders (SOC)				
Thrombosis Arterial	29 (1.5)	18 (0.9)	0.6 (-0.1, 1.3)	1.6 (0.9, 2.9)
Thrombosis	30 (1.5)	23 (1.2)	0.4 (-0.4, 1.1)	1.3 (0.8, 2.2)
Anemia	12 (0.6)	10 (0.5)	0.1 (-0.4, 0.6)	1.2 (0.5, 2.8)
Cardiac disorders (SOC)				
Myocardial ischemia	28 (1.4)	18 (0.9)	0.5 (-0.2, 1.2)	1.6 (0.9, 2.8)
Acute coronary syndrome	25 (1.3)	16 (0.8)	0.5 (-0.2, 1.1)	1.6 (0.8, 2.9)
Myocardial infarction	17 (0.9)	14 (0.7)	0.2 (-0.4, 0.7)	1.2 (0.6, 2.5)
Heart failure	27 (1.4)	32 (1.7)	-0.3 (-1.0, 0.5)	0.8 (0.5, 1.4)
Infections and infestations (SOC)				
Viral infection	11 (0.6)	11 (0.6)	-0.0 (-0.5, 0.5)	1.0 (0.4, 2.3)
Bacterial infection	39 (2.0)	45 (2.3)	-0.3 (-1.2, 0.6)	0.9 (0.6, 1.3)
Pneumonia	24 (1.2)	36 (1.9)	-0.6 (-1.4, 0.2)	0.7 (0.4, 1.1)
Musculoskeletal and connective tissue disorders (SOC)				
Fracture	12 (0.6)	5 (0.3)	0.4 (-0.1, 0.8)	2.4 (0.8, 6.8)
Renal and urinary disorders (SOC)				
Acute kidney injury	40 (2.1)	21 (1.1)	1.0 (0.2, 1.8)	1.9 (1.1, 3.2)
Renal and urinary tract infection	9 (0.5)	18 (0.9)	-0.5 (-1.0, 0.1)	0.5 (0.2, 1.1)
Vascular disorders (SOC)				
Hemorrhage	18 (0.9)	13 (0.7)	0.3 (-0.3, 0.8)	1.4 (0.7, 2.8)

Source: adsl.xpt, adae.xpt; Software: R

Treatment-emergent adverse events defined as any AEs occurring or worsening between the first dose and the end of the follow-up period.

Serious adverse events defined as any untoward medical occurrence that, at any dose that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.

Abbreviations: AE, adverse event; CI, confidence interval; FMQ, FDA medical query; ITT, intent-to-treat; N, number of patients in treatment arm; n, number of patients with adverse event; RD, risk difference; rhEPO, recombinant human erythropoietin; RR, risk ratio; SOC, system organ class; TIA, transient ischemic attack

Table 103 shows the incidence of adjudicated CV safety events for various windows around the time of dialysis initiation in those patients transitioning to dialysis during ASCEND-ND. There do not appear to be notable imbalances in myocardial infarction or stroke between treatment arms within 30 or 60 days before or after initiating dialysis. There are slight imbalances not favoring daprodustat in myocardial infarction within 90 or 180 days after transitioning to dialysis and for thromboembolic events within 60 days before or after initiating dialysis or within 90 or 180 days after initiating dialysis.

These analyses of a subpopulation of ASCEND-ND are limited by their exploratory nature, post-randomization event analyses, and low number of events to draw definitive conclusions.

Table 103. Patients With Adjudicated Cardiovascular Safety Events Before and After Dialysis Initiation, ITT Population, ASCEND-ND

Adjudicated Event Type	ASCEND-ND Daprodustat N=1937 n (%)	ASCEND-ND rhEPO N=1935 n (%)	ASCEND-ND RD (%) (95% CI)	ASCEND-ND RR (95% CI)
Myocardial infarction				
All MI	105 (5.4)	99 (5.1)	0.3 (-1.1, 1.7)	1.1 (0.8, 1.4)
MI within 30 days before or after ID	29 (1.5)	30 (1.6)	-0.1 (-0.8, 0.7)	1.0 (0.6, 1.6)
MI within 60 days before or after ID	34 (1.8)	35 (1.8)	-0.1 (-0.9, 0.8)	1.0 (0.6, 1.5)
MI within 90 days after ID	21 (1.1)	16 (0.8)	0.3 (-0.4, 0.9)	1.3 (0.7, 2.5)
MI within 180 days after ID	31 (1.6)	24 (1.2)	0.4 (-0.4, 1.1)	1.3 (0.8, 2.2)
Stroke				
All stroke	50 (2.6)	35 (1.8)	0.8 (-0.2, 1.7)	1.4 (0.9, 2.2)
Stroke within 30 days before or after ID	5 (0.3)	9 (0.5)	-0.2 (-0.6, 0.2)	0.6 (0.2, 1.7)
Stroke within 60 days before or after ID	7 (0.4)	9 (0.5)	-0.1 (-0.5, 0.3)	0.8 (0.3, 2.1)
Stroke within 90 days after ID	7 (0.4)	9 (0.5)	-0.1 (-0.5, 0.3)	0.8 (0.3, 2.1)
Stroke within 180 days after ID	12 (0.6)	11 (0.6)	0.1 (-0.4, 0.5)	1.1 (0.5, 2.5)
Thromboembolic events				
All TEE	64 (3.3)	53 (2.7)	0.6 (-0.5, 1.6)	1.2 (0.8, 1.7)
TEE within 30 days before or after ID	8 (0.4)	6 (0.3)	0.1 (-0.3, 0.5)	1.3 (0.5, 3.8)
TEE within 60 days before or after ID	14 (0.7)	6 (0.3)	0.4 (-0.0, 0.9)	2.3 (0.9, 6.1)
TEE within 90 days after ID	14 (0.7)	9 (0.5)	0.3 (-0.2, 0.7)	1.6 (0.7, 3.6)
TEE within 180 days after ID	28 (1.4)	15 (0.8)	0.7 (0.0, 1.3)	1.9 (1.0, 3.5)

Source: adsl.xpt, adevent.xpt; Software: R

Risk difference (with 95% confidence interval) is shown between treatment and comparator.

Abbreviations: CI, confidence interval; ID, incident dialysis; ITT, intent-to-treat; MI, myocardial infarction; N, number of patients in treatment arm; n, number of patients with adverse event; RD, risk difference; rhEPO, recombinant human erythropoietin; RR, risk ratio; TEE, thromboembolic event

Conclusion

It is unclear whether the cardiovascular safety of daprodustat in the ID population more closely resembles that of the DD population or the NDD population. The available cardiovascular safety data from ASCEND ID are too limited to draw definitive conclusions. Exploratory analyses from the subset of patients transitioning to dialysis during ASCEND-ND show some signals but also have limitations that preclude definitive conclusions. Therefore, (b) (4)

(b) (4) we cannot reliably differentiate these patients from the ND population. With regard to initiating daprodustat after chronic dialysis has been started, ASCEND-D enrolled patients who had been on dialysis for more than 90 days prior to the screening visit, and were treated with daprodustat after a 4-week run-in period (totaling 118 days). (b) (4)

7.7.7. Gastric Erosions and Gastrointestinal Bleeding

Issue

A higher incidence of serious investigator reported treatment emergent AEs related to gastric erosions and gastrointestinal bleeding was observed in the daprodustat arm compared to the ESA comparator arm in ASCEND-ND and ASCEND-D.

Background

The Applicant's nonclinical data demonstrated gastric erosions/ulcerations in mice, rats, dogs, and monkeys with a possible basis for erosions and ulcers being compromised vascular perfusion associated with marked increases in hematocrit in non-anemic animals. Potential upper gastrointestinal erosive events were identified and collected prospectively in the ASCEND-D and ASCEND-ND trials as an AESI.

The Applicant prespecified a list of MedDRA PTs pertaining to the AESI of gastric erosions. This list is available publicly¹⁹ on page 636 of the protocol provided as supplementary material. Our review of the individual PTs used in the Applicant's query determined that the breadth of the queries was reasonable for a risk specific to gastric erosion. The adverse events were not adjudicated and diagnostic confirmation with upper endoscopy was not required in the protocols.

Assessment

There was a higher incidence of Applicant-defined AESIs for serious gastric/esophageal erosions reported in the daprodustat arm compared to ESA with a more notable difference in the ASCEND-ND population (HR [95% CI]: 1.63 [1.17, 2.27]) than in ASCEND-D (HR [95% CI]: 1.16 [0.78, 1.73]) (Table 104). The incidence of all TEAEs of gastric/esophageal erosions (serious plus non-serious) was similarly increased with daprodustat compared to ESA in ASCEND-ND (HR [95% CI]: 1.96 [1.24, 3.09]) but not in ASCEND-D (HR [95% CI]: 0.84 [0.63, 1.13]).

¹⁹ <https://www.nejm.org/doi/full/10.1056/NEJMoa2113379>

Table 104. Summary of Results for the Prespecified Queries Related to Gastric Erosions—ASCEND-D and ASCEND-ND and Pooled, (On-Study Analysis)

	Daprodustat N=1487	ESA N=1477	Rate Diff per 100 PY (95% CI)	Hazard Ratio (95% CI)
ASCEND-D	n [IR]	n [IR]		
All erosions	84 [2.5]	98 [2.9]	-0.4 (-1.2, 0.3)	0.84 (0.63, 1.13)
Serious erosions	53 [1.5]	45 [1.3]	0.2 (-0.3, 0.7)	1.16 (0.78, 1.73)
	Daprodustat N=1937	Darbepoetin alfa N=1935	Rate Diff per 100 PY (95% CI)	Hazard Ratio (95% CI)
ASCEND-ND	n [IR]	n [IR]		
All erosions	91 [2.5]	56 [1.5]	1.0 (0.3, 1.6)	1.63 (1.17, 2.27)
Serious erosions	55 [1.5]	28 [0.8]	0.7 (0.3, 1.2)	1.96 (1.24, 3.09)
	Daprodustat N=3424	ESA N=3412	Rate Diff per 100 PY (95% CI)	Hazard Ratio (95% CI)
Pooled				
All erosions	175 [2.4]	154 [2.2]	0.3 (-0.2, 0.8)	1.13 (0.91, 1.40)
Serious erosions	108 [1.5]	73 [1.0]	0.5 (0.1, 0.8)	1.47 (1.09, 1.98)

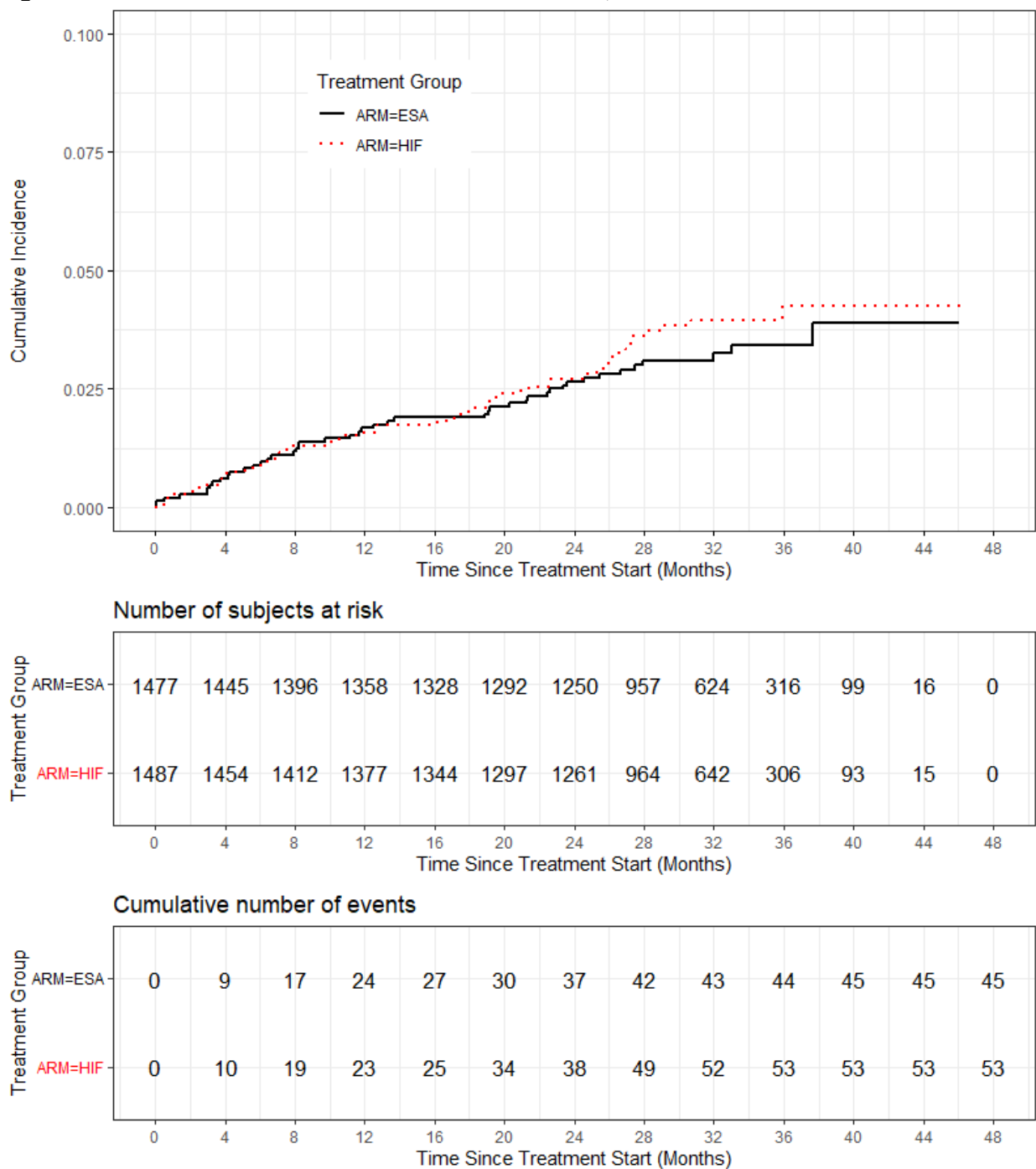
Source: Clinical Reviewer, ADAE.xpt, ADSL.xpt, R v. 4.2, ITT population.

This analysis treats deaths and administrative censoring the same.

Abbreviations: CI, confidence interval; Diff, difference; ESA, erythropoiesis-stimulating agent; IR, incidence rate per 100 PY; N, number of subjects; n, number of subjects with event; PY, person-year

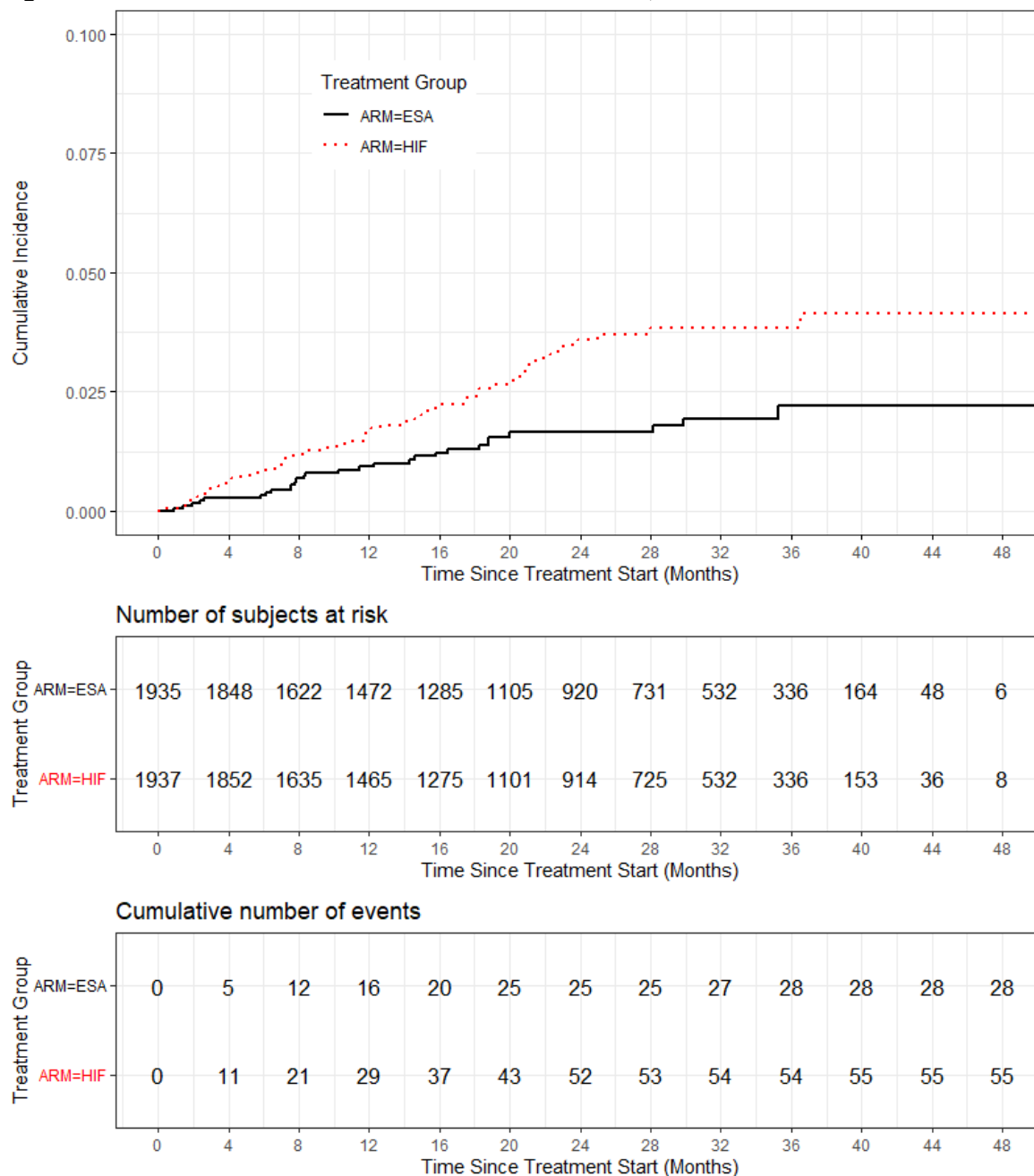
The differences in risk are driven by treatment differences in serious gastrointestinal hemorrhages, rather than ulcerations ([Figure 34](#) and [Figure 35](#)). The Kaplan-Meier curves for serious gastrointestinal hemorrhages are superimposed for the first 24 months of ASCEND-D and then separate slightly. In contrast, the Kaplan-Meier curves for serious gastrointestinal hemorrhages in ASCEND-ND separate early. Importantly, the treatment arms in ASCEND-D and ASCEND-ND were balanced with respect to anti-platelet, anti-coagulant use, prophylactic drugs (e.g., antacids), and risk factors (gastroesophageal reflux disease, peptic ulcer disease, smoking).

Figure 34. Time to First TESAE for Gastrointestinal Bleed, ASCEND-D



Source: Clinical Reviewer, ADAE.xpt, ADSL.xpt, R Version 4.2, ITT population.
 This analysis treats deaths and administrative censoring the same.
 Abbreviations: ESA, erythropoiesis-stimulating agent; HIF, hypoxia-inducible factor, ITT, intent-to-treat; TESAE, treatment-emergent serious adverse event

Figure 35. Time to First TESAE for Gastrointestinal Bleed, ASCEND-ND



Source: Clinical Reviewer, ADAE.xpt, ADSL.xpt, R Version 4.2, ITT population.

This analysis treats deaths and administrative censoring the same.

Abbreviations: ESA, erythropoiesis-stimulating agent; HIF, hypoxia-inducible factor, ITT, intention-to-treat; TESAE, treatment-emergent serious adverse event

The Applicant provided an external, blinded, review of the serious AESIs of gastric or esophageal erosions from ASCEND-D and ASCEND-ND. This review found that most included events were overt gastrointestinal bleeding, with over half requiring transfusion. However, the external blinded review also noted several limitations related to the fact that the AE reports did not trigger confirmatory testing (e.g., *Helicobacter pylori* testing and confirmatory endoscopy).

As a potential risk mitigation strategy, we considered known risk factors which were also reported in the subject level dataset. These included smoking history, use of aspirin, clopidogrel, and vitamin K antagonists (e.g., warfarin) at baseline. In the pooled ASCEND-D and ASCEND-ND dataset, aspirin was independently associated with serious gastrointestinal erosions/bleeds (HR 1.52 [95% CI: 1.1 – 2.1]), as was clopidogrel (HR 1.16 [95% CI: 0.7 – 1.9]), and warfarin (HR 2.0 [95% CI: 1.1 – 3.4]). Smoking history was not strongly related.

Subgroup analyses for serious gastric erosions/bleeds did not suggest a substantial treatment interaction with concomitant aspirin, warfarin, or clopidogrel ([Table 105](#)). It is important to note that the warfarin and clopidogrel subgroups were limited in the trial population (less than 10% of subjects used warfarin or clopidogrel). Approximately one-half used aspirin. These products (as well as others, such as NSAIDs) may increase the risk of GI bleeding. The data from ASCEND-D and ASCEND-ND are too limited to reliably assess the extent of the role these agents played in the GI bleeding risk with daprodustat.

Table 105. Summary of Subgroup Results for the Prespecified Queries* Related to Serious Gastric Erosion Events—ASCEND-D and ASCEND-ND (On-Study Analysis)

	Subgroup Totals					ESA n (%) [IR]	IRD Incidence Per 100 PY
	All	HIF	ESA	Daprodustat n (%) [IR]			
ASCEND-D							
No Vitamin K antagonist	2813	1407	1406	50 (3.6) [1.4]	42 (3) [1.2]		0.2 [-0.3-0.8]
Vitamin K antagonist	151	80	71	3 (3.8) [1.7]	3 (4.2) [2.0]		-0.3 [-3.2-2.7]
No clopidogrel	2670	1358	1312	48 (3.5) [1.4]	40 (3) [1.2]		0.2 [-0.4-0.8]
Clopidogrel	294	129	165	5 (3.9) [1.6]	5 (3) [1.3]		0.3 [-1.6-2.2]
No aspirin	1910	965	945	30 (3.1) [1.3]	27 (2.9) [1.2]		0.1 [-0.5-0.7]
Aspirin	1054	522	532	23 (4.4) [1.8]	18 (3.4) [1.4]		0.4 [-0.6-1.4]
ASCEND-ND							
No Vitamin K antagonist	3746	1866	1880	49 (2.6) [1.4]	26 (1.4) [0.7]		0.7 [0.2-1.1]
Vitamin K antagonist	126	71	55	6 (8.5) [4.3]	2 (3.6) [1.7]		2.6 [-1.6-6.9]
No clopidogrel	3526	1763	1763	52 (2.9) [1.6]	22 (1.2) [0.7]		0.9 [0.4-1.4]
Clopidogrel	346	174	172	3 (1.7) [0.9]	6 (3.5) [1.9]		-1 [-2.8-0.8]
No aspirin	2713	1348	1365	34 (2.5) [1.3]	13 (1) [0.5]		0.8 [0.3-1.3]
Aspirin	1159	589	570	21 (3.6) [1.9]	15 (2.6) [1.4]		0.6 [-0.5-1.6]

Source: Clinical Reviewer, ADAE.xpt, ADSL.xpt, R v. 4.2, ITT population.

Abbreviations: ESA, erythropoiesis-stimulating agent; HIF, hypoxia-inducible factor, ITT, intention-to-treat; n, number of events with characteristic; %, percentage of events with characteristic; IR: incidence rate (per 100 PY of follow-up); IRD, incidence rate difference

[Table 106](#) presents a summary of all serious gastric erosion events identified by the Applicant’s prespecified query for the ASCEND-D and ASCEND-ND populations (i.e., events occurring more than once in a single subject were included).

Table 106. Summary of Clinical Outcomes for Serious Gastric Erosions (Prespecified MedDRA Term) Pooled ASCEND-D and ASCEND-ND (On-Study Analysis)

	Daprodustat N=118 Events	ESA N=82 Events
Outcome n (%)		
Fatal	8 (6.8)	3 (3.7)
Not recovered/not resolved	14 (11.9)	10 (12.2)
Recovered/resolved	92 (78.0)	66 (80.5)
Recovered/resolved with sequelae	4 (3.4)	2 (2.4)
Recovering/resolving	0 (0.0)	1 (1.2)
Severity n (%)		
Mild	6 (5.1)	3 (3.7)
Moderate	42 (35.6)	40 (48.8)
Severe	70 (59.3)	39 (47.6)
Duration (days) (median [IQR])	8.00 [5.00, 12.50]	8.00 [6.00, 16.50]
Action taken n (%)		
Dose increased	2 (1.7)	1 (1.2)
Dose not changed	67 (56.8)	56 (68.3)
Drug interrupted	9 (7.6)	2 (2.4)
Drug withdrawn	7 (5.9)	1 (1.2)
Not applicable	33 (28)	22 (26.8)
Life threatening n (%)	16 (13.6)	10 (12.2)
MedDRA PTs selected by query (only events occurring >4 times shown overall) n (%)		
Duodenal ulcer	6 (5.1)	4 (4.9)
Duodenal ulcer hemorrhage	7 (5.9)	2 (2.4)
Gastric ulcer	6 (5.1)	9 (11)
Gastric ulcer hemorrhage	7 (5.9)	6 (7.3)
Gastritis erosive	11 (9.3)	5 (6.1)
Gastrointestinal hemorrhage	41 (34.7)	24 (29.3)
Upper gastrointestinal hemorrhage	23 (19.5)	15 (18.3)

Source: Clinical Reviewer, ADAE.xpt, ADSL.xpt, R v. 4.2, ITT population.

All treatment emergent events are counted in this analysis, individual subjects may be counted more than once.

Includes all reported events.

Abbreviations: ESA, erythropoiesis-stimulating agent; IQR, interquartile range; ITT, intent-to-treat; MedDRA, Medical Dictionary for Regulatory Activities; N, number of events; n, number of events with characteristic; %, percentage of events with characteristic; PT, preferred term

Conclusion

In summary, there was a treatment difference in serious esophageal and gastric erosions disfavoring daprodustat that was more pronounced in ASCEND-ND than ASCEND-D. Whether there is a true risk in the ASCEND-D population is unclear as there are some inconsistencies in the data. For example, all TEAEs of esophageal and gastric erosions (serious plus non-serious) did not disfavor daprodustat in ASCEND-D. Also, the risk of serious esophageal and gastric erosions in the on-study analysis of ASCEND-D was not apparent or became weaker in the OT analyses. Other limitations include the lack of confirmatory testing, including endoscopy. Nonetheless, most of these reported serious events were related to gastrointestinal bleeding and had important clinical consequences such as transfusions and at this time we cannot rule out a drug effect based on the nonclinical findings and signal in both ASCEND-D and ASCEND-ND populations. Therefore, we will include a Warning and Precaution in the label, recommending the healthcare provider consider this risk, particularly in patients who have risk factors for erosions.

7.7.8. Acute Kidney Injury

Issue

A higher incidence of serious investigator reported treatment emergent AEs related to AKI was observed in the daprodustat arm compared to the ESA comparator arm in ASCEND-ND.

Background

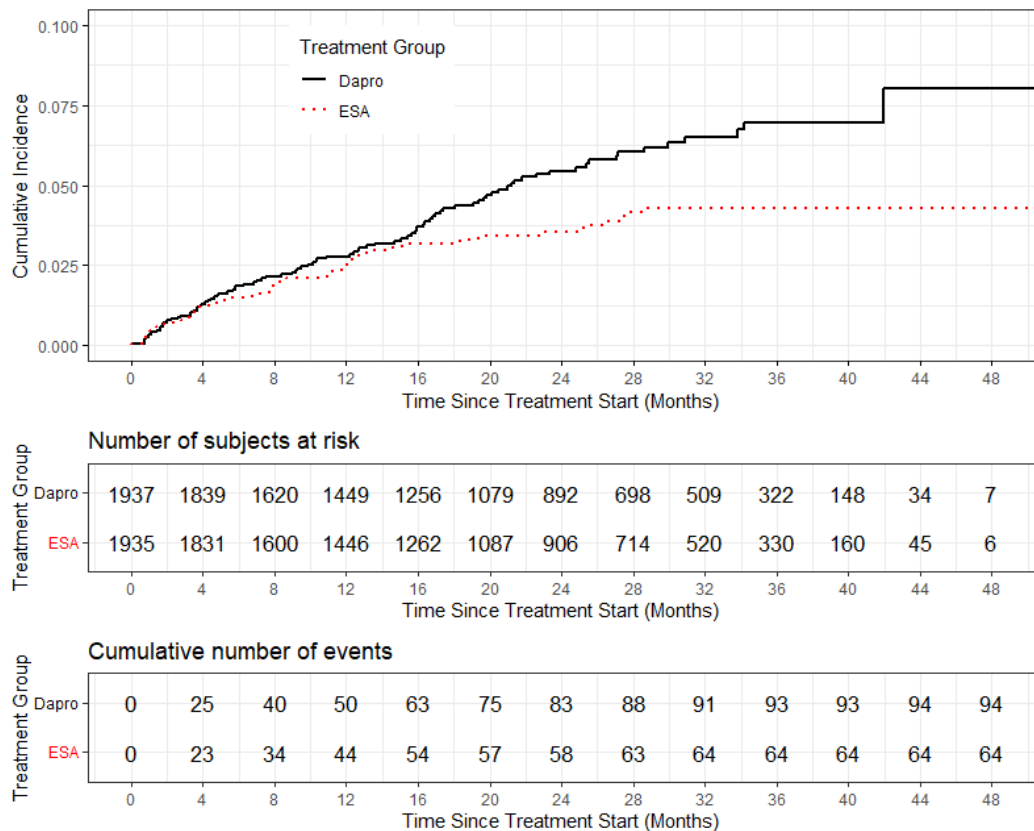
Direct daprodustat-mediated nephrotoxicity was not observed in the nonclinical program (although there were effects secondary to non-physiologic Hb levels and blood viscosity, leading to compromised blood flow and vascular perfusion, including in the kidney). In ASCEND-ND, the time to first occurrence of CKD progression was a pre-specified principal secondary endpoint, which was assessed in participants with preserved kidney function at baseline (eGFR ≥ 15 mL/min/1.73 m², N=2,485). This endpoint was a composite of a confirmed (through repeat eGFR) 40% decline in eGFR, clinical indication for dialysis, transition to chronic dialysis, or receipt of a renal allograft. CKD progression was generally similar between the treatment groups and daprodustat did not significantly reduce the time to progression of CKD compared with darbepoetin (IRD [95% CI] -0.2 [-2.8, 2.4] events per 100 PY, HR 0.98, 95% CI: 0.84, 1.13). Routine safety laboratory results (collected at least every 12 weeks) in ASCEND-ND (e.g., aggregate longitudinal plots and shift tables) for serum creatinine, eGFR, and BUN was similar between treatment groups.

In contrast, the FDA analyses using treatment-emergent serious adverse events (TESAEs) of AKI shows a potential increased risk for serious AKI for daprodustat compared to the ESA treatment arm in the NDD population.

Assessment

We performed exploratory analyses to further refine the safety signal of AKI. Adverse event analyses of serious AKI (as a nFMQ) in ASCEND-ND showed a relative risk of 1.47 (95% CI 1.07, 2.00) and risk difference of 1.54% [0.30, 2.79]). The risks were attenuated when considering all AKI TEAEs regardless of seriousness (relative risk of 1.2, 95% CI [-0.4, 2.7]). We note that the treatment difference appears after 16 months on study, so we preferred cumulative incidence differences to quantify risk. A Kaplan-Meier curve for time to first treatment-emergent serious adverse event (TESAE) of AKI for the ASCEND-ND study is presented in [Figure 36](#). At 2 and 3 years, there was a cumulative incidence difference of 2.1% (95% CI: 0.5, 3.5) and 2.7% (95% CI 0.8, 4.6), respectively.

Figure 36. Time to First TESAE of Acute Kidney Injury (Narrow FMQ) in ASCEND-ND, (On-Study Analysis)



Source: Clinical Reviewer, ADAE.xpt, ADSL.xpt, R v. 4.2, ITT population; TESAEs of acute kidney injury (narrow) per FMQ v. 2.0. This analysis treats deaths and administrative censoring the same. Abbreviations: Dapro, daprodustat; ESA, erythropoietin-stimulating agent; FMQ, FDA medical query; ITT, intent-to-treat; TESAE, treatment-emergent serious adverse event

There was a higher percentage of participants randomized to the daprodustat arm with more progressed CKD (i.e., stage 4 or 5), and advanced age (≥ 75 -year-old age), which may have contributed, to some degree, to the observed treatment difference (both are risk factors for AKI). However, an analysis provided by the Applicant, adjusting for baseline CKD stage, did not meaningfully change the estimate of harm (Table 46 of advisory committee briefing document).

The Applicant's key secondary endpoint, by design, does not capture acute AKI events, because a repeat measurement of eGFR was required to confirm a 40% decline in eGFR. A sensitivity analysis, which included a single measurement of a 40% decline in eGFR as a CKD progression event, did capture an unfavorable treatment difference. Lastly, the occurrence of a serious event is clinically important, regardless of whether it contributed to long term outcomes (such as progression of CKD or ACM). We acknowledge that the predefined adjudicated secondary safety analyses such as ACM (HR 1.03, 95% CI: 0.87, 1.20) and CKD progression (HR 0.98, 95% CI: 0.84, 1.13) were balanced between arms.

We considered whether the AKI TESAE imbalance could be related to the increased risk for HHF with daprodustat compared to darbepoetin alfa. However, the AKI risk was still evident in

subjects without a history of heart failure at baseline (data not shown); a population that did not appear to have an increased risk of HHF with daprodustat compared to darbepoetin alfa.

Most patients with TESAE AKI events recovered, with most events lasting 14 to 16 days. Most events occurred while receiving study drug (16 to 19% occurred off treatment) (see [Table 107](#)).

Table 107. Severity and Outcome of TESAEs of AKI (FMQ-FDA Query) in ASCEND-ND, (On-Study Analysis)

	Daprodustat N=104	Darbepoetin N=73
Outcome		
Fatal	4 (3.8)	3 (4.1)
Not recovered	9 (8.7)	6 (8.2)
Recovered	78 (75.0)	59 (80.8)
Recovered with sequelae	13 (12.5)	5 (6.8)
Severity		
Mild	5 (4.8)	5 (6.8)
Moderate	34 (32.7)	35 (47.9)
Severe	65 (62.5)	33 (45.2)
AE duration, days (mean, SD)	14.4 (20.5)	16.1 (29.8)
AE action		
Dose not changed	70 (67.3)	57 (78.1)
Drug interrupted	11 (10.6)	1 (1.4)
Drug withdrawn	3 (2.9)	3 (4.1)
Off treatment	20 (19.2)	12 (16.4)

Source: Clinical Reviewer, ADAE.xpt, ADSL.xpt, R v. 4.2, On study (ITT population), TESAEs of AKI (narrow) per FMQ v. 2.0 Includes all reported events.

Abbreviations: AE, adverse event; AKI, acute kidney injury; FMQ, FDA Medical Dictionary for Regulatory Activities query; ITT, intent-to-treat; SD, standard deviation; TESAE, treatment-emergent serious adverse event

Conclusion

In summary, there are discrepant lines of evidence regarding kidney injury with daprodustat in the NDD population. There were no differences detected for all-cause mortality or CKD progression, which are important sequelae of AKI. Since this risk is specific to the NDD population, risk mitigation strategies are premature for our planned DD-only approval (b) (4)

7.7.9. Malignancy

Issue

Tumor progression, recurrence, and cancer-related mortality are theoretical risks based on the hypoxia-inducible factor biology.

Background

Hypoxia inducible factors are transcription factors involved in the hypoxia response and impact the expression of a number of survival genes in cancer cells and the tumor microenvironment. Daprodustat increases erythropoietin as part of its mechanism of action and drug labels for

marketed erythropoiesis stimulating agents include an increased risk of tumor progression as a boxed warning. Whether daprodustat shares this risk of ESA products is uncertain.

Lifetime studies in rodents did not identify a carcinogenic effect of daprodustat. However, based on the findings with ESAs and the theoretical risk related to the mechanism of action of daprodustat, the Applicant prespecified malignancy as an AESI. The Applicant’s prespecified analysis approach for non-CV events was OT, defined by the last dose given plus 1 day. The ITT/on study analysis is more sensitive than the OT for long-latency events (such as malignancy), and less confounded by differences in dosing frequency (such as the darbepoetin alfa comparator in ASCEND-ND)(see Section 7.6.1.1.2).

The ASCEND-D and ASCEND-ND protocols required treatment discontinuation following a diagnosis of cancer (new or recurrent), with the exception of squamous cell or basal cell carcinoma of the skin. Therefore, in the on-treatment assessment a malignancy diagnosis that was made two or more days after the last dose was not counted as an event, because per-protocol, subjects would not be dosed again. This scenario would be expected to exclude more events occurring with a drug dosed less frequently, but that are still very proximal to randomized treatment.

Assessment

For the above reasons, we considered the OT analysis as prespecified in the protocol but focused mainly on the on-study estimates for assessment of malignancy risk. We used a pooled analysis of ASCEND-ND and ASCEND-D and included treatment-emergent events regardless of reported seriousness. A summary of these results is presented in [Table 108](#).

The on-study analysis did not suggest a treatment group difference in the incidence of malignancy (152 of 3419 [4.4%] daprodustat and 161 of 3407 [4.7%] ESA control with a relative risk of 0.9 (95% CI 0.8, 1.2). There did not appear to be notable imbalances in any pattern type or location of cancer between treatment groups. Fatal outcomes due to malignancy in both arms were infrequent.

Table 108. Pooled ASCEND-D and ASCEND-ND Adverse Events Related to Malignancy (SMQ and Prespecified Terms) – (On-Study Analysis)

Adverse Event	Daprodustat at N=3424 n (%) [Events]	ESA N=3412 n (%) [Events]	Relative Risk [95% CI]	Risk Difference, % [95% CI]
Applicant AESI – malignancy	152 (4.4) [187]	161 (4.7) [211]	0.9 [0.8 - 1.2]	-0.3 [-1.3 - 0.7]
Reported as life-threatening	23 (0.7) [27]	19 (0.6) [21]	1.2 [0.7 - 2.2]	0.1 [-0.3 - 0.5]
Reported as contributing to death	17 (0.5) [17]	26 (0.8) [26]	0.7 [0.4 - 1.2]	-0.3 [-0.6 - 0.1]
Malignancies ^a	136 (4) [169]	149 (4.4) [193]	0.9 [0.7 - 1.1]	-0.4 [-1.3 - 0.6]
Non-hematological malignant tumors ^a	115 (3.4) [142]	116 (3.4) [154]	1 [0.8 - 1.3]	0 [-0.9 - 0.8]
Hematological malignant tumors ^a	5 (0.1) [5]	18 (0.5) [18]	0.3 [0.1 - 0.7]	-0.4 [-0.7 - 0.1]

Premalignant disorders ^a	75 (2.2) [86]	73 (2.1) [79]	1 [0.7 - 1.4]	0.1 [-0.6 - 0.7]
Blood premalignant disorders ^a	8 (0.2) [8]	8 (0.2) [8]	1 [0.4 - 2.7]	0 [-0.2 - 0.2]
Gastrointestinal premalignant disorders ^a	46 (1.3) [55]	55 (1.6) [61]	0.8 [0.6 - 1.2]	-0.3 [-0.8 - 0.3]
Skin premalignant disorders ^a	16 (0.5) [16]	7 (0.2) [7]	2.3 [0.9 - 5.5]	0.3 [0 - 0.5]
Hypoplastic anemias HLT	11 (0.3) [12]	16 (0.5) [16]	0.7 [0.3 - 1.5]	-0.1 [-0.4 - 0.1]
Skin neoplasms, malignant and unspecified ^b	40 (1.2) [51]	36 (1.1) [54]	1.1 [0.7 - 1.7]	0.1 [-0.4 - 0.6]
Skin malignant tumors ^a	32 (0.9) [39]	30 (0.9) [43]	1.1 [0.6 - 1.7]	0.1 [-0.4 - 0.5]
Breast malignant tumors ^a	11 (0.3) [11]	9 (0.3) [10]	1.2 [0.5 - 2.9]	0.1 [-0.2 - 0.3]
Colorectal neoplasms malignant HLT	9 (0.3) [9]	8 (0.2) [8]	1.1 [0.4 - 2.9]	0 [-0.2 - 0.3]
Renal neoplasms malignant HLT	13 (0.4) [13]	10 (0.3) [11]	1.3 [0.6 - 3]	0.1 [-0.2 - 0.4]
Prostate neoplasms, malignant and unspecified ^a	6 (0.2) [7]	9 (0.3) [9]	0.7 [0.2 - 1.9]	-0.1 [-0.3 - 0.1]

Source: Clinical Reviewer, ADAE.xpt, ADSL.xpt, R v. 4.2, ITT population. Includes SMQ queries or MedDRA HLTs with 15 or more occurrences.

^a MedDRA SMQ narrow query.

^b MedDRA SMQ broad query.

Abbreviations: AESI, adverse event of special interest; CI, confidence interval; ESA, erythropoiesis-stimulating agent; HLT, high-level term; ITT, intent-to-treat; MedDRA, Medical Dictionary for Regulatory Activities; N, number of subjects; n, number of subjects reporting at least one event; %, percentage of subjects reporting at least one event; SMQ, standardized medical query

Estimates of harm specific to the dialysis dependent population are required for the purposes of product labeling. Serious and nonserious malignancies (prespecified AESI query) were observed in 4.4% (n=65,) in ASCEND-D treated with daprodustat and 5.2% (n = 77,) of patients treated with rhEPO. Related queries (i.e., narrow FMQ, narrow SMQ for Malignancy) estimated similar incidences and treatment differences. The clinical reviewer reproduced the Applicant's estimated incidence rates for each treatment arm (1.9 per 100 PY for daprodustat, and 2.3 per 100 PY for subjects treated with ESA), using the submitted time-to-event datasets (adtte3.xpt).

Conclusion

In summary, the risk for tumor progression does not appear to be further increased compared to currently labeled ESAs, however, the short latency period of the studies conducted to date and the low event rates for specific cancers do not allow for definite conclusions on the long-term risk of daprodustat on tumor progression or development of new malignancies. For this reason, we are requiring a post-marketing observational study to assess long term outcomes of daprodustat on malignancy incidence. In addition, a risk for malignancy will be included in the warnings and precautions section of the label. Refer to the postmarketing requirement (PMR) in Section [24](#) for additional details.

7.7.10. Drug Induced Liver Injury

Issue

We assessed if there was a clinically significant hepatocellular injury risk with the use of daprodustat in subjects with CKD.

Background

There was no significant hepatotoxicity signal identified by the Applicant during development of daprodustat. Early during our exploratory review of individual patient data, an imbalance of potential Hy's law cases, not favoring daprodustat, was observed in routinely collected study site labs (pooled ASCEND-D and ASCEND-ND: 4/3424 cases in the daprodustat arm, 2/3412 cases in the ESA comparator arm). Inspection of mean-level hepatic injury biomarkers versus time (aspartate aminotransferase, alanine aminotransferase, bilirubin) as well as shift tables did not reveal a population-wide signal. However, out of an abundance of caution, we consulted the Drug Induced Liver Injury (DILI) team to assess whether there is a DILI risk with daprodustat (Refer to DILI review dated November 29, 2022 in DARRTs).

Assessment

Our initial exploratory DILI analysis only included routine, study-site laboratory data. Per protocol, non-study site lab data was missing from the integrated laboratory dataset (i.e., the ADLB.xpt dataset). On August 5, 2022, the Applicant submitted an updated ADLB dataset for each ASCEND study and the Integrated Summary of Safety including central and non-study site lab results (importantly, the Applicant conducted a manual search in case report forms to locate non-study site data and update the ADLB dataset, as requested). For all Integrated Summary of Safety subjects, there were no clear imbalances in incidence of alanine aminotransferase/aspartate aminotransferase elevations, jaundice, or cases meeting Hy's Law liver test criteria between subjects receiving daprodustat versus those receiving rhEPO ([Table 109](#)).

Table 109. Overall Liver Biochemistry by Treatment in ISS

ISS	Daprodustat N=4154	Placebo N=306	rhEPO N=1610	Darbepoetin alfa N=2088
Hy's Law quadrant (ALT/AST $\geq$ 3 x ULN & BILI $\geq$ 2 x ULN)	18 (0.4%)	2 (0.7%)	6 (0.4%)	7 (0.3%)
ALT/AST $\geq$ 3 x ULN	71 (1.7%)	4 (1.4%)	49 (3.0%)	40 (1.9%)
ALT/AST $\geq$ 5 x ULN	47 (1.1%)	2 (0.7%)	25 (1.6%)	26 (1.2%)
ALT/AST $\geq$ 10 x ULN	26 (0.6%)	1 (0.3%)	18 (1.1%)	16 (0.8%)
Total BILI $\geq$ 2 x ULN	27 (0.6%)	3 (1.0%)	13 (0.8%)	12 (0.6%)

Source: DILI team analyses results

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILI, bilirubin; ISS, Integrated Summary of Safety; rhEPO, recombinant human erythropoietin; ULN, upper limit of normal

eDISH plotting had numerous cases in the Hy's Law quadrant but no imbalance between daprodustat and comparator arms. The DILI team assessed the 25 subjects exposed to daprodustat that fell in the Hy's Law quadrant. None were considered probable DILI due to daprodustat. Only one was considered possible with alcohol as a competing cause.

Conclusion

The NDA data for daprodustat do not identify a significant liver injury risk that would preclude a favorable benefit-risk assessment. However, there were issues regarding missing data (e.g., alkaline phosphatase) that may have impaired a more thorough evaluation. The DILI team recommends labeling to monitor liver enzymes and bilirubin at baseline and as clinically indicated and recommends including liver injury in a post market pharmacovigilance strategy.

7.8. Risk and Evaluation Mitigation Strategy

The Division of Risk Management (DRM) and Office of Medication Error Prevention and Risk Management (OMEPRM) were consulted. The conclusions from their review are summarized below.

Based on the available data, a risk evaluation and mitigation strategy is not necessary to ensure the benefits of daprodustat in the DD population outweigh the risks. The safety concerns associated with daprodustat use are similar to ESAs including the risk of MACE and healthcare providers who treat anemia of CKD are familiar with and able to appropriately monitor these risks.

If an Applicant resubmits daprodustat for use in the NDD population, a re-evaluation of the need for a risk evaluation and mitigation strategy will be conducted at that time.

For additional details, refer to the review by Carla Darling, PharmD, BCPS, Jacqueline Sheppard, Pharm D and Cynthia LaCivita, Pharm D, dated January 26, 2023 in DARRTs.

8. Therapeutic Individualization

8.1. Intrinsic Factors

Hepatic Impairment

The Applicant did not propose any dose modifications for patients with mild or moderate hepatic impairment. Patients with severe hepatic impairment (Child Pugh C) were not studied, and the Applicant recommends that treatment with daprodustat be avoided in that population.

Mild and Moderate Hepatic Impairment

A dedicated hepatic impairment study (Study 200231) was conducted where participants with mild hepatic impairment (Child-Pugh Class A (Score 5 to 6)), moderate hepatic impairment (Child-Pugh Class B (Score 7 to 9)) and their matched healthy controls with normal hepatic function were administered a single 6 mg oral dose of daprodustat in a fasted state. Results are shown below in [Table 110](#).

Daprodustat C_{max} and AUC geometric least square mean ratios [90% CI] were 1.9-fold [1.06, 3.71] and 2.0-fold [1.10, 3.61] higher, respectively, in subjects with moderate hepatic impairment (Cohort 1) compared with their matched healthy control subjects (Cohort 2). All metabolites exposure geometric least square mean ratios were between 1.2-fold and 1.7-fold

higher in subjects with moderate hepatic impairment (Cohort 1) compared with their matched healthy control subjects (Cohort 2).

C_{max} for daprodustat was similar between subjects with mild hepatic impairment (Cohort 3) and their matched healthy controls (Cohort 4). The C_{max} geometric least square mean ratio [90% CI] was 1.01 [0.71, 1.43]. The daprodustat AUC geometric least square mean ratio [90% CI] was 1.46-fold [1.03, 2.05] higher in subjects with mild hepatic impairment compared with their matched healthy control subjects. C_{max} and AUC geometric least square mean ratios for all metabolites were between 1.3-fold and 2.0-fold higher in subjects with mild hepatic impairment compared with their matched healthy control subjects.

Daprodustat is highly protein bound. The fraction unbound (f_u) in the 3-hour post-dose plasma samples was 0.0034 in the participants with moderate hepatic impairment and 0.0028 in their matched healthy controls. Unbound AUC and C_{max} of daprodustat were 2.2-fold to 2.3-fold higher in subjects with moderate and mild hepatic impairment compared to their matched healthy cohorts. However, high variability (33.2-578% CV) was noted for the unbound PK parameters. In general, time to maximum concentration (T_{max} , median ~1.5 hours for daprodustat and 3 to 4 hours for the metabolites) and the $t_{1/2}$ (mean ~4 to 4.5 h for daprodustat and 2.7 to 5.6 h for the metabolites) was similar across Cohorts 1 to 4.

Table 110. Statistical Assessment of Daprodustat PK Parameters After Single-Dose (6 mg) Daprodustat Administered in Mild and Moderate Hepatic Impaired Subjects, Compared to Administration in Healthy Adults With Normal Hepatic Function

Analyte Parameter	Cohort	n	Geometric LS Means	Cohort Comparison	Ratio of Geometric LS Means	90% CI of the Ratio
Daprodustat						
AUC _(0-t) (h•ng/mL)	1	8	296.10			
	2	8	148.25	1/2	2.00	(1.11, 3.61)
	3	8	299.53			
	4	7	205.63	3/4	1.46	(1.03, 2.05)
AUC _(0-∞) (h•ng/mL)	1	8	296.24			
	2	8	148.32	1/2	2.00	(1.11, 3.61)
	3	8	299.88			
	4	7	205.76	3/4	1.46	(1.04, 2.05)
C_{max} (ng/mL)	1	8	139.71			
	2	8	70.61	1/2	1.98	(1.06, 3.71)
	3	8	113.23			
	4	7	112.14	3/4	1.01	(0.71, 1.43)
$t_{1/2}$ (h)	1	8	3.99			
	2	8	4.41	1/2	0.91	(0.69, 1.19)
	3	8	4.53			
	4	7	4.28	3/4	1.06	(0.79, 1.42)
Free AUC _(0-t) (h•ng/mL)	1	8	0.96			
	2	8	0.42	1/2	2.28	(1.1, 4.72)
	3	7	2.67			
	4	6	1.20	3/4	2.23	(0.43, 11.68)
Free AUC _(0-∞) (h•ng/mL)	1	8	0.96			
	2	8	0.42	1/2	2.28	(1.1, 4.72)
	3	7	2.68			
	4	6	1.20	3/4	2.23	(0.43, 11.7)

Analyte Parameter	Cohort	n	Geometric LS Means	Cohort Comparison	Ratio of Geometric LS Means	90% CI of the Ratio
Daprodustat						
Free C _{max} (ng/mL)	1	8	0.45			
	2	8	0.20	1/2	2.25	(1.05, 4.83)
	3	7	0.99			
	4	6	0.62	3/4	1.59	(0.31, 8.04)

Source: Study 200231 CSR Table 15.

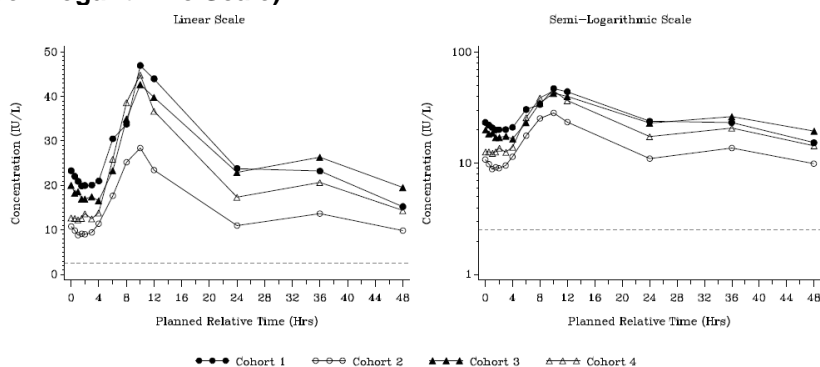
Cohort 1 = Moderate hepatic impairment, Cohort 2 = Matched healthy controls for moderate hepatic impairment, Cohort 3 = Mild hepatic impairment, Cohort 4 = Matched healthy controls for mild hepatic impairment n = number of subjects with evaluable values.

Note: Analysis of variance with cohort as a fixed effect was performed on the natural log-transformed parameters total/free AUC_(0-t), total/free AUC_(0-∞), total/free C_{max}, and t_{1/2}.

Abbreviations: AUC_(0-∞), area under the concentration-time curve from time zero to infinity; AUC_(0-t), area under the concentration-time curve from time 0 to last quantifiable concentration; C_{max}, maximum plasma concentration; CI, confidence interval, LS, least squares, PK, pharmacokinetic; t_{1/2}, half-life

Following single oral administration of 6 mg of daprodustat, arithmetic mean peak and systemic EPO levels (C_{max}, EPO and AUC_[0-t,EPO]) in subjects with hepatic impairment were between 1.2-fold (mild) and 1.8-fold (moderate) those of matched healthy controls. This increase in EPO observed in hepatically impaired subjects is consistent with the increased daprodustat exposure.

Figure 37. Mean Erythropoietin Plasma Pharmacodynamic Concentration-Time Plots (Semilogarithmic Scale)



Source: Study 200231 CSR Figure 2.

Note: LLOQ = 2.50 IU/L. Dashed line represents LLOQ

Cohort 1 = Moderate hepatic impairment, Cohort 2 = Matched healthy controls for moderate hepatic impairment Cohort 3 = Mild hepatic impairment, Cohort 4 = Matched healthy controls for mild hepatic impairment

Daprodustat is titrated to attain and maintain a target hemoglobin level of 11 g/dL. It is important to prevent a rapid increase in Hb or attaining too high a Hb level (>11 g/dL) in patients with CKD as rapid or excessive Hb increases have been associated with the risk of severe adverse cardiovascular events and death in patients treated with ESAs, another treatment for anemia of CKD. A 2-fold increase in exposure of daprodustat in patients with moderate hepatic impairment may be associated with too large an increase in Hb. Therefore, a reduction of the starting dose of daprodustat in patients with moderate hepatic impairment is recommended to account for the increase in the systemic exposure of daprodustat.

We recommend reducing the starting dose of daprodustat by approximately half when initiating treatment in patients with moderate hepatic impairment (Child-Pugh Class B) except in patients

whose starting dose is already 1 mg, the lowest available strength of daprodustat. No adjustment of the starting dose is required in patients with mild hepatic impairment.

Severe Hepatic Impairment

The Applicant has not evaluated the effect of severe hepatic impairment (Child-Pugh Class C) on daprodustat PK. Subjects with severe hepatic impairment and history of chronic liver disease (e.g., cirrhosis or fibrosis of the liver) were excluded from the phase 3 studies. The unbound exposure of daprodustat is expected to further increase in patients with severe hepatic impairment compared to moderate hepatic impairment due to more progressive hypoalbuminemia. As there is no PK and clinical safety experience for daprodustat in patients with severe hepatic impairment, use of daprodustat in patients with severe hepatic impairment is not recommended.

Other Intrinsic Factors

Renal Impairment

Daprodustat PK was evaluated in single dose (Study PHI112843) study in subjects with moderate to severe renal impairment and matched healthy cohort, and in a multiple dose study (Study PHI115573) in subjects with NDD renal impairment (Stages 3/4/5 CKD), and subjects with Stage 5 CKD on hemodialysis. Daprodustat PK following single or repeat doses was similar between subjects with moderate to severe renal impairment (CKD Stages 3 to 4) and their matched healthy controls (see Section [14.2](#) for details). Daprodustat PK was similar between subjects on hemodialysis on a non-dialysis day and subjects with moderate to severe renal impairment. Daprodustat is not significantly cleared by hemodialysis. For subjects with Stage 3/4 non-dialysis and Stage 5 CKD on hemodialysis (dialysis day), accumulation ratios for metabolites were up to 1.8-fold and 1.4-fold for the dialysis day and non-dialysis day, respectively, except for M13, for which accumulation ratio values were 2.6-fold and 1.5-fold, respectively.

The efficacy and safety of daprodustat in patients with CKD on dialysis or not on dialysis is primarily supported by the pivotal five global Phase 3 trials (ASCEND-ND, ASCEND-D, ASCEND-TD, ASCEND-ID and ASCEND NHQ) in these populations. No dose adjustment is needed for daprodustat in renal impairment.

8.2. Extrinsic Factors

Drug Interactions

Metabolic Pathway

In vitro studies demonstrated that daprodustat is primarily metabolized by CYP2C8 (95% contribution), with a minor contribution of CYP3A4 (5% contribution). producing 6 predominant circulating metabolites, of which M2, M3, M13, are major metabolites. ($\geq 10\%$).

Effect of Other Drugs on Daprodustat

Effect of Strong CYP2C8 Inhibitors on Daprodustat

The drug-drug interaction (DDI) of daprodustat with gemfibrozil, a strong CYP2C8 inhibitor, was evaluated in a dedicated DDI study in healthy adults. Gemfibrozil 600 mg BID was dosed to steady state and its effects were evaluated on a single-dose of daprodustat 100 mg. Co-administration of a single-dose 100 mg daprodustat with gemfibrozil resulted in a 3.92-fold and 18.6-fold increase in daprodustat C_{max} and $AUC_{(0-inf)}$, respectively. This marked increase in both rate and extent of systemic exposure of daprodustat when co-administered with gemfibrozil is consistent with daprodustat being primarily metabolized by CYP2C8. The decrease in CYP2C8 metabolism of daprodustat is reflected by its ~4-fold increase in $t_{1/2}$, 2.51 to 9.26 h when co-administered with gemfibrozil. T_{max} was also delayed from 1.00 to 4.00 h.

Co-administration of daprodustat with strong CYP2C8 inhibitors (e.g., gemfibrozil) was contraindicated in the global Phase 3 studies. The review team agrees with the Applicant that the use of daprodustat should be contraindicated with strong CYP2C8 inhibitors in labeling.

Effect of Moderate CYP2C8 Inhibitors on Daprodustat

The Applicant did not conduct a dedicated in vivo DDI study of daprodustat with a moderate CYP2C8 inhibitor. However, the phase 2/3 trials including ASCEND-D allowed the use of clopidogrel, a moderate CYP2C8 inhibitor. The Applicant states that based on the Phase 2/3 PopPK analyses, co-administration of daprodustat with clopidogrel at steady state in 70 subjects showed that clopidogrel increases the exposure of daprodustat by 1.59-fold (90% CI: 1.39-1.82). PopPK analyses from previous Phase 2 and 3 clinical studies (GSK Reports 2019N405983_00 and 2019N405417_00) demonstrated up to 1.75- to 2.65-fold increase in daprodustat exposure when co-administered with clopidogrel (n=54 Phase 2 and n=22 Japan Phase 3 subjects).

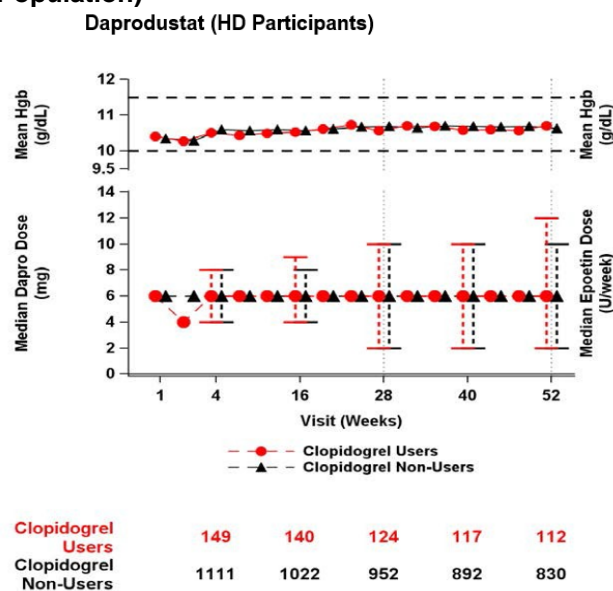
However, the Applicant's current PopPK based DDI assessment is not adequate to accurately characterize the effect of concomitant medications on the PK of daprodustat in the pivotal Phase 3 clinical trials due to the following limitations: (a) A binary covariate analysis was performed in the Applicant's PopPK modeling; (b) An adequate number of daprodustat PK samples during the elimination phase were not available in the data from pivotal clinical trials; (c) There was a lack of data on medication history prior to study entry and dosing records for concomitant medications, such as, dose level, dose frequency, formulation, route of administration, start and stop date and clock time, duration, dose modification, etc. in the PopPK dataset.

Physiological based pharmacokinetic (PBPK) analyses showed that daprodustat AUC was increased by approximately 7.6-fold following coadministration with a single dose of 300-mg clopidogrel and approximately 4-fold following coadministration with maintenance doses of clopidogrel (75 mg). However, the effects of clopidogrel on daprodustat may be underestimated because the clopidogrel PBPK models used by the Applicant were not reliably validated and these clopidogrel models significantly underpredicted the clinically observed interactions between clopidogrel and repaglinide (see Section 14.6 for details). This suggests that the effect of clopidogrel on daprodustat exposure is expected to be no less than the model-predicted effects.

Although at least a 4-fold or higher increase in the AUC of daprodustat can be expected with concomitant administration of clopidogrel, no obvious trends were observed in the number of Hb increases per participant by baseline clopidogrel use, new clopidogrel use, or clopidogrel non-users in pooled data from studies 200807/ASCEND-D and 200808/ASCEND-ND.

Figure 38 shows the mean evaluable hemoglobin and median assigned daprodustat dose by visit and by clopidogrel co-administration at baseline in patients from 200807/ASCEND-D. The mean Hb profile at each visit among clopidogrel users was similar to that in patients who are clopidogrel non-users. While these results support concomitant clopidogrel use with daprodustat, it is important to also compare daprodustat doses between clopidogrel users and non-users, since daprodustat is titrated based on Hb response. Interestingly, there was no consistent trend for a dose reduction of daprodustat in the clopidogrel users group compared to the non-users, as observed from the closely overlaying median and the corresponding distribution bars for daprodustat dose at each visit between the two groups, except for one step median dose decrease at the second visit (Figure 38).

Figure 38. Line Plot of Mean Evaluable Hemoglobin and Median Assigned Daprodustat Dose by Visit by Clopidogrel Co-Administration in Patients on Hemodialysis (Study 200807/ASCEND-D, ITT Population)



Source: Study 200807/ASCEND-D CSR Figure 3.054

Error bars indicate first and third quartiles. The horizontal reference lines in the figures represent the Hgb analysis range (10 to 11.5 g/dL). The Hgb target range for dose changes is 10 to 11 g/dL. The dashed vertical lines represent the EP (Week 28 to Week 52). Error bars indicate 1st and 3rd quartiles

Abbreviations: Dapro, daprodustat; HD, hemodialysis; Hgb, hemoglobin; ITT, intent-to-treat

Although, a decrease in the daprodustat dose is expected based on the predicted 4-fold or higher increase in exposure of daprodustat on concomitant clopidogrel administration, this was not reflected in the clinical experience with daprodustat in the pivotal Phase 3 trial. The reason for this observation is unclear, but the clinical experience from more than 100 patients who are clopidogrel users does support concomitant use of clopidogrel with daprodustat. That said, because of an expected 4-fold increase in daprodustat AUC on a background of clopidogrel at 75 mg, the review team recommends initiating clopidogrel users at a lower starting dose of

daprodustat to avoid exposure to high daprodustat concentrations during initiation except in patients whose starting dose is already 1 mg, the lowest available strength of daprodustat. For patients who may initiate clopidogrel while on daprodustat, the regular monitoring of Hb every 4 weeks should be adequate to inform the need for any dose adjustment of daprodustat.

Effect of CYP2C8 Inducers on Daprodustat

CYP2C8 is the primary CYP enzyme involved in the oxidative metabolism of daprodustat. Concomitant administration of drugs that are inducers of CYP2C8 may decrease daprodustat exposure. The Applicant proposed to monitor hemoglobin and adjust the dose of daprodustat as appropriate when initiating or stopping therapy with CYP2C8 inducer. The review team agrees with the Applicant's proposal.

Effect of BCRP Inhibitors on Daprodustat

In vitro data indicate daprodustat is a substrate of the breast cancer resistance protein (BCRP) (efflux ratio ~4) and therefore has the potential for in vivo drug interactions due to BCRP-mediated inhibition (see Section 14.2 for details). BCRP inhibition-mediated drug interactions are known to primarily occur at the intestine, affecting absorption, and at the liver bile canalicular membrane, affecting hepatobiliary secretion. Oral daprodustat is well-absorbed, with a calculated absorption fraction of 80%. Any intestinal BCRP inhibition would not be expected to lead to a 20% increase in absorption. Daprodustat has limited hepatobiliary clearance and is cleared primarily by oxidative metabolism. In the human ADME study following an IV infusion, daprodustat only represented 0.5% of total radioactivity in the duodenal bile, with oxidation metabolites accounting for the rest, and approximately 99.5% of the oral dose was excreted as oxidation metabolites, with daprodustat accounting for 0.5%. Based on the disposition profile of daprodustat, a clinically significant effect of BCRP inhibitors on daprodustat PK is not expected.

Effect of Acid Reducing Agents on Daprodustat

Daprodustat has a pH dependent solubility. Co-administration of daprodustat with acid reducing agents could increase solubility and thus potentially increase the systemic exposure of daprodustat. The Population PK model predicted up to 1.06-fold increase in C_{max} and 0.996-fold change in AUC of daprodustat with concomitant administration with acid reducing agents (N=526). Because oral daprodustat is well-absorbed, with a calculated fraction absorbed of 80%, any increase in absorption would not be expected to exceed 20%. A clinically significant effect of acid reducing agents on daprodustat PK is not expected.

Effect of Phosphate Binders and Oral Iron on Daprodustat

There is a potential for commonly co-administered medications such as oral iron and phosphate binders to affect the rate and extent of absorption of drugs including daprodustat. Co-administered oral iron (N=84) and phosphate binders (N=415), including sevelamer and aluminum, calcium, magnesium, and bicarbonate-based binders, were tested as potential covariates in the Phase 2/3 PopPK analysis. However, the Applicant's current PopPK based DDI assessment is not adequate to accurately characterize the effect of concomitant medications on the PK of daprodustat in the pivotal Phase 3 clinical trials due to the limitations of analysis

method and concomitant medication dosing history and records as detailed under a previous section discussing drug interaction with clopidogrel.

While a dedicated DDI study with oral iron or phosphate binders was not conducted, there is clinical experience from ASCEND-D for concomitant use of daprodustat with phosphate binders. Data from ASCEND-D shows that the phosphate binder user group in the daprodustat arm had a median dose of 6 mg compared to a one-step lower dose of 4 mg for the non-users. This likely reflects a potential interaction of daprodustat with phosphate binders per the expectation that a decrease in daprodustat exposure could lead to a higher maintenance dose to maintain Hb in the target range. That said, the extensive clinical experience from ASCEND-D supports the concomitant use of phosphate binders with daprodustat as it is shown that regular Hb monitoring and dose titration is adequate to manage a potential interaction. Although, a similar analysis has not been conducted for concomitant administration of oral iron, the recommended hemoglobin monitoring and the corresponding daprodustat dose titration algorithm seem adequate to support concomitant administration of oral iron with daprodustat.

Table 111. Summary of Assigned Daprodustat Dose by Baseline Phosphate Binder Use at Week 28 and Week 52 (200807/ASCEND-D, ITT Population)

Planned Relative Time	Baseline Phosphate Binder Use	200807/ASCEND-D Daprodustat N=1487
Week 28	Yes, n	956
	Mean (SD)	7.0 (5.99)
	Median	6.0
	No, n	264
	Mean (SD)	5.9 (5.09)
	Median	4.0
Week 52	Yes, n	843
	Mean (SD)	7.3 (6.86)
	Median	6.0
	No, n	227
	Mean (SD)	6.0 (5.88)
	Median	4.0

Source: Study 200807/ASCEND-D CSR Table 2.160
Abbreviations: ITT, intent-to-treat

8.3. Plans for Pediatric Drug Development

The Applicant submitted an initial pediatric study plan (iPSP) on April 6, 2016 and proposed a waiver in children less than 1 year of age for daprodustat for the treatment of anemia associated with chronic kidney disease due to uncertainty of the ability to demonstrate therapeutic benefit in this age cohort. An amended agreed initial pediatric study plan letter was issued on May 24, 2021. The Division agreed with the Applicant's request regarding deferring the start of the clinical portion of the pediatric development program until after the adult population NDA has been submitted.

The Applicant submitted a proposed pediatric study request on June 29, 2021, to conduct an Integrated Pharmacokinetic and Safety Open-label Basket Trial of Daprodustat for the Treatment of Anemia Associated with Chronic Kidney Disease in Male and Female Children and

Adolescents Aged 3 Months to Under 18 Years Requiring or Not Requiring Dialysis (ASCEND-P) where sixty participants will receive daprodustat for one year to assess its safety and tolerability.

An inadequate study request letter was issued on October 12, 2021, where the Agency recommended commencing pediatric clinical studies (through a deferral request) after the NDA submission in adults to ensure that sufficient efficacy and safety data (including data on CV risk) have been collected and reviewed by the Agency in order to establish an acceptable risk: benefit profile prior to its exposure to children. In addition, the Division recommended longer follow-up safety in the pediatric population (b) (4)

(b) (4) The Division is recommending one Pediatric Research Equity Act PMR for daprodustat in the dialysis dependent pediatric population. Details of the PMR can be found in Section 24.

8.4. Pregnancy, Lactation, and Females/Males of Reproductive Potential

Animal Data

The following nonclinical data are used to support the label. Additional detailed information is available in Section 13 for nonclinical data.

Table 112. Nonclinical Data Supporting Labeling on Fertility, Pregnancy, and Lactation

Labeling Section	Nonclinical Data
8.1 Pregnancy	Daprodustat was orally administered to pregnant rats at 0.5, 7, or 60 mg/kg/day from gestation day 6 to gestation day 17 during the period of organogenesis. No adverse effects were observed at doses less than or equal to 7 mg/kg/day (3 times the MRHD based on body surface area). Daprodustat administration resulted in post-implantation loss, increased embryofetal death, and reduction in skeletal ossification in rats at a dose of 60 mg/kg/day (24 times the MRHD based on body surface area), which was associated with maternal toxicity (reduced body weight gain or weight loss). Maternal toxicity occurred at doses associated with polycythemia. Daprodustat was orally administered to pregnant rabbits at doses of 4, 30, or 60 mg/kg/day from gestation day 7 until gestation day 19 during the period of organogenesis. No adverse effects were observed at doses less than or equal to 30 mg/kg/day (24 times the MRHD based on body surface area). Daprodustat administration was associated with low incidence of abortions and fetal skeletal malformations (irregularly shaped anterior fontanelle, manubrium, fused sternal centra, and microphthalmia) at a dose of 60 mg/kg/day (49 times the MRHD based on body surface area) in the presence of maternal toxicity (reduced body weight gain or weight loss) and polycythemia. In a pre- and postnatal development study, pregnant rats were dosed orally with daprodustat from implantation until weaning (gestation day 6 to lactation day 21) at 0.8, 7, or 40 mg/kg/day concomitantly with 3 major human metabolites of daprodustat. No adverse effects were observed at doses less than or equal to 7 mg/kg/day (3 times the MRHD based on body surface area). Maternal toxicity was noted at 40 mg/kg/day, which represents 16-times the MRHD based on body surface area, which was associated with dystocia and increased pup deaths and decreased pup weights.

Labeling Section	Nonclinical Data
8.2 Lactation	In a pre- and postnatal development study in rats, when daprodustat was orally administered to maternal rats during the lactation period, the drug was detected in plasma of suckling pups on postnatal day 10. The plasma concentration of daprodustat in pups was 2.3% to 3.7% of daprodustat detected in the plasma of dams when dosed at 40 mg/kg/day.
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility	Daprodustat was not carcinogenic in two-year carcinogenicity studies when administered orally at doses of 0.02, 0.1, 0.8, or 4 mg/kg/day (males)/ 7 mg/kg/day (females) in rats and 0.2, 0.8, or 3 mg/kg/day (including subcutaneous injection of major human metabolites of daprodustat) in mice. Daprodustat was negative for mutagenic or clastogenic potential in the in vitro bacterial reverse mutation assay, the in vitro human lymphocyte chromosomal aberration assay, and the in vivo rat bone marrow micronucleus assay. In a fertility and early embryonic development study in rats, daprodustat was administered orally at doses of 2, 7, or 100 mg/kg/day in females, resulting in maternal toxicity of reduced body weight gain and decreased mean uterine weight, associated with decreased number of corpora lutea, implantations, and live fetuses, and increased post-implantation loss at the dose of 100 mg/kg/day (41 times the MRHD based on body surface area).

Source: FDA Reviewer

Abbreviations: MRHD, maximum recommended human dose

Details of above information and dose multiples shown are based on body surface area compared between animals and humans.

Human Data

The Division of Pediatric and Maternal Health (DPMH) was consulted for advice on labeling text for Section 8.1 to 8.3 (Pregnancy and Lactation Labeling).

There are limited human pregnancy outcome data for daprodustat from the Sponsor's Pharmacovigilance Database. There were 9 pregnancies exposed to daprodustat identified in the clinical trials. The outcomes for these 9 pregnancies were

- 7 spontaneous abortions (SABs) at ages 6 to 19 weeks gestation (including both fetuses of one twin pregnancy)
- 2 elective abortions
- One normal live birth

There was no human pregnancy outcome data found in the published English language literature. The findings from animal studies suggest potential for fetal harm from exposure to daprodustat. Labeling will note that CKD is associated with adverse pregnancy outcomes, that animal data suggest harm, and that there are inadequate human pregnancy data.

Pregnancy would be expected to be uncommon in women in dialysis and a traditional pregnancy registry or a claims database study would likely be unsuccessful due to low enrollment. Therefore, DPMH recommends a PMR Descriptive Pregnancy Safety Study to follow-up on maternal and infant outcomes of pregnancies that do occur. Refer to Section 24 for a detailed summary of the PMR.

DPMH also recommends that the Applicant submit annual reports of daprodustat utilization rates amongst females of reproductive potential (females aged 15 to 50 years) calculated cumulatively

from the time of initial approval. If there appears to be substantial use of daprodustat in females of reproductive potential, then this would be considered new safety information that would trigger FDAAA and would result in the Agency issuing a PMR for a pregnancy registry study at that time.

There are no data on the presence of daprodustat in human milk, its effect on a breastfed infant or milk production. Parameters such as daprodustat's low molecular weight (~393 Daltons) and the presence of daprodustat in rat milk suggest it is likely that daprodustat will be present in human milk. The high protein binding (99%) suggest that the amount present is likely to be low. However, given the spectrum of serious adverse events that can be seen with daprodustat in adults, breastfeeding is not recommended during treatment with daprodustat and for one month after the last dose.

Given the low incidence of pregnancy and live births in the proposed population and recommendation against use during breastfeeding, a PMR for a lactation study would not be feasible and the alternative of performing a lactation study in healthy volunteers would not be ethically permissible in view of DPMH because a single dose of daprodustat could potentially cause thrombosis in a predisposed population of recently pregnant/lactating women.

There were no clinical studies of the adverse effect of daprodustat on fertility. Recommendations for pregnancy testing or recommendations for contraception while taking daprodustat would not be appropriate given that the fetal harm warning is based on adverse effects in a single animal species at multiples greater than 10 times the maximum recommended human dose. Therefore, DPMH recommends that subsection 8.3 be omitted from the labeling.

For additional details regarding the DPMH review, refer to the consult review by Jane Liedkta M.D, Tamrar Johnson MD, MS and Lynne P. Yao, MD.

9. Product Quality

The Office of Pharmaceutical Quality (OPQ) review team has assessed NDA 216951 with respect to chemistry, manufacturing, and controls (CMC) and has determined that it meets all applicable standards to support the identity, strength, quality, and purity that it purports. As such OPQ recommends approval of this NDA from a quality perspective.

For additional details, refer to the OPQ Integrated Quality Assessment dated December 28, 2022.

9.1. Device or Combination Product Considerations

Not applicable.

10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure Review

In relation to good clinical practice (GCP) compliance, the Applicant stated that the clinical trial protocols, informed consent forms (ICF) and other information that required pre-approval were reviewed and approved by national, regional, or investigational center ethics committees or institutional review boards, in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use GCP and applicable country-specific requirements, including US 21 Code of Federal Regulations (CFR) 312.3(b) for constitution of independent ethics committees. Ethics committee or institutional review board approvals are maintained in the Sponsor's study file.

Investigators were trained to conduct the study in accordance with GCPs and the study protocol, as defined in ICH E3, Section 9.6. Written commitments were obtained from investigators to conduct the study in accordance with ICH GCP and all applicable subject privacy requirements, and the ethical principles that are outlined in the Declaration of Helsinki, and to conduct the study in accordance with the protocol.

The study was monitored in accordance with ICH E6, Section 5.18. If significant findings (e.g., potential serious misconduct or noncompliance with GCP, including potential Serious Breaches) were identified during monitoring or auditing of a site, these are presented in Section 5.2, Protocol Deviations, of this report.

A discussion of Financial Disclosures is in Section 25.

11. Advisory Committee Summary

The Division of Non-Malignant Hematology (DNH) convened a virtual advisory committee meeting to discuss daprodustat on October 26, 2022. DNH sought the advisory committee's advice and recommendations on the safety issues discussed in this review and whether the benefits of daprodustat outweigh its risks in both the non-dialysis and dialysis populations.

The advisory committee members voted 5 yes vs. 11 no on the question of whether the benefits outweigh the risks for anemia of chronic kidney disease in patients not on dialysis. For the dialysis population, the vote was 13 yes vs. 3 no. Committee members recognized that the oral route of administration would provide convenience to some patients and another treatment option. However, the committee was concerned about the potential further increase in some cardiovascular risks seen only in the non-dialysis population when compared to the erythropoietin stimulating agent control, which itself carries these risks. See the Minutes and Transcript available on the external FDA website ([fda.gov](https://www.fda.gov))²⁰ for further details. Our actions on

²⁰ <https://www.fda.gov/advisory-committees/advisory-committee-calendar/updated-contact-information-october-26-2022-meeting-cardiovascular-and-renal-drugs-advisory>

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this NDA – [REDACTED] ^{(b) (4)} Approval for the DD
population – are consistent with the advisory committee’s recommendations and advice.

III. Additional Analyses and Information

12. Summary of Regulatory History

Daprodustat was granted approval for the treatment of adults with anemia due to chronic kidney disease (CKD) on or not on dialysis on June 29, 2020, in Japan.

Investigational New Drug Application IND 101291 was received on June 3, 2008, to study GSK1278863 (also referred to as daprodustat) for the treatment for anemia of chronic kidney disease. The initial IND included the protocol titled *PHX111427: A Phase I, Randomized, Single-Blind, Placebo-Controlled, Dose-Escalation Study to Evaluate the Safety, Pharmacokinetics, and Pharmacodynamics of Single Oral Doses of GSK1278863A in Healthy Subjects*. The IND became active on July 3, 2008.

Transfer of IND 102191 ownership from GlaxoSmithKline to Octagon Research Solutions, Inc. was made on February 4, 2009. The ownership was transferred back to GlaxoSmithKline on March 1, 2011.

A Type C End of Phase 2A meeting was held on July 23, 2012, to discuss questions related to dosing. In addition, the Applicant proposed to conduct separate phase 3 trials to assess efficacy in two groups – patients requiring hemodialysis (HDD) and patients not requiring hemodialysis (non-dialysis dependent [NDD]), and planned to assess cardiovascular safety in a prospectively defined meta-analysis across their phase 3 trials. In terms of dosing, the Agency stated the Applicant's approach seemed reasonable and encouraged them to consider requesting another End of Phase 2A meeting with the Agency to discuss the details of their dosing modeling approaches to select the appropriate dose for their phase 3 trials. The Agency also commented that separate meta-analyses should be performed for NDD and HDD patients, instead of combining both populations, and recommended the Applicant to consider a superiority design for cardiovascular safety.

A carcinogenicity Special Protocol Assessment request was submitted for the proposed carcinogenicity protocol entitled *GSK1278863: A 2-year Oral (Gavage) Carcinogenicity Study in Rats* on August 16, 2013, and an agreement letter was issued on September 20, 2013. Another carcinogenicity Special Protocol Assessment request was submitted for the proposed carcinogenicity protocol entitled *GSK1278863: A 2 Year Carcinogenicity Study in Mice* on August 27, 2014, and an agreement letter was issued on October 8, 2014.

The Applicant submitted various meeting requests to obtain feedback and guidance in preparation for submission of a new marketing application for daprodustat. An End of Phase 2 meeting was held on February 22, 2016, to discuss the non-clinical and clinical program conducted to date and the phase 3 clinical development plan for daprodustat in the context of supporting a future NDA submission for daprodustat.

The Applicant's initial proposed proprietary name, (b) (4) was found unacceptable on August 14, 2019. Another proposed proprietary name review request was submitted with the proposed name of (b) (4), which was found to be conditionally acceptable on January 15, 2021.

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Subsequently, the proposed proprietary name, (b) (4) was withdrawn by the Applicant on August 16, 2021.

A pre-NDA meeting took place on September 7, 2021, during which the Agency provided feedback on the Applicant's proposals for the content and format of the planned NDA submission and the Agency also provided comments on the high-level results for the daprodustat ASCEND phase 3 program.

The Applicant submitted another proposed proprietary name request with the proposed name of Jesduvroq, which was found to be conditionally acceptable on October 14, 2021.

The Applicant submitted the NDA on February 1, 2022, with the indication for the treatment of anemia due to chronic kidney disease in adult patients on dialysis and not on dialysis.

A summary of key meetings for IND 101291 and NDA 216951 with the Division of Non-Malignant Hematology is presented below:

Table 113. Summary of Key Meetings for IND 101291 and NDA 216951 With DNMH

Date	Event Type	Purpose
March 31, 2008	Pre-IND submission	To discuss early development plan for the treatment of anemia of CKD
June 30, 2008	IND 101291 Phase 1, single-blind, randomized, placebo-controlled, single dose escalation study to determine the safety, tolerability, and pharmacokinetics (PK) of GSK1278863 after single doses in healthy adult subjects	30 day safety review of phase 1 trial
February 4, 2009	Change sponsorship from GSK to Octagon Research Solutions, Inc	Change in sponsorship
March 29, 2011	Change sponsorship from Octagon Research Solutions, Inc to GSK	Change in sponsorship
March 5, 2012	Genotoxicity studies paused based on the uncertainty about the genotoxic potential of the M13 metabolite of GSK 1278863 following the results of mouse lymphoma assay and the initial mouse liver comet assay	The Division concluded that the M13 metabolite was not genotoxic and agreed with the Sponsor to re-initiate clinical trials of GSK 1278863
July 18, 2012	Type C Meeting (End of Phase 2a)	Discuss the development plan on the Phase 2b program (selecting starting dose in Phase 3 trial in DD and NDD patients and CV composite endpoint based on non-inferiority)

Date	Event Type	Purpose
July 15, 2014	Type C Meeting	Updates to preliminary dose-response modelling conducted at the end of Phase 2A and to inform selection of the starting dose (s) and optimization of the dose adjustment regimen(s) for Phase 3 clinical trials. Agency's feedback on their proposed global phase 3 development program in DD and HDD programs which will include multi-center Hb efficacy studies and a large cardiovascular safety study relative to rhEPO.
October 8, 2014	Special Protocol Assessment (SPA)	Agreement letter issued for the proposed carcinogenicity protocol entitled GSK1278863: A 2 Year Carcinogenicity Study in Mice
January 14, 2015	Type C Meeting	Approach to assess the comparative CV safety of GSK1278863 to include the proposed MACE NI margin of 1.25 was discussed. The MACE event number of 1200 for each event driven trial will provide greater than 90% power to rule out the Sponsor's proposed NI margin of 1.25. The proposed NI margin (-0.75 g/dL) to assess comparative Hb efficacy of GSK1278863 was found to be reasonable. Durability of mean change in Hb was discussed.
February 24, 2016	Type C Meeting	Analysis of a subgroup with hyporesponsiveness to rhEPO would be exploratory Dose selection should be appropriate for patients across a range of body weights Use of modeling and simulation (b) (4) of GSK12778863. Sponsor proposed to change the MACE NI to 1.2 and Division agreed.
April 4, 2016	Submission of Initial Pediatric Study Plan (iPSP) Randomized, open-label, active-controlled study in DD and NDD pediatric patients	Waiver in children less than 1 year of age. Deferral request until marketing approval in adults has been obtained. Division recommended extrapolation of efficacy from adult population.
December 20, 2016	Agreed iPSP	Deferral of pediatric studies was granted. Extrapolation of efficacy from adult population as well as PK extrapolation for the younger cohort. Waiver was not applicable.
September 15, 2017	Type C (Pre-Phase 3) Meeting	Once-daily daprodustat administration to characterize the CV safety profile of three-times a week daprodustat dosing requiring exploratory analyses to evaluate the impact of hemoglobin level (early responder versus late responder, rate of change of hemoglobin) on the MACE event rates.
October 5, 2018	Type C Meeting	Discuss the adequacy of the scientific bridging strategy between US and EU-licensed Aranesp being used in the daprodustat clinical development program
July 12, 2019	Type C Meeting (WRO)	Discussion of acceptability of pooling clinical data would be contingent upon the demonstration of an adequate analytical bridge between US and non-US Aranesp used in the clinical studies.

Date	Event Type	Purpose
		(b) (4) Concerns with single historical study not being sufficient to quantify between-study heterogeneity of the underlying true control MACE risk. Upper bound of the 95% credible interval rather than the proposed upper bound of the 80% credible interval to assess clinically meaningful difference in MACE risk (b) (4)
August 2, 2019	Type C Meeting	Significant concerns with conducting an interim analysis (as a result of positive results for the coprimary endpoints (i.e., futile for MACE superiority, but NI for MACE and Hb) that may result in stopping the studies for non-inferiority for MACE in ASCEND-ND and ASCEND-D studies
December 4, 2019	Type C Meeting	Concerns with the use of historical data from the TREAT trial in the analysis of the secondary endpoints in Study 205270 Concerns with the interpretation of PRO data utilized from historical placebo data from the TREAT trial in the analysis of the principal secondary endpoint (mean change in SF-36 vitality sub-score between baseline and Week 28) in Study 205270.
July 1, 2020	Type C Meeting	Discuss the CKD-AQ to be used as secondary endpoints in the ASCEND-D and ASCEND-ND trials.
July 22, 2020	Type C Meeting	The proposal to change the MACE non-inferiority from 1.2 to 1.25 in the phase 3 studies ASCEND-D and ASCEND-ND (NI) margin to 1.25 and reducing the number of target events to 664 in each individual trial, ASCEND-D and ASCEND-ND, respectively was granted based on impact of the COVID-19 pandemic.
September 28, 2020	Type C Meeting	Model Informed Drug Development (MIDD) paired meetings to discuss proposed MIDD approaches to refine dosing strategies that can be used to evaluate the performance of dose transition from ESAs to daprodustat.
March 3, 2021	Type C Meeting	Model Informed Drug Development (MIDD) paired meetings to discuss proposed MIDD approaches to refine dosing strategies in the DOSING and ADMINISTRATION section of drug label.
September 7, 2021	Type B Meeting (Pre NDA)	Discuss agreement on the planned NDA and high-level results from the Phase 3 ASCEND program.

Source: FDA

Abbreviations: CKD, chronic kidney disease; CKD-AQ, Chronic Kidney Disease Anemia Questionnaire; CV, cardiovascular; DD, dialysis dependent; DNMH, Division of Non-Malignant Hematology; ESA, erythropoiesis-stimulating agents; EU, European Union; Hb, hemoglobin; HDD, hemodialysis; MACE, major adverse cardiovascular events; NDD, non-dialysis dependent; NI, noninferiority; PRO, patient-reported outcome; rhEPO, recombinant human erythropoietin; TIW, three times a week; US, United States

13. Pharmacology Toxicology

13.1. Summary Review of Studies Submitted With the Investigational New Drug Application

13.1.1. Primary pharmacology

Daprodustat is a reversible inhibitor of hypoxia-inducible factor (HIF)-PH1, PH2, and PH3 which results in the stabilization and nuclear accumulation of HIF-1 α and HIF-2 α transcription factors, leading to increased transcription of the HIF-responsive genes, including erythropoietin. Treatment of daprodustat in vitro and in vivo pharmacology studies showed that daprodustat increases erythropoietin (EPO) levels (mRNA and protein), which results in an increase of red blood cell (RBC) parameters. Tabulated summary of the in vitro and in vivo pharmacology studies in animals conducted in support of daprodustat's mechanism of action are listed below. Daprodustat was coded as GSK1278863A in the pharmacology study reports.

Table 114. Summary of the In Vitro and In Vivo Pharmacology Studies

Study/ Study No.	Findings
Mechanism of action:	
Biochemical Activity of the Prolyl Hydroxylase Inhibitor GSK1278863A/UH2008/00036/01	The effects of daprodustat on in vitro enzymatic activity of HIF PH3 and PH2, referred to as egg-laying deficiency protein nine-like protein 3 and 1 (EGLN3 and EGLN1) in the study report, were measured using a LANCE™ (Homogeneous Time-Resolved Fluorescence Quenching Assay based on lanthanide chelates) format, a high-throughput homogeneous FRET assay. To evaluate the selectivity of daprodustat, two close homolog PHs, α -ketoglutarate dependent iron dioxygenases CP4H and factor inhibiting HIF (FIH) were tested for inhibition. Daprodustat competitively inhibited enzymatic activity of HIF-prolyl-hydroxylases PH3 and PH2 ($K_i=1.8$ and 7.3 nM, respectively). Daprodustat inhibited PHD3 with reversibility in a time dependent manner. The dissociation half-life ($t_{1/2}$) of daprodustat was 132 min, suggesting that daprodustat does not instantaneously dissociate and prolonged effect of daprodustat is possible. Daprodustat did not strongly inhibit the CP4H and FIH with IC_{50} values in the μ M range $>200\mu$ M and 9.8μ M, respectively.
Activity of Prolyl Hydroxylase Inhibitor GSK1278863A and its Metabolites in Various Primary, Selectivity and Liability Assays/ 2011N121507/00	The inhibition of enzymatic activity of other α -ketoglutarate dependent iron dioxygenases and hERG and hNav1.2 channels (patch clamp assays) by daprodustat and its metabolites [M2; M3, M4, M5, M6 (both stereoisomers); M13] were tested to further investigate the pharmacological activity of metabolites of daprodustat. Daprodustat and its metabolites were also tested against eXP. eXP represents pharmacological targets from six target classes, comprising functional and binding assays. The constituent assays in eXP are mostly associated with clinical adverse events and/or drug side-effects. All data are derived from 11-point concentration-response curves. PH2, PH1, and PH3 were referred to as EGLN1, EGLN2, and EGLN3, respectively, in the study report.

Study/ Study No.	Findings
	Daprodustat exhibited similar potency in inhibition of PHs in rats, dogs, and humans and selectivity against close homologs, collagen CP4H and factor FIH based on IC ₅₀ (nM) and K _{iapp} (nM) values.

Table 115. Daprodustat Inhibition of Daprodustat on PH1, PH2, and/or PH3 of Human, Rat, and Dog

Enzyme	IC ₅₀ ^a (nM)	K _{iapp} ^b (nM)
Human PH1	3.5	1.8
Human PH2	3.3	1.7
Human PH3	8.9	4.4
Rat PH2	2.4	1.2
Rat PH3	4.3	2.2
Dog PH3	4.1	2.0
Human CP4H	>200µM	--
Human FIH	9.8µM	--

Source: Reviewer's analysis

^a IC₅₀ values were determined with 30-minute enzyme-inhibitor pre-incubation.

^b K_{iapp} values were calculated using Cheng-Prusoff equation for competitive inhibition.
- Not measured.

Abbreviations: CP4H, collagen prolyl hydroxylase; FIH, factor inhibiting hypoxia inducible factor; IC₅₀, half maximal inhibitory concentration; K_{iapp}, apparent inhibitory constant; PH, prolyl hydroxylase

The metabolites tested exhibited comparable pharmacological activity to the parent compound inhibiting PH1, PH2, and/or PH3 and comparable selectivity against CP4H and FIH with more than 800-fold selectivity for PHs vs. FIH; and, even higher for CP4H (IC₅₀ >100µM).

The hERG inhibition by daprodustat (tested up to 75µM) and its metabolites (tested up to 30µM) was weak with 24.4% and 12% inhibition, respectively.

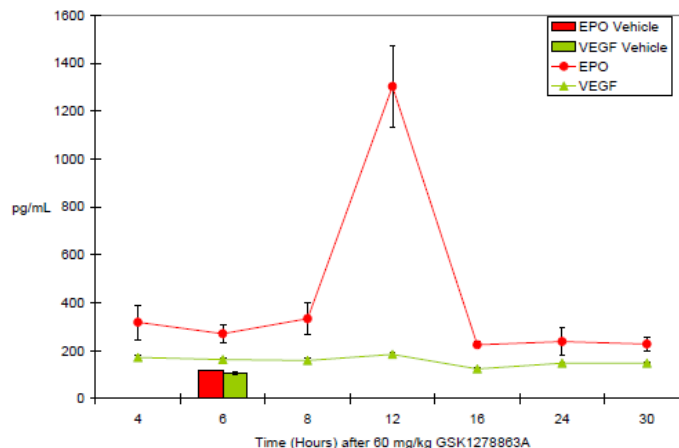
Daprodustat and metabolites did not show any significant activity on any liability target included in the enhanced eXP panel including the surrogate measures of cellular toxicity, genotoxicity, and phospholipidosis assays, defined as a mean half maximal effective concentration of less than 1µM.

HIF Stabilization in Hep3B Cells after GSK1278863A Treatment/ UH2008/00026/00	To investigate the stabilization of HIF1α and HIF2α as a result of inhibition of PHs, Hep3B cells, a hepatocellular carcinoma cell line, were incubated with 25 and 50 µM daprodustat or 100 µM deferoxamine (DFX, positive control) for 6 hours at 37°C. Nuclear protein was isolated, denatured, transferred to a nitrocellulose membrane and incubated overnight with mouse monoclonal HIF1α or rabbit polyclonal HIF2α antibodies. Antibody-protein complexes were then detected using ECL-plus and visualized (qualitative assay only) using x-ray film. HIF1α and HIF2α were detected with daprodustat or DFX treatment, while there was none detected in the vehicle group. The result shows that daprodustat induced HIFα accumulation in the nucleus which indicates stabilization of both HIF1α and HIF2α.

Study/ Study No.	Findings
Drug activity related to proposed indication:	
Erythropoietin and VEGF Protein Production in Hep3B Cells Treated with GSK1278863A/ UH2008/00024/00	To investigate the effect of daprodustat on EPO and VEGF, Hep3B cells were incubated in duplicate with 0.026 to 100 μM (2.5-fold increase/concentration) daprodustat for 48 hours at 37°C. Media EPO and VEGF concentrations were then determined using an ELISA assay. The average EPO EC₅₀ was 3.3 μM and the average VEGF EC₅₀ was 2.2 μM over 5 separate experiments. The average “maximum fold induction” relative to vehicle controls produced over the 5 experiments was 5.8 for EPO and 2.2 for VEGF. A bell-shaped concentration-response curve was observed with maximum EPO and VEGF levels consistently occurring at the 6.4 or 16 μM concentrations. Less than 75% viability was observed at 40 and 100 μM, suggesting toxicity at those concentrations.
Message Production in Hep3B Cells Treated with GSK1278863A/ UH2008/00027/00	To investigate the downstream effects of PH inhibition by daprodustat, the changes in mRNA and/or protein levels of HIF-responsive genes, including EPO and VEGF-A were evaluated. Hep3B cells were incubated with 2.5, 12.5, and 25 μM daprodustat or 100 μM deferoxamine (DFX, positive control) for 16 hours at 37°C. TaqMan RT-PCR was then used to measure mRNA expression. EPO mRNA expression was increased by 1.7-, 5.0-, and 7.4-fold, respectively, and HIF1α-specific PGK1 mRNA expression was increased by 1.7-, 2.6-, and 2.9-fold, respectively, in daprodustat treated cells compared to vehicle control. DFX induced EPO and PGK1 mRNA expression 14- and 6.6-fold, respectively, compared to vehicle control. mRNA levels of PH2, PH1, and PH3 were not affected by daprodustat treatment, but were increased by 2.7-, 0.8, and 7.2 fold in DFX-treated cells. Daprodustat treatment also increased the level of EPO protein secreted from the cells and, to a lesser extent, secretion of VEGF protein.
Mouse Plasma Erythropoietin and VEGF Production after a Single Dose of GSK1278863A/ UH2008/00023/00	To evaluate the effect of daprodustat on EPO and VEGF in PPP from normal female B6D2F1 mice, EPO and VEGF levels were determined using ELISA (n=6/timepoint) at 6 hours after treatment with vehicle and at 4, 6, 8, 12, 16, 24, and 30 hours after administration of 60 mg/kg daprodustat via oral gavage. PPP was prepared by centrifugation of plasma samples for ELISA analysis. Daprodustat treatment increased plasma EPO levels by 11-fold (vs. control) which peaked at 12 hours after daprodustat treatment. A 1.2- to 1.8-fold increase in VEGF compared to vehicle control was observed at all timepoints.

Study/ Study No.	Findings

Figure 39. Mouse EPO and VEGF Plasma Time Course Following a Single 60 mg/kg Oral Dose of Daprodustat (Excerpted From Submission)



Source: Applicant's submission
Abbreviations: EPO, erythropoietin; VEGF, vascular endothelial growth factor

Mouse Erythropoietin Message in Liver and Kidney after a Single Dose of GSK1278863A/UH2008/00022/00

Normal female B6D2F1 mice were dosed with 60 mg/kg daprodustat, and blood samples were collected (n=5/timepoint) at 2, 4, 6, 8, 10, 12, 14, and 16 hours after administration of daprodustat (vehicle controls collected at the 4, 8, and 12 hour timepoints) via oral gavage. To evaluate the effect of daprodustat on EPO protein level in plasma, PPP samples were prepared and analyzed by ELISA. Additionally, liver and kidneys were collected at the 2, 4, 6, 8, 10, and 12 hours after administration timepoints to evaluate EPO and PGK1 mRNA expression using TaqMan RT-PCR (normalized to GAPDH).

Daprodustat treatment at 60 mg/kg in mouse induced EPO liver mRNA expression and plasma protein levels exhibited a biphasic response with peaks at 8 and 12 hours after dosing. EPO liver mRNA expression increased from 279-fold (relative to vehicle control) observed at 2 hours after dosing to 449-fold at the 6-hour time point. Liver EPO mRNA expression then decreased to 31-fold at the 8-hour time point, then was increased again at the 12-hour timepoint (279-fold; 10-hour timepoint was not evaluated). Kidney EPO mRNA expression was increased by 56-fold and 35-fold, respectively, at the 8-hour and 12-hour timepoints. PGK1 expression was minimally affected by daprodustat-treatment.

Mouse Hemoglobin Increases after 8 Doses of GSK1278863A/UH2008/00028/00

Female B6D2F1 mice (n=5/group) were treated with single daily oral doses of 0 (vehicle), 3, 10, and 30 mg/kg daprodustat for 8 consecutive days to evaluate the effects of daprodustat on erythropoiesis. Animals were euthanized 24 hours after the last dose and blood was collected for hematology parameter analyses.

Daprodustat treatment increased RBC parameters including RBC count, hemoglobin, hematocrit, and reticulocytes (up to 17%, 17%, 25% and 673%, respectively vs. control) and decreased platelet/white cell counts with dose-relation compared to the vehicle control.

Study/ Study No.	Findings
Rat Hemoglobin Increases after GSK1278863A Treatment In Vivo/ UH2008/00025/00	Male Sprague-Dawley rats (n=5/group) were treated with oral doses of daprodustat at 0 (vehicle), 0.3, 1, 3, or 10 mg/kg for 21 consecutive days to evaluate the effect of daprodustat on erythropoiesis. Blood was collected on Day 8, 15, and 22 for hematology parameter analyses. Daprodustat treatment resulted in dose-related increases in RBC parameters (up to 18%, 22%, and 26% of RBC, hemoglobin, and hematocrit, respectively) that were evident by Day 8 and further increased on Day 22. There were no significant drug-related effects on white cell counts.

Source: Reviewer's analysis

Abbreviations: CP4H, collagen prolyl hydroxylase; DFX, deferoxamine; EC₅₀, half maximal effective concentration; ECL, electrochemiluminescent; ELISA, Enzyme-Linked Immunosorbent Assay; EPO, erythropoietin; eXP, enhanced Cross-screen Profiling panel; FIH, factor inhibiting HIF; FRET, Fluorescence Resonance Energy Transfer; GADPH, glyceraldehyde 3-phosphate dehydrogenase; hERG, human ether-a-go-go-related gene; HIF, hypoxia-inducible factor; IC₅₀, half maximal inhibitory concentration; mRNA, messenger ribonucleic acid; PGK1, phosphoglycerate kinase-1; PH, prolyl hydroxylase; PPP, platelet-poor plasma; RBC, red blood cell; VEGF, vascular endothelial growth factor

13.1.2. Secondary Pharmacology

To characterize any potential activity associated with daprodustat beyond its understood mechanism of action, secondary pharmacology studies were conducted screening for the activity in vitro of daprodustat on a range of binding uptake sites expressed on tissues/organs.

Table 116. Summary of Secondary Pharmacology Studies

Study/ Study No.	Findings
Study of GSK1278863A in Various Receptor Binding and Enzyme Assays (CEREP Screen)/ UH2008/ 00031/00	To assess the selectivity of daprodustat in 50 receptor binding, ion channel and transporter assays unrelated to direct PH inhibitory activity, CEREP profiling (in vitro assessment of the effect of drug on 50 targeted receptors/ion channels/enzymes) was performed at the selected concentration of 10 µM daprodustat in order to screen for off-target potential. There was no significant (<20%) inhibition observed in any of the 50 assays, indicating that daprodustat has low potential to cause unintended effects through any of the receptors and enzymes in the panel.

Study/ Study No.	Findings
Effect of GSK1278863A on the Activity of Rat Cyclooxygenase/ CH2008/ 00030/00 & Selectivity against protein kinases/ UH2008/00036/00	To investigate any potential inhibitory activity of daprodustat for the protein kinase enzymes, daprodustat was evaluated against cyclooxygenase 1 and 2 enzymes (COX1 and COX2) and 7 protein kinases [CDK-2/Cyclin A, PLK1, 70 kDa ribosomal protein S6 kinase 1 (p70S6K), SRC1, DYRK3 (also known as YAK3), VEGFR-2 and rat RSK1]. Daprodustat weakly and reversibly inhibited COX1 activity with an IC₅₀ value of 66 µM, which suggests low likelihood of functional inhibition in vivo. Daprodustat had no effect on COX2 activity. It is unlikely that the COX1 inhibition by daprodustat is directly contributing to the adverse effects on stomach, considering the unbound concentration of daprodustat needed to inhibit COX1 by 50% is 764-fold higher than the maximum free drug concentration (C_{max}) at the maximum recommended human dose. The IC₅₀ values for all protein kinases tested were >10µM which suggests that daprodustat selectively inhibits PHs (with lower IC₅₀ values in nM range) vs. the protein kinases.

Source: Reviewer's analysis

Abbreviations: CKD-2, human-cell division kinase-2; COX, cyclooxygenase; DYRK3, dual specificity tyrosine phosphorylation-regulated kinase 3; IC₅₀, half maximal inhibitory concentration; PH, prolyl hydroxylase; PLK1, polo-like kinase 1; RSK1, ribosomal s6 kinase-1; SRC1, steroid receptor coactivator 1; VEGFR-2, vascular endothelial growth factor receptor-2; YAK3, Dual specificity tyrosine phosphorylation-regulated kinase 3

13.1.3. Safety Pharmacology

- Daprodustat had no effect on hERG channel activity (up to 47.66µM), neurobehavioral, and respiratory activity (up to 20 mg/kg).
- Mild cardiovascular effects of daprodustat including small magnitude of QT and QRS prolongation was observed in ex vivo study with rabbit left ventricular wedge at concentrations ≥10µM.
- Mild reversible increase in heart rate was observed in rats after repeated oral dosing of daprodustat for 5 days at doses of ≥30 mg/kg and did not alter the mean arterial pressure.

13.1.4. ADME/PK

Absorption

Pharmacokinetics of daprodustat (coded as GSK1278863A or GSK1278863 in the pharmacokinetic study reports) and its predominant human metabolites were evaluated in mice, rats, dogs, and monkeys. For the species that do not generate the metabolites (mice and rats), the animals were treated with major human metabolites of daprodustat [GSK2391220 (M2), GSK2506104 (M3), and GSK2531401(M13)].

- Single dose studies were conducted with daprodustat and its metabolites in mouse, rat, dog, and monkeys for characterization of pharmacokinetics of daprodustat.

Table 117. Absorption of Daprodustat Studies in Different Species

Species	Compound	Report #
Mouse (CD-1)	Daprodustat	UH2008/00002
	Daprodustat	2012N156583
	M13	
	M13	2011N130731
	M13	2012N137345
	Metabolite cocktail (M2, M3, M13)	2013N174038
Rat (Sprague Dawley)	Daprodustat	UH2008/00002
	Daprodustat	CD2008/01285
	Daprodustat	2013N184197
	M13	2012N145868
Dog (Beagle)	Daprodustat	UH2008/00002
Monkey (Cynomolgus)	Daprodustat	

Source: Reviewer's analysis

- Bioavailability of daprodustat after oral dosing of daprodustat in solution was higher in the mouse and rat and moderate in the dog and monkey.
 - There were no significant differences in oral exposures to different formulations of daprodustat. The crystalline free acid and two crystalline salts (potassium and Tris monohydrate) exhibited similar oral exposures after dosing crystalline free acid as a powder in a capsule and the solution, and similar (within 2-fold) oral exposures were observed for the crystalline free acid, potassium and Tris monohydrate following capsule dosing to dogs.

Table 118. Oral Bioavailability After Oral Dosing of Daprodustat in Solution

Mouse	89% at 1.9 mg/kg
Rat	78% at 1.6 mg/kg
Dog	45% at 3.2 mg/kg (solution form) 15% at 5 mg/kg (crystalline form)
Monkey	34% at 3 mg/kg

Source: Reviewer's analysis

Oral bioavailability of M13 was 0.85% in rats.

- Daprodustat had a low volume of distribution (V_{ss}), 0.3, 0.4, 0.5, and 0.8 L/kg in the mouse, rat, dog, and monkey, respectively. The steady state V_{ss} was less than total body water.
 - Low clearance (<1% in mouse and rat, ~3% in dog, and ~19% in monkey) was observed after IV administration of daprodustat.

Table 119. Mean PK or TK Parameter Estimates for Daprodustat After Single Intravenous Injection of Daprodustat

Species	Dose (mg/kg)	CL (mL/min/kg)	Vss (L/kg)	MRT (h)	t _{1/2} (h)
Mouse – Non-fasted	1.3	0.7	0.3	7.8	-
Rat - Non-fasted	0.6	0.2	0.4	36.7	33.5
Dog - Fasted	1.2	0.9	0.5	8.8	6.6
Monkey - Fasted	1.1	8.4	0.8	1.6	1.9

Note: Report UH2008/00002, m4.2.2.2.

Source: Applicant's submission

Abbreviations: CL, clearance; MRT, mean residence time; PK, pharmacokinetic; t_{1/2}, half-life; TK, toxicokinetic; Vss, volume of distribution at steady-state

- In repeat dose studies, the exposure (based on the area under the concentration-time curve [AUC] and the maximum plasma concentration [C_{max}]) to daprodustat increased with dose increment in a less than dose proportional manner, and a sex difference in exposure was not evident in single or repeat dose studies. There was no accumulation noted with repeated dosing.

Table 120. PK Parameters of Daprodustat and its Major Metabolites (M2, M3, and M13) in Mouse, Rat, Dog, and Monkeys After Repeated-Dose of Daprodustat

Species	Dosing Compound	Duration	Daprodustat Dose (mg/kg/day)	C _{max} (µg/mL)	AUC _{0-t} (µg.h/mL)	T _{max} (h)	
Mouse (CD-1)	Daprodustat	14 days 4 weeks	30	68.9	905	1-4	
			3	14.5	181	2	
			30	50.7	668.5	2-4	
			60	60.5	832.5	4-8	
	M2 ^a M3 ^a M13 ^a	14 days	2	2.67	1.61	0.25	
			2.5	3.03	1.68	0.08	
			1.3	2.04	1.26	0.25	
	Daprodustat + Metabolite cocktail (M2+ M3+ M13 ^a)	13 weeks	6	24.9	369	2	
			20	49	711	1-8	
			6	M2	2.6	1.4	0.25
			M3	3.1	1.6		
			M13	2	1.2		
			20	M2	2.4	1.4	
M3	2.9	1.7					
M13	1.9	1.3					

Species	Dosing Compound	Duration	Daprodustat Dose (mg/kg/day)	C _{max} (µg/mL)	AUC _{0-t} (µg.h/mL)	T _{max} (h)	
Rat (Sprague Dawley)	Daprodustat	5 days	30	112	1980	1	
		14 days	10	54.8	946		
			30	64.2	1241	2	
			60	93.8	1745		
			100	187	2994	1	
	250	147	3184	1.5			
	Daprodustat + Metabolite cocktail (M2+ M3+ M13 ^a)	5 days (GD6-GD10) ^d	60	Daprodustat	126	2300	2
				M2 ^b	1.68	3.33	
				M3 ^b	1.87	3.27	0.25
		60	M13 ^b	1.34	2.79		
			Daprodustat	141	2510	2	
			M2 ^c	6.63	9.61		
		M3 ^c	7.84	9.92	0.25		
		M13 ^c	4.99	8			
		Daprodustat	14 days	20	110	1689	--
	40			109	2022	--	
	4 weeks		2	28.9	449	--	
			7	72.4	1141	--	
			20	103	1813	--	
13 weeks	0.8		11.7	159.5			
	4		52.1	653.5	1		
	20		120	1590			
Monkey (Cynomolgus)	Daprodustat		14 days	10	1.9	9.5	1
		30		3.3	22.8	1	
		100		5.9	76	1-8	
		13 weeks	5	0.8	4.6	0.5	
			20	1.4	14.8	3	
			100	3.3	43.3	2-6	
	5		M2	0.04	0.43	1-4	
		M3	0.01	0.12	1-4		
		M13	0.01	0.07	4		
	20	M2	0.07	0.96	4		
		M3	0.03	0.31	4		
		M13	0.01	0.19	4		
		100	M2	0.16	2.84	4-8	
			M3	0.06	0.94	4-8	
M13			0.03	0.52	8		

Source: Reviewer's analysis

-- not applicable

^a as cocktail of M2, M3, and M13 (2, 2.5, 1.3 mg/kg/day)

^b as cocktail of M2, M3, and M13 at 2.5, 3.2, and 1.8 mg/kg/day, respectively

^c as cocktail of M2, M3, and M13 at 7.5, 9.6, and 5.4 mg/kg/day, respectively

^d In pregnant rats between gestation day (GD) 6-10

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; IV, intravenous; PK, pharmacokinetic; SC, subcutaneous; T_{max}, time to maximum concentration

Distribution

Plasma Protein Binding

- The in vitro plasma protein binding of daprodustat was high ($\geq 98\%$ in dog and monkey and $>99\%$ in mouse, rat, rabbit, and human) (UH2008/00002/00, CD2010/00340).
- In rats, there was variability in the amount of daprodustat measured in plasma and free (pharmacologically active) daprodustat could not be detected in multiple samples likely due to lack of precision in the assay as the % bound approaches 100%.

Table 121. Plasma Protein Binding of Daprodustat in Rats

Nominal Plasma Concentration ($\mu\text{g/mL}$)	Measured Concentration ($\mu\text{g/mL}$)		% Bound in Plasma	Average % Bound
	Plasma	Dialysate (Free Drug)		
1	0.947	0.00107	99.89%	$>99.9\%$
	0.972	NQ*	NC**	
	0.923	NQ*	NC**	
10	10.8	0.00140	99.99%	$\sim 100\%$
	10.4	0.00138	99.99%	
	10.9	0.00142	99.99%	

Source: Reviewer's analysis

Note: % bound in human plasma=99.4%, representing ~ 600 -fold freer drug than in rat plasma, *BLQ: $<0.001 \mu\text{g/mL}$

Abbreviations: **NC, not calculated

- Extremely high protein binding in rats (99.99%), as well as in rabbits ($>99.9\%$), precludes an accurate estimation of free (pharmacologically active) drug in rat (and rabbit) plasma.
- Plasma protein binding for the 3 major circulating human metabolites (M2, M3, and M13) was $<23.5\%$ in human plasma.

Table 122. Plasma Protein Binding of Circulating Human Metabolites in Human Plasma

Nominal Plasma concentration (ng/mL)	% Bound in Plasma							
	GSK2391220 (M2)	GSK2506104 (M3)	GSK2531403 (M3)	GSK2499166 (M4)	GSK2531399 (M5)	GSK2531398 (M6)	GSK2531401 (M13)	GSK2531400 (M13)
10	2.2	13.2	1.0	68.3	74.0	25.9	0.1	0.3
100	13.9	16.9	3.8	86.9	90.8	33.5	2.7	0
250	12.1	17.0	5.9	97.5	97.9	33.7	3.9	0.7
500	10.5	23.5	3.2	97.7	98.3	23.5	0	0.5

Source: Applicant's submission (2012N131897)

Association to RBC and Stability

- Daprodustat exhibited minimal association to red blood cells in humans and animals (UH2008/00002/00).

Table 123. Blood Cell Association of Daprodustat in Mouse, Rat, Dog, Monkey, and Human In Vitro

Species	Cb/Cp	
	1 µg/mL	10 µg/mL
Mouse	0.45 ± 0.10	0.71 ± 0.09
Rat	1.64 ± 0.23	1.15 ± 0.03
Dog	1.05 ± 0.24	0.85 ± 0.16
Monkey	0.84 ± 0.23	0.61 ± 0.01
Human	1.23 ± 0.11	0.75 ± 0.16

Source: Applicant's submission
 Mean ± SD (n=6)
 Abbreviations: Cb/Cp, concentration in blood to concentration in plasma ratio

Blood to Plasma Ratio

- The blood to plasma and blood to liver ratios were tested in Sprague Dawley rats with a single oral dose of 10 mg/kg [¹⁴C]-radiolabeled daprodustat (CD2008/00100). The highest mean concentrations of radioactivity of blood, plasma, and liver (37.4-76.1 and 22.2-30.7 µg equiv/g, respectively) were observed at 1 hour post dose in males and females. Low association of radioactivity with blood cells were noted with blood to plasma ratio 0.505-0.560 and mean liver to blood ratios (0.319-0.825) decreased through 24 hours post dose.
- The blood to plasma ratio was 0.84 µg equiv/g in dogs (intact) (CD2008/00025) and the average blood to plasma concentration ratios were 0.64 µg equiv/g for both intact and BDC monkeys.

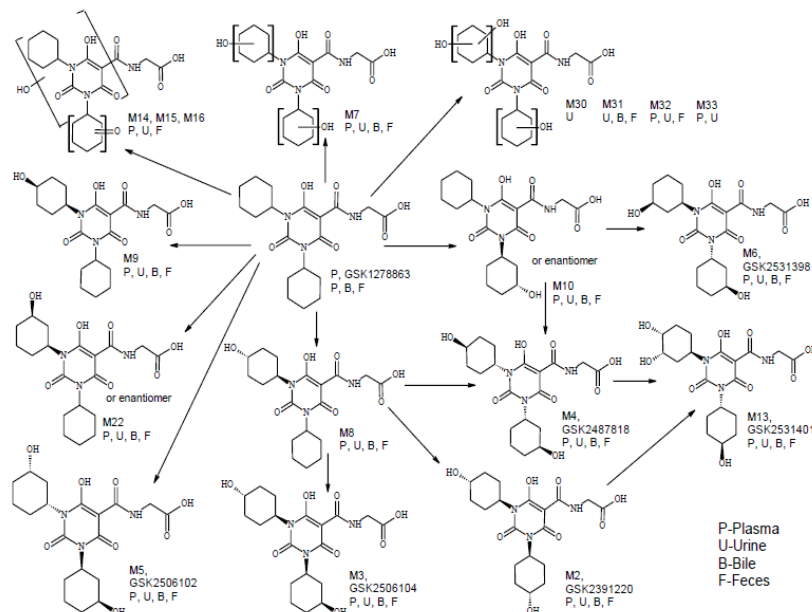
Whole Body Distribution

- In the Long Evans rat, the tissue radioactivity was evaluated with [¹⁴C]-radiolabeled daprodustat (CD2008/00271/00) and the result exhibited that daprodustat was widely distributed into the tissues tested (CNS [Brain, choroid plexus, meninges, peripheral nerve, and spinal cord], glands [adrenal, pineal, pituitary, thyroid, lachrymal, harderian, pancreas, salivary], fat [brown or abdominal], skin [non-pigmented or pigmented], reproductive organs [prostate gland, epididymis, and testis], skeletal muscle [skeletal and heart], respiratory tract [lung and nasal turbinates], GI tract mucosa [esophagus, cecum, small/large intestine, and stomach], and ocular tissue [lens and uveal tract]).
 - The highest radioactivity concentrations at any timepoint were observed in the GI tract. For most non-GI tract tissues, the highest radioactivity concentrations were observed at 1 h, including in the blood, aorta, lung, and meninges.
 - The radioactivity concentrations were low in the brain suggesting little penetration of the blood brain barrier.
 - The only radioactivity detected by Day 7 was low levels observed in peripheral nerves and large intestine contents.
 - Radioactivity concentrations in the uveal tract, pigmented skin, and meninges peak at 1h or 4h declining steadily afterwards until undetectable by Day 7. This finding suggests no irreversible binding to melanin-containing tissues.

Metabolism

- Intrinsic clearance of daprodustat was low in the liver microsomes and hepatocytes of mouse, rat, dog, monkey, and human based on clearance (CL_i) data (UH2008/0018).
- Total of 19 metabolites of daprodustat were identified, of which 14 metabolites were detected in human hepatocytes.

Figure 40. Possible Metabolic Pathway of Daprodustat



Source: Applicant's submission

- Daprodustat is metabolized mainly to products of di- and tri-oxidation via hydroxylation of the cyclohexane rings in humans.
- The major human metabolites [$>10\%$ of DRM] of daprodustat detected in patients include M2 (GSK2391220), M3 (GSK2531403 and GSK2506104), and M13 (GSK2531400 and GSK2531401), of which M3 and M13 exist as enantiomers. The predominant circulating stereoisomers of the major human metabolites M3 and M13 are GSK2506401 and GSK2531401, respectively.
- The 3 major circulating human metabolites (M2, M3, and M13) were present in rabbit and monkey plasma with an animal:human AUC ratio of at least 1X in monkeys (comparing the 50 mg/kg MTD dose in the 39-week toxicity study and human exposure at the 48 mg three times a week (TIW) human dose).
- The metabolites were not detected in mouse, rat, or dog plasma, and were administered SC as a metabolite cocktail to mice and rats in a mouse carcinogenicity study and rat pre- and post-natal development study to achieve at least 1X human exposure.
- The intrinsic clearance values for daprodustat were low (<0.5 - 1.1 mL/min/g liver) in liver microsomes and hepatocytes of all species examined including mouse, rat, dog, monkey, and human. (UH2008/00018/00).

Excretion

Single oral administrations of [¹⁴C] daprodustat to intact male and female rats and dogs and to male monkeys, and to BDC male rats, dogs, and monkeys (CD2008/00100, CD2008/00025, and 2018N355713) showed that feces is the major route of elimination of daprodustat in intact animals. Urinary and biliary recoveries indicated that they are minor routes of elimination and the highest blood radioactivity concentrations were observed at 1 hour after dosing, and the majority of recovered radioactivity was excreted within the first 24 hours.

Table 124. Excretion of Radioactivity After a Single Dose of [¹⁴C] Daprodustat in Rat, Dog and Monkey With/Without Bile Duct Cannulation

Report No. (Module)	Species/ Status	Sex/ Number	Route	Dose (mg/kg)	Percent of Dose Recovered in:			
					Feces	Urine ^a	Bile	Total ^a
CD2008/00100 (m4.2.2.5)	Rat / Intact	15M	Oral	10	86.3	10.5	-	97.6
		15F	Oral	10	87.5	8.74	-	96.9
	Rat / BDC	3M	Oral	10	45.7	6.61	42.0	96.4
CD2008/00025 (m4.2.2.5)	Dog / Intact	3M	Oral	20	86.38	1.79	-	90.16
		3F	Oral	20	81.41	2.03	-	87.95
	Dog / BDC	3M	Oral	20	76.29	0.52	7.77	86.80
2018N355713 (m4.2.2.5)	Monkey / Intact	3M	Oral	10	77.5	13.4	-	90.9
	Monkey / BDC	2M	Oral	10	24.1	15.8	53.0	93.9

Source: Applicant's submission

^a Includes, as appropriate, radioactivity recovered in cage rinses/washes/wipes/debris GI tract and residual carcass.

Abbreviations: -, not determined, BDC, bile duct cannulated, F, female; GI, gastrointestinal; M, male

13.1.5. Toxicology

13.1.5.1. General Toxicology

General toxicology studies of 4- and 13-weeks duration were conducted in Sprague Dawley rats and Beagle Dogs with rats receiving daily oral doses by gavage and dogs by capsule. A 13-week toxicity study was also conducted in the cynomolgus monkey with daily dosing by oral gavage. Toxicities in rats were overall consistent with toxicities observed in the 26-week rat study and were mainly due to exaggerated pharmacology (see Section 13.2). Higher doses were tolerated in the 4- and 13-week rat studies compared to the 26-week rat study. The 100 mg/kg/day dose administered to rats in the 13-week study toxicity resulted in severe toxicities to the heart (valvulopathy, atrial thrombus, valvular thrombus, endocardial hyperplasia, and cardiomyopathy), lung (chronic passive congestion), kidney (infarct, thrombus, cortical tubular pigment and tubular basophilia), brain (focal degeneration) and stomach (glandular erosion/ulceration, submucosal inflammatory cell infiltrate, hemorrhage, and vascular medial hypertrophy). Multifocal erosions, ulcers, hemorrhage, edema, and/or inflammation in the glandular region of the stomach were observed at doses as low as 7 mg/kg/day in the 4-week study. Toxicities in dogs were due to the exaggerated pharmacology of daprodustat related to polycythemia and hyperviscosity of the blood leading to poor tissue perfusion and anoxia. In monkeys, there were fewer organs of toxicity (only bone marrow [erythroid hypercellularity], lung [alveolar hemorrhage], thymus [lymphoid atrophy], and stomach [hemorrhage and effects

on body weight and clinical signs/chemistry]) in the 13-week study compared to the 39-week study, but the toxicities in the 13-week study were more severe at the higher dose tested of 100 mg/kg/day (see Section [13.2](#)).

13.1.5.2. Genetic Toxicology

Table 125. Summary of Genotoxicity Studies With Daprodustat

Study No./ Study Title	Key Study Findings
In vitro	
WD2007/01575/00./ GSK1278863A: Bacterial Mutation Assay (Ames Test) with Salmonella typhimurium and Escherichia coli	<i>Ames test with bacterial strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537) and Escherichia coli WP2 uvrA (pKM101) were conducted at concentrations up to 500 µg/plate daprodustat in the absence or presence of an in vitro metabolic activation system (S9-mix).</i>
GLP compliance: Yes Study is valid: Yes	The 1500 µg/plates were not evaluated due to the marked precipitation and bactericidal activity. Daprodustat was not mutagenic in the bacterial mutation assay, when tested in either the presence or absence of S9-mix
WD2007/01578/00/ GSK1278863A: In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus	<i>L5178Y (TK+/-) mouse lymphoma cells were treated with daprodustat up to 70 µg/mL for 3 hours in the presence or absence of rat liver S9-mix, or up to 30 µg/mL for 24 hours in the absence of S9-mix.</i>
GLP compliance: Yes Study is valid: Yes	Daprodustat was not genotoxic either in the presence or absence of S9-mix. Cytotoxicity was observed at 70 µg/mL daprodustat (+/- S9-mix) with 3 hour-incubation and 30 µg/mL daprodustat (-S9-mix) with 24 hour-incubation.

Study No./ Study Title	Key Study Findings
In vivo	
WD2007/01574/00/ GSK1278863A: Oral Bone Marrow Micronucleus Assay in Rats GLP compliance: Yes Study is valid: Yes	<i>Sprague Dawley male rats (n=3/group) were administered 2 oral doses of 1000 or 2000 mg/kg, approximately 24-hours apart.</i> The mean % proportion of immature polychromatic erythrocytes (PCE) was reduced by ~23% at ≥1000 mg/kg/day daprodustat. In addition, a statistically significant 2-fold increase in micronucleated PCE (MPCE) frequency that exceeded the upper 98% tolerance limit of historical controls was observed in the 2000 mg/kg/day daprodustat group. There was also a slight increase (approximately 1.6-fold) in group mean reticulocyte count observed in treatment groups compared to the control group. The result was positive, but it is not considered to be a direct genotoxic response based on the small magnitude of the response and the mechanism of action of GSK1278863A which can contribute to the positive result.

Table 126. Result of Bone Marrow Micronucleus Assay in Rats

Treatment	Dose (mg/kg/day)	Number of animals	MPCE	%PCE
GSK1278863	100	3M	2.3	32.7
GSK1278863	300	3M	3.7	34.9
GSK1278863	1000	3M	2.3	25.1
GSK1278863	2000	3M	3.3	25.5
GSK1278863	100	3F	2.7	39.5
GSK1278863	300	3F	1.7	39.5
GSK1278863	1000	3F	2.3	28.3
GSK1278863	2000	2F	2.0	23.6

Vehicle 1% Methylcellulose (w/v) in deionized water (400 cps)
 MPCE Number of micronucleated cells observed per 2000 PCE examined (see results for individual animals in appendices for actual numbers of cells examined)
 %PCE Proportion of immature (Polychromatic) Erythrocytes (PCE) in the total population of erythrocytes (PCE+Mature (Normochromatic) Erythrocytes(NCE))
 M = male, F = female

Source: Applicant's submission

Source: Reviewer's analysis

Abbreviations: GLP, good laboratory practice; MPCE, micronuclei in polychromatic erythrocytes; PCE, polychromatic erythrocytes

13.2. Individual Reviews of Studies Submitted With the New Drug Application

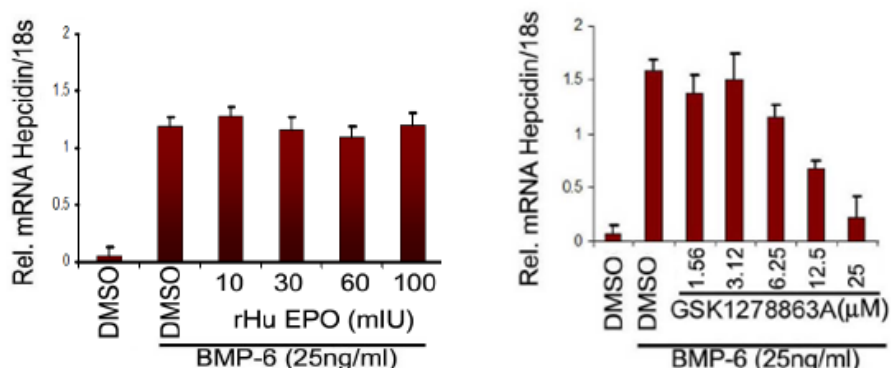
13.2.1. Primary Pharmacology

Table 127. Summary of Primary Pharmacology Studies

Study/ Study No.	Findings
Mechanism of action:	
GSK1278863A: Effects on Hepcidin Expression in Hep3B Cells/ UH2009/00011/00	Reduced hepcidin level is known to increase the utilization of the iron to produce hemoglobin and HIF and hypoxia have been shown to suppress hepatic hepcidin expression in mice. To investigate the effects of daprodustat on the iron regulation protein hepcidin, Hep3B cells were treated with BMP-6 to induce levels of hepcidin for 6 hours. Cells were then treated with daprodustat at 1.56-25 µM or recombinant human EPO at 0-100 mIU/mL for 48 hours. TaqMan RT-PCR was used to measure hepcidin mRNA expression.

Study/ Study No.	Findings
	Hepcidin mRNA level was induced by BMP-6, and this induction was reduced with daprodustat-treatment by 55% at 12.5 μ M (the highest non-toxic concentration) while EPO did not affect the hepcidin induction.
	See Figure 41 . Effect of EPO vs. Daprodustat (GSK1278863A) on Hepcidin mRNA Expression at 48 hrs in BMP-6 Treated Hep3B Cells (excerpted from submission).

Figure 41. Effects of Daprodustat on Hepcidin Expression in Hep3B Cells



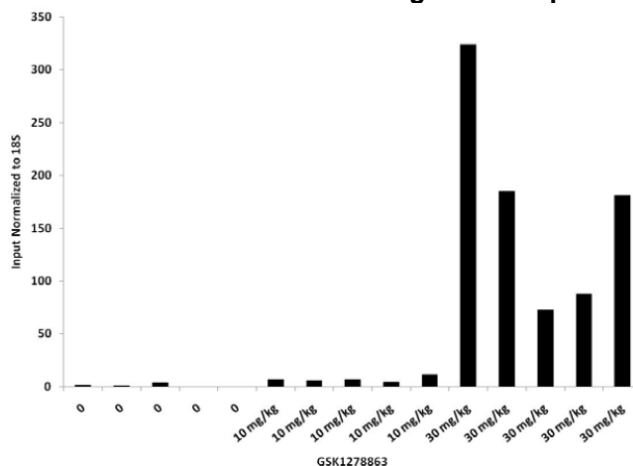
Source: Applicant's submission
Abbreviations: BMP-6, bone morphogenic protein; EPO, erythropoietin

Drug activity related to proposed indication:

Effect of GSK1278863A and its metabolites on EPO Expression in Hep3B Cells/ 2012N134715/00	To evaluate the effects of daprodustat and its metabolites on induction of EPO protein, Hep3B cells were incubated with 3 and 30 μ M daprodustat and its predominant metabolites (M2; M4; M3, M4, M5, M6 (both stereoisomers); M13) for 24 hours. Human EPO levels were measured using the Meso Scale Discovery Assay. The experiment was conducted twice with confluent and sub-confluent cell density.
GSK1278863A: Erythropoietin Response Following a Single Oral Dose in Sprague Dawley Rats/ 2011N126015/00	Daprodustat elevated EPO levels in a dose dependent manner, but EPO induction was not shown by the metabolites tested at 3 or 30 μ M. Male Sprague-Dawley rats (n=3-5/group) were treated with doses of daprodustat at 0 (vehicle), 1, 3, 10 or 30 mg/kg as a single dose or once daily for 3 consecutive days via oral gavage. Plasma EPO and VEGF levels and EPO, VEGF, PDK1, and GLUT-1 mRNA in the liver were measured. EPO and VEGF levels in plasma were not increased with single or 3 daily oral doses of daprodustat. Although, the EPO mRNA expression in liver was dose-dependently increased at $\geq$ 10 mg/kg 6 hours post-dose (up to 324X vs. control). HO-1 mRNA expression (up to 18X vs. control), was also increased at 30 mg/kg, 6 hours post-dose.

Study/ Study No.	Findings

Figure 42. Effect of Daprodustat on EPO mRNA Expression in Liver Individual Rats Treated With Single Oral Daprodustat



Source: Applicant's submission
Abbreviations: EPO, erythropoietin; mRNA, messenger ribonucleic acid

GSK2391220A, GSK2506104A and GSK2531401A (M2, M3 and M13 Metabolites of GSK1278863): Subcutaneous Repeat Dose Pharmacodynamic and Toxicokinetic Study in Female Mice (GlaxoSmithKline Study No. M70590N)/ 2014N208198	To evaluate the effect of major metabolites of daprodustat on erythropoiesis, female ICR mice (n=10/group) were treated subcutaneously once daily for 28 days with a metabolite cocktail consisting of GSK2391220A (M2), GSK2506104A (M3) and GSK2531401A (M13) at 0 (vehicle of 25mM phosphate buffer, pH 7.4), 2.0, 2.5, and 1.3 mg/kg/day, respectively. Metabolite cocktail treatment resulted in increased RBC parameters (RBC, hemoglobin, and hematocrit) by ~10% compared to control on Day 29. The result shows that the exposure is achieved for the metabolites which are comparable to clinical exposure and the metabolite cocktail has pharmacology effect on their own with delayed response compared to the parent compound.

Source: Reviewer's analysis
Abbreviations: BMP-6, bone morphogenic protein; EPO, erythropoietin; GLUT-1, glucose transporter 1; HIF, hypoxia-inducible factor; HO-1, heme oxygenase-1; mRNA, messenger ribonucleic acid; PDK1, pyruvate dehydrogenase kinase 1; RBC, red blood cell; VEGF, vascular endothelial growth factor

13.2.2. Secondary Pharmacology

Table 128. Summary of Secondary Pharmacology Studies

Study/ Study No.	Findings
Effect of GSK1278863A on γ-hemoglobin Expression in Human Primary CD34+Derived Erythroid Progenitor Cells/ 2012N145308/00	This study is to investigate the effect of daprodustat on 1) γ-hemoglobin for the hypothesis that γ-hemoglobin could be induced by PH inhibition and 2) the cell viability. Erythroid progenitor cells (human primary bone marrow CD34+ derived) and KU812 (human leukemia) cells were treated with daprodustat for 3 days and 2 days, respectively. The γ-hemoglobin levels were determined by ELISA assay and KU812 human leukemia cell line viability was assessed by CellTiter-GloTM. No daprodustat-related effects on viability were observed in both CD34+ cells derived erythroid progenitor cells and human leukemia cell line, KU812.

Source: Reviewer's analysis
Abbreviations: ELISA, Enzyme-Linked Immunosorbent Assay

13.2.3. General Toxicology

Title/ Study No.: GSK1278863A: A 26-week Oral (Gavage) Toxicity Study in the Rat/ 2011N126130

Key Study Findings

- Daprodustat (GSK1278663A) produced drug-related mortality at 10 mg/kg/day. Seven main study and four toxicokinetic (TK) males were found dead or euthanized. Due to mortality and toxicity observed at 10 mg/kg/day, all surviving males in this group were pre-terminally euthanized during Week 20 (Day 135/134). Cause of death appeared related to hemorrhage, systemic thrombosis and/or necrosis and thrombosis of various organs including the heart, lung, kidney, and cecum. These findings are consistent with the drug's primary pharmacology and secondary impact on hemodynamics.
- Moderately higher reticulocyte (~70% ↑) and red blood cell counts (29-41% ↑) were observed by week 26/27 in males and females at 10 mg/kg/day. Females could tolerate the elevations (reticulocytes up to 82% ↑, red blood cell counts 39% ↑), but males could not.
- Systemic thrombosis, hemorrhage, and necrosis were observed at 10 mg/kg/day and in the heart and lung of males at 10 mg/kg/day.
- The no observed adverse effect level (NOAEL) was determined at 4 mg/kg/day based on the mortality and drug-related toxic effects observed at 10 mg/kg/day.

Table 129. Methods of the 26-Week Oral (Gavage) Toxicity Study in the Rat/ 2011N126130

Study Feature	Method Details
GLP compliance:	Yes
Dose and frequency of dosing:	0 (vehicle control), 0.8, 4, and 10 mg/kg/day daprodustat, 10 mL/kg, once daily
Route of administration:	Oral gavage
Formulation/vehicle:	Suspension/1% (w/v) Methylcellulose (400 cps) in ultra pure water
Species/strain:	Rat/Crl:CD(SD)
Number/sex/group:	12/sex/group
Age:	7 weeks
Satellite groups/unique design:	Toxicokinetics: 3/sex/group (0, 0.8, and 4 mg/kg daprodustat); 9/sex/group (10 mg/kg/day)
Deviation from study protocol affecting interpretation of results:	Due to the reduced survival rate observed in males given 10 mg/kg/day daprodustat (Group 4 males), all surviving Group 4 males were pre-terminally euthanized during Week 20 (Day 135/134).

Source: Reviewer's analysis

Abbreviations: GLP, good laboratory practice; SD, standard deviation

Table 130. Observations and Results of the 26-Week Oral (Gavage) Toxicity Study in the Rat/ 2011N126130

Parameter	Major Findings
Mortality	The following is the summary of the animals of the early mortality.

Table 131. Mortality of Rats During the 26-Week Treatment of Daprodustat

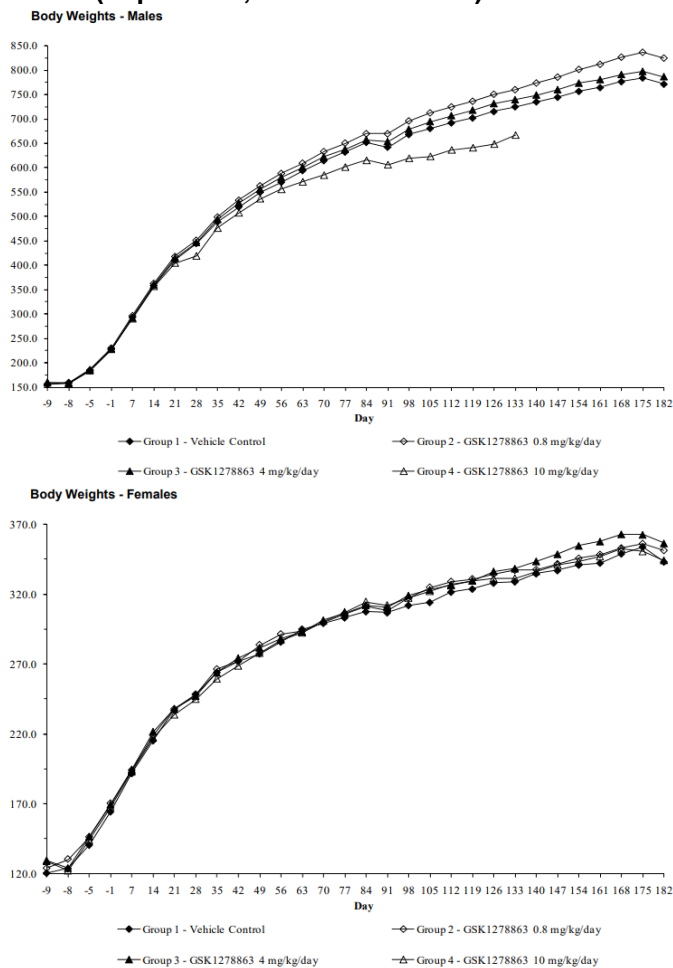
Daprodustat Animal (mg/kg/day) #	Sex	Found Dead or Euthanized	Day	Potential Cause of Mortality or Moribundity/ Macroscopic Findings	
Main study					
0.8	2509	F	Found Dead	22	Undetermined, death possibly associated with pathology blood collection
10	4002	M	Found Dead	135	Kidney, necrosis, unilateral, grade 3
	4003	M	Found Dead	105	Kidney, necrosis, grade 4
	4005	M	Found Dead	131	Heart, thrombosis, atrial/ventricular, grade 4
	4007	M	Euthanized	130	Kidney, necrosis, grade 4
	4009	M	Found Dead	59	Systemic, thrombosis
	4010	M	Found Dead	104	Kidney, necrosis, grade 3
	4011	M	Found Dead	128	Heart, thrombosis, atrial/ ventricular, grade 3
TK animals					
10	4013	M	Euthanized	99	Cecum, necrosis, grade 3
	4019	M	Found Dead	55	Systemic, thrombosis
	4020	M	Euthanized	87	Undetermined, dark areas in whole body, dilatation of heart (ventricle left, coronary)
	4021	M	Found Dead	59	Lung, thrombosis, grade 4

Source: Reviewer's analysis
Abbreviations: F, female; M, male; TK, toxicokinetic

Clinical signs	The primary daprodustat-related clinical sign was skin reddening of the extremities (forelimb, hindlimb, pinna, tail, and/or muzzle). HD animals started presenting skin reddening as early as Week 6 in males and Week 7 in females and most of the HD animals presented skin reddening by Week 12. Skin reddening also occurred in males given 4 or 0.8 mg/kg/day, starting at Weeks 10 and 11, respectively.

Parameter	Major Findings
Body weights and Food Consumption	HD males exhibited decreased mean body weight gain (33% ↓ compared to controls) from approximately Week 9 until their early termination in Week 20 with corresponding food consumption decrease of 19%↓.

Figure 43. Body Weight Changes of Rats With Daprodustat Treatment for 26 Weeks (Top: Males, Bottom: Females)



Source: Applicant's submission

Ophthalmoscopy	Congestion of retinal vessels were observed in HD females (5/12) in Week 25 and the ophthalmic evaluation was not conducted in HD males due to early termination.
Hematology	- Daprodustat-related findings includes increase of red blood cell (RBC) mass (RBCs, hemoglobin, and hematocrit), reticulocytes, and neutrophils and decrease of platelet number. - The increase of RBC parameters peaks at Week 13 and the increase remains through Week 26/27. The hematology of HD males was not evaluated at the end of dosing due to premature termination. - HD females had elevated hematocrit but no mortality occurred which suggests females have higher tolerance to daprodustat-related effect on hemodynamics. - Platelet number decreased at ≥MD which is associated with the mechanism of action of daprodustat. Overexpression of HIF1-α is known to result in low platelet number

Parameter	Major Findings

Table 132. Hematology Parameters Percent Change (vs. Controls) in Rats After Daprodustat-Treatment for 26 Weeks

Parameter	Week	Daprodustat (mg/kg/day)					
		Male			Female		
		0.8	4	10	0.8	4	10
RBC (10 ⁶ /μL)	4	+4	+2	+16 ^c	-2	-1	+17 ^c
	13	+5	+7	+41 ^c	-2	+4	+39 ^c
	26/27	+6	+10 ^c	-	+2	+3	+29 ^c
Hemoglobin	4	+2	+5 ^a	+26 ^c	-1	-1	+20 ^c
	13	+1	+8 ^b	+48 ^c	-2	+2	+46 ^c
	26/27	+4	+15 ^c	-	+1	+2	+34 ^c
Hematocrit %	4	+3	+3	+24 ^c	-1	-1	+23 ^c
	13	+2	+6	+43 ^c	-2	+1	+43 ^c
	26/27	+5	+14 ^c	-	+2	+2	+33 ^c
RDW %	4	-2	-5 ^a	+8 ^c	0	0	+12 ^c
	13	-2	-5	+7 ^a	-1	-2	+7 ^c
	26/27	0	-6	-	-1	+2	+9 ^b
Reticulocyte (10 ⁶ /μL)	4	-5	-7	+48 ^b	-8	-14	+82 ^b
	13	-5	+2	+72 ^c	-6	-16	+48 ^c
	26/27	+6	-2	-	-6	+11	+71 ^b
Platelet	4	-1	-1	-17	+9	-4	-12
	13	-1	-11	-48 ^c	0	-14	-23 ^a
	26/27	-5	-17 ^b	-	+2	-12	-29 ^c
Neutrophil	4	+41	-8	+62	-8	-18	-13
	13	+11	0	+59 ^a	-4	+9	+32
	26/27	+22	-5	-	+15	+16	+97

Source: Reviewer's analysis

^a p≤0.05

^b p≤0.01

^c p≤0.001

+/- increase/decrease

Abbreviations: RBC, red blood cells, RDW, red blood cell distribution width

Coagulation APTT was increased without affecting PT in males and PT was increased in HD females, associated with decreased platelets.

Table 133. Coagulation Parameters Percent Change (vs. Controls) After Daprodustat-Treatment for 26 Weeks

Coagulation Parameter	Week	Daprodustat (mg/kg/day)					
		Male			Female		
		0.8	4	10	0.8	4	10
APTT	26/27	+11	+15	-	+8	+1	+4
PT	26/27	+2	+4	-	+2	+2	+9

Source: Reviewer's analysis

- = coagulation was not assessed in males with early mortality

Abbreviations: APTT, activated partial thromboplastin time; PT, prothrombin time

Parameter	Major Findings
Serum cardiac troponin	At doses of 0.8 and 4 mg/kg/day, increased serum troponin (cTnl) values in individual treated males at Week 13 were between 3.7- and 5.8-fold, relative to the highest concurrent control value of 0.051 µg/L. On Week 26, serum cTnl values in males ranged from 2.3- to 14-fold, relative to the highest concurrent control value of 0.026 µg/L. The increases in cTnl were not dose-related and all rats with serum cTnl values greater than 0.028 µg/L had microscopic evidence of cardiomyopathy, a background finding in rats.
ECG	Not conducted.
Clinical chemistry	Daprodustat-related effects include dose-dependent increase of triglycerides, iron and total iron binding capacity and decrease of cholesterol. • During week 26/27, one female (Animal No. 4504) at 10 mg/kg/day had elevated ALT (613 U/L) and AST (501 U/L), a 6- and 3-fold increase compared to the control mean, respectively, that were associated with microscopic findings of decreased liver weight and extramedullary hematopoiesis. • Potassium (K) concentrations were decreased in males and females at 10 mg/kg/day during Week 4 (down to 0.81X) and at ≥ 4 mg/kg/day (down to 0.86X) during Week 13, which seems to be a daprodustat effect on HIF.

Table 134. Clinical Chemistry Parameters Percent Change (vs. Controls) With Daprodustat-Treatment for 26 Weeks

Parameter	Week	Daprodustat (mg/kg/day)					
		Male			Female		
		0.8	4	10	0.8	4	10
Cholesterol	4	+5	-9	-35 ^c	-6	-4	-22
	13	-7	-40 ^c	-43 ^c	-4	-8	-27 ^a
	26/27	-13	-43	--	+1	-20	-31 ^b
Triglycerides	4	+27	+52	+114 ^c	+10	+54 ^a	+69 ^a
	13	+38 ^a	+47 ^a	+203 ^c	-14	+48	+73
	26/27	+29	+10	--	-10	+17	+64 ^a
Iron	4	-11	+24	26	-3	+22	+25
	13	-2	+36 ^c	84 ^c	+15	+42 ^b	-2
	26/27	-8	+34	--	+24	+37	+10
TIBC	4	+8	+28 ^c	+48 ^c	+9	+23 ^c	+40 ^c
	13	+18 ^c	+26 ^c	+33 ^a	+6	+21 ^c	+22 ^c
	26/27	+25 ^a	+28 ^c	--	+10	+21 ^b	+38 ^c

Source: Reviewer's analysis

^a p≤0.05

^b p≤0.01

^c p≤0.001

Control group= mean values, Daprodustat-treated groups= % difference vs. control

Abbreviations: TIBC, total iron binding capacity

Urinalysis	Significant increases (59%) in creatine and glucose (102%) were observed in the urine of females in the 10 mg/kg/day group (compared to control females) during week 4, which normalized thereafter.
Gross pathology	Daprodustat-related findings include dark discoloration, clots, and/or dark fluid in the abdomen, adrenal glands, digestive content, eye, GI tract, kidneys, liver, lymph nodes, pancreas, prostate (males), skeletal muscle, thorax, trachea, urinary bladder, thymus and/or whole body, mostly observed at HD. These findings correspond with histopathological findings of congestion/hemorrhage in multiple organs. HD males presented dilatation of blood vessels/heart chambers (1/12) and spongy and uncollapsed lungs (6/12) which corresponds to microscopic finding of congestion. In addition, splenomegaly (4/12, corresponds to extramedullary hematopoiesis),

Parameter	Major Findings
	enlarged adrenal glands (2/12, corresponds to cortical hypertrophy), and pale/depressed area of kidney (5/12, corresponds to necrosis/infarction) were observed in HD males. Dark/depressed areas in the stomach was noted in males (HD 6/12) and females (HD 2/12), which corresponds to erosion of the glandular stomach or hemorrhages.

The majority of findings occurred in males with early mortality.

Organ weights	Daprodustat-related organ weight changes of liver and spleen (absolute and relative to body weight) were noted at MD (note: HD males were prematurely terminated and no organ weight data available). A decrease in liver weights in both sexes was observed, which is aligned with the microscopic finding of atrophy at HD males. Spleen weights decreased in LD and MD males but spleen weight increase was noted in HD females.

Table 135. Organ Weights Relative to Body Weight Percent Change (vs. Controls) in Rats After Daprodustat Treatment for 26 Weeks

Organ Weights Relative to Body Weight (%)	Daprodustat (mg/kg/day)					
	Male			Female		
	0.8	4	10	0.8	4	10
Liver	-10	-25	-	+1	-12	-15
Spleen	-7	-18	-	+2	-5	+26

Source: Reviewer's analysis

Histopathology Adequate battery: Yes	Most of the histological findings related to daprodustat-treatment were noted in HD males (main study and TK) with early mortality. Vascular inflammation and thrombosis with minimal to marked level of severity were observed with hemorrhages, edema, congestion, and/or necrosis/infarction in several organs including adrenals, bone marrow, brain, heart, kidneys, liver, lung, lymph nodes, stomach, duodenum, jejunum, pancreas, testes, eye, and thymus of males. The observed microscopic findings are associated with either direct hematopoietic effect or secondary effect of daprodustat on hemodynamics.

Table 136. Microscopic Findings in Rats After Daprodustat-Treatment for 26 Weeks

Treatment-Related Microscopic Findings Main Study Animals	Dose (mg/kg)	Number of animals examined	Organ	Finding	Severity	Number of Animals Affected									
						Males					Females				
						Main Study		TK	Main Study		Main Study		Main Study		TK
						0	0.8 ^c	4	10	10	0	0.8 ^c	4	10	
						0/12	0/12	0/12	7 ^a /5 ^b	3 ^a	0/12	1 ^a /11	0/12 ^d	0/12 ^e	
Abdomen	Necrosis	Slight				0/1									
Bone marrow	Hyperplasia: erythroid	Min Slight						2 ^a /2						0/1	
								4 ^a /1		3 ^a					
Brain	Thrombosis	Min						1 ^a /0		1 ^a		0/1			
Heart	Hemorrhage	Min Slight				0/1		2 ^a /0							
						0/1		2 ^a /0		2 ^a					
	Inflammation: subacute	Slight				4 ^a /1									
	Degeneration valvular	Min				0/1									
	Thrombosis: atrial/ventricular	Min				1 ^a /1									
Kidney	Thrombosis	Min						1 ^a /0		1 ^a		0/1			
	Deposits: pigment	Slight				0/1									
	Necrosis	Mod						2 ^a /1		1 ^a		0/1			
	Hemorrhage	Slight				1 ^a /0									

Parameter	Major Findings									
Liver	Hyaline droplet: tubular	Min	0/1	1 ^a /2		1 ^a				
	Hematopoiesis: extramedullary	Min		0/1				0/3		
Lung	Hemorrhage	Min	0/1	2 ^a /0		1 ^a				
	Inflammation: vascular	Slight		0/1						
	Macrophage accumulation	Mod		0/1						
Lymph node	Erythrocytosis/ erythrophagocytosis	Min		1 ^a /1						
LN, mesenteric	Erythrocytosis/ erythrophagocytosis	Min	0/1	1 ^a /1				0/1		
		Slight		3 ^a /1						
Muscle skeletal	Regeneration: myofiber	Min		0/2						
	Hemorrhage	Mod						0/1		
Pancreas	Atrophy: acinar cell	Min				1		0/2		
Spleen	Hematopoiesis/ extramedullary	Min	0/1	0/1	0/1	1 ^a /2				
		Slight				2 ^a /1				
	Fibrosis: capsular	Mod				1 ^a /0		2 ^a		
	Hemorrhage	Min				0/1				
Stomach	Thrombosis	Min				1 ^a				
		Slight				1 ^a				
	Erosion/ ulceration: glandular mucosa	Min				1 ^a /0				
		Slight				0/1		0/2		
		Mod				1 ^a /0				
	Hemorrhage	Min	0/1			0/2				0/1
	Slight				3 ^a /1		2 ^a			
	Mod				1 ^a /1					
	Inflammation: vascular	Min				0/1				
		Slight				1 ^a				
Thymus	Hemorrhage	Min	0/7	0/2	0/3	0/3	1 ^a	0/5	1a/0	0/4
		Slight				2 ^a /0				0/1
		Mod				5 ^a /0				0/1
Urinary bladder	Inflammation: chronic	Min								0/1
		Slight	0/1							0/1
		Mod				1 ^a /0				0/1
Whole body	Congestion	Min		0/3		0/2				
		Slight				5 ^a /3		2 ^a		

Source: Reviewer's analysis

Number of animals examined and affected: Early deaths/Terminal necropsy (Note: only 3 out of 4 early deaths in TK animals were examined microscopically.)

^a Early deaths

^b Scheduled necropsies, however, they occurred six weeks prior to completion of the study. Only main study animals were examined macroscopically or microscopically.

^c The list of tissues identified as having test article-related changes in Group 3 (adrenals, bone marrow, brain, heart, kidney, liver, lymph nodes [mandibular and mesenteric], spleen, stomach, and thymus as well as blood vessels in some of the preceding organs and in the cecum, colon, duodenum, epididymis, eyes, ileum, jejunum, lung, pancreas, seminal vesicles and testes) were examined in Group 2.

^d One female in Group 2 was also examined microscopically for the following organs: aorta, bone (femur and sternum), cavity nasal/sinuses, cervix, mammary gland, nerves (optic and sciatic), ovary, parathyroid gland, salivary glands, skin, spinal cord lumbar, thyroid, tongue, trachea, urinary bladder, uterus, and vagina.

^e The subcutaneous tissue of only one high dose female was examined

Abbreviations: Min, minimal; Mod, moderate; TK, toxicokinetic

Toxicokinetics	The C _{max} of GSK1278663 increased with an increase in dose, and T _{max} ranged from 0.5-2 hours after dosing. The increase in exposure (AUC ₀₋₂₄) to GSK1268663 was approximately dose proportional. There was higher exposure in females than males

Parameter	Major Findings
	at doses ≥ 4 mg/kg/day, but differences were less than 2-fold, and accumulation (~30%) was noted in females.

Table 137. Toxicokinetic Parameters in Rats After GSK128863A-Treatment for 26 Weeks

Study Type: Repeat Dose Toxicity				GSK Reference No.: G10276					
Daily Dose (mg/kg/day)		Male				Female			
		0	0.8	4	10	0	0.8	4	10
Numbers of Animals:									
Main		12	12	12	12	12	12	12	12
Toxicokinetic ^a		3	3	3	9	3	3	3	9
AUC ₀₋₄ (µg.h/mL)	Week 4	NC	173	639	941	NC	179	707	1034
	Week 13	NC	197	636	807	NC	258	857	1233
	Week 26	NC	208	607	NA ^b	NC	259	964	1377
C _{max} (µg/mL)	Week 4	NA	10.0	37.7	49.5	NA	11.0	40.9	58.5
	Week 13	NA	13.1	41.0	45.1	NA	15.3	50.8	71.4
	Week 26	NA	13.3	38.4	NA ^b	NA	15.7	55.3	75.0

Source: Applicant's submission

^a Results are reported as mean.

^b Week 26 parameter values are from female animals only. Surviving males were euthanized during Week 20 due to poor survival.

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration

Source: Reviewer's analysis

Abbreviations: ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; GI, gastrointestinal; HD, high dose; HIF, hypoxia-inducible factor; LD, low dose; MD, mid dose; PT, prothrombin time; BC, red blood cell; TK, toxicokinetic

Title/ Study No.: GSK1278863A: A 39-week Oral (Gavage) Toxicity Study in the Cynomolgus Monkey with a 6-week Recovery Period /2011N126131

Key Study Findings

- One mortality (unscheduled termination due to moribundity) occurred at LD but the cause of death is not determined.
- Lower body weight gain was noted in all daprodustat-treated groups, but the findings resolved after the recovery period.
- Daprodustat-related increase of RBC mass, reticulocytes, and serum iron parameters at ≥ 10 mg/kg/day was observed, related to the direct pharmacological effect of daprodustat, and corresponding evidence of erythropoiesis were noted including congestion in multiple organs (kidneys, liver, brain, and eye) or whole body, skin reddening, and bone marrow erythroid hypercellularity.
- Other drug-related microscopic findings include gastric hemorrhage, erosions and/or ulcerations in the stomach, pigment deposits in kidney, mononuclear cell infiltration of choroid plexus, and degeneration of skeletal muscle, and these findings persisted after the recovery period.
- One high dose (HD) female presented partly closed right eye (ptosis of the right upper eyelid) and anisocoria (mydriasis of the right pupil) and congestion of the retinal vessels at $\geq$ mid dose and optic disc hyperemia at HD were observed at the end of dosing period which persisted with decreased incidence/severity at the end of recovery period.
- The NOAEL is determined at 3 mg/kg/day based on the microscopic findings in choroid plexus, kidney, and stomach at ≥ 10 mg/kg/day.

Table 138. Methods of the 39-Week Oral (Gavage) Toxicity Study in the Cynomolgus Monkey With a 6-Week Recovery Period /2011N126131

Study Feature	Method Details
GLP compliance:	Yes
Dose and frequency of dosing:	0 (vehicle control), 3, 10, and 50 mg/kg/day daprodustat, 4 mL/kg, once daily
Route of administration:	Oral gavage
Formulation/vehicle:	Suspension/1% (w/v) Methylcellulose (400 cps) in ultra pure water
Species/strain:	Monkey/Cynomolgus, <i>Macaca fascicularis</i> (Chinese origin)
Number/sex/group:	4/sex/group for main study, 2/sex for 0 and 50 mg/kg/day group for recovery study
Age:	2.7-3.9 years old at onset of dosing
Satellite groups/unique design:	None
Deviation from study protocol affecting interpretation of results:	None of the deviations were considered to have impacted the overall integrity of the study or the interpretation of the study results and conclusions.

Source: Reviewer's analysis
Abbreviations: GLP, good laboratory practice

Table 139. Observations and Results of the 39-Week Oral (Gavage) Toxicity Study in the Cynomolgus Monkey With a 6-Week Recovery Period /2011N126131

Parameter	Major Findings
Mortality	One LD male was terminated due to moribundity on Day 262 (Week 38). Diarrhea and liquid feces, significant body weight loss and changes in clinical chemistry (decreased neutrophil count, decreased urea, albumin, total protein, total calcium, phosphorus and cholesterol concentrations, increased potassium concentrations) were consistent with protein loss and poor clinical condition. The cause of death could not be determined at the time of necropsy. This animal had bone marrow hypocellularity, thickening of the wall of the small intestine with moderate muscular hypertrophy/hyperplasia in the jejunum, mild multifocal acinar cell atrophy in the pancreas, marked thymus lymphoid atrophy, and minimal fibrosis of the lung and spleen. TK analysis shows that this animal was not exposed to higher levels of test article compared to other animals in the group. Metabolite levels were not measured in this animal. This mortality appears unrelated to the test article (lack of dose response and comparable findings to animals at higher doses).
Clinical signs	Daprodustat related findings were noted: • Skin reddening, primarily on the cranium, with dose dependence was observed in all monkeys with higher incidence. Other affected locations include but are not limited to: gums and/or mouth, fore limbs, hind limbs, abdominal area and ventral thoracic and/or cervical region. Scabby skin was more frequently observed in higher number of HD males. • Dose-related changes in feces (soft and/or liquid, color change to green/pale) was noted and liquid vagina discharge was observed in females treated with daprodustat. • Red skin at the right periorbital area and right eyelid, partly closed eyes (right eye, ptosis and mydriasis) was observed in one HD female (No. 453) between dosing Days 138-149. Eye position, movements and pupillary light reflexes were normal. These observations led to the hypothesis of an oculomotor nerve palsy supported by the histological brain findings (localized inflammation [superficial brainstem/nerve root] and concurrent changes including edema and hemorrhage). Findings were fully or partially reversible.

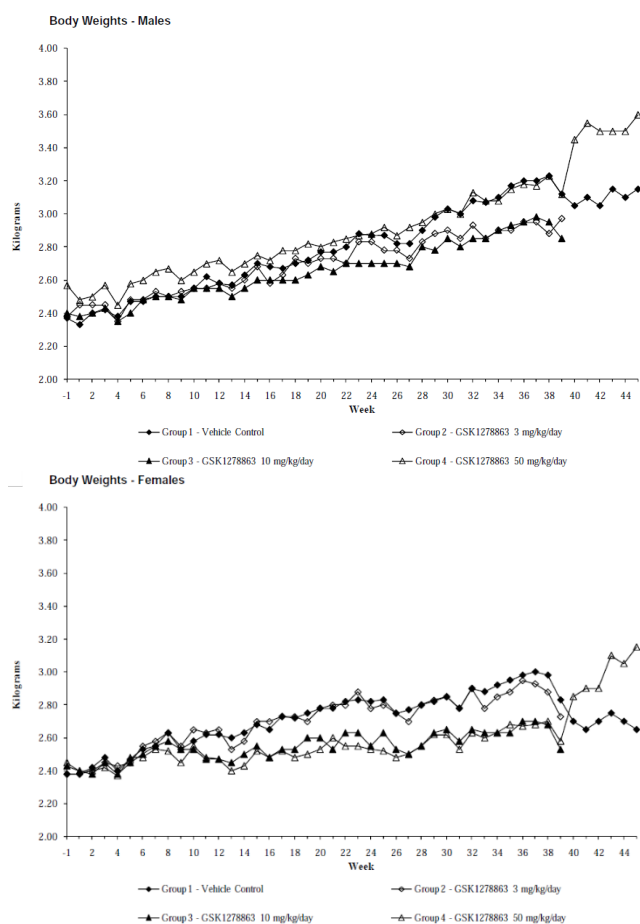
Parameter	Major Findings
Body weights	Daprodustat-related lower body weight gain was observed in daprodustat-treated groups. The drug-effect on the body weight gain resolved after the 6-week recovery period. After this recovery period, the body weight of HD males was comparable to the controls and HD females recovered from minor differences in body weight gain by the end of dosing

Table 140. Body Weight Gain Percent Changes (vs. Controls) in Monkeys With Daprodustat-Treatment for 39 Weeks Followed by 6-Week Recovery Period

Daprodustat (mg/kg/day)	Male			Female		
	3	10	50	3	10	50
Dosing period weeks 1-39	-7%	-40%	-27%	-22%	-78%	-71%

Source: Reviewer's analysis
+/- Increase/decrease

Figure 44. Body Weight Changes of Monkeys With Daprodustat-Treatment for 39 Weeks Followed by 6-Week Recovery Period (Top: Males, Bottom: Females)



Source: Applicant's submission

Food consumption	No-drug related findings were noted.
Ophthalmoscopy	Drug-related ophthalmic findings were noted at the end of dosing period: Congestion of the retinal vessels were observed at MD (4/4 males, 3/4 females) and HD (6/6 males, 6/6 females), and optic disc hyperemia was noted at HD (5/6 males, 5/6 females). The findings persisted with reduced severity after the recovery period.

Parameter	Major Findings				
Hematology	For the following RBC parameters, the levels start increasing at Week 4, peak at 26 weeks and stabilize until the end of dosing. The changes showed trend of recovery at the end of recovery period.				
Table 141. Hematology Parameters in Monkeys With Daprodustat-Treatment for 39 Weeks Followed by 6-Week Recovery Period					
Parameters	Dose	Male		Female	
		Week 4	Week 39	Week 4	Week 39
RBC (10 ¹² /L)	0	5.687	5.745	5.758	5.71
	3	+11	+16	+2	+3
	10	+29	+52	+17	+40
	50	+40	+77	+35	+74
Hemoglobin (g/L)	0	135	141.7	135.2	137.2
	3	+9	+15	+5	+8
	10	+30	+56	+22	+47
	50	+30	+62	+36	+77
Hematocrit (%)	0	40.77	42.35	41.22	41.62
	3	+11	+15	+8	+10
	10	+36	+55	+26	+44
	50	+42	+71	+45	+77
RBC distribution width (%)	0	12.53	12.33	12.37	11.87
	3	0	+2	-1	0
	10	+10	+4	+7	+6
	50	+22	+27	+20	+19
Reticulocyte (10 ⁹ /L)	0	52.02	59.77	65.45	66.87
	3	+29	+35	-16	-18
	10	+333	+122	+156	+79
	50	+562	+226	+436	+179
Platelet (10 ⁹ /L)	0	401.3	456.5	456.2	482.2
	3	-1	-10	-3	-16
	10	+5	-29	-15	-31
	50	+26	-39	-2	-43
Monocyte (10 ⁹ /L)	0	0.24	0.385	0.325	0.44
	3	+21	-11	+18	+13
	10	+25	+1	-33	-11
	50	+78	+130	-5	+43
Coagulation	Dose-dependent increase in PT and APTT were observed at the end of dosing period which resolved after the recovery period. These changes in coagulation factors are indirectly drug-related, which may be linked to the decreased platelet counts, high hematocrit and over-citrated plasma observed in daprodustat-treated groups.				

Source: Reviewer's analysis

+/- increase/decrease

-- not available as these groups were not assessed during recovery

Abbreviations: RBC, red blood cell

Parameter	Major Findings

Table 142. Coagulation Parameters Percent Change (vs. Controls) in Monkeys After Daprodustat-Treatment for 39 Weeks

Coagulation Parameter	Week	Daprodustat (mg/kg/day)							
		Male				Female			
		0 (sec)	LD	MD	HD	0 (sec)	LD	MD	HD
APTT (% change)	39	22.4	-8	+14	+40	23.37	-2	+2	+23
PT (% change)	39	10.98	+6	+23	+43	11.33	+4	+13	+33

Source: Reviewer's analysis

+/- increase/decrease

Abbreviations: APTT, activated partial thromboplastin time; HD, low dose; LD, low dose; MD, mid dose; PT, prothrombin time

ECG	No daprodustat-related effects were observed. Cardiac troponin levels were measured in serum with no test article-related findings.

Urinalysis	No daprodustat-related changes were observed

Clinical Chemistry	- Decreased total iron concentration at Weeks 4 and 13 at HD with increase of unsaturated and/or total iron binding capacity were observed which is likely from the pharmacological effects of daprodustat. Findings were reversible and during the recovery period total iron concentration was increased at the HD compared to controls. - Decreased glucose level at HD throughout the dosing period is likely from the decreased food consumption and stomach lesion (hemorrhage and erosion), which showed recovery (or trend of recovery) at the end of recovery period. - Dose related increase of ALP and total bilirubin were observed throughout the dosing period at $\geq$ MD and showed trend of recovery after the recovery period. This finding may reflect liver damage and corresponds to the findings of slight increase of liver weight, fibrosis and dark discoloration at HD. - Creatinine concentrations were increased which corresponds to the macro/microscopic findings in kidney, and it resolved after the recovery period.

Table 143. Clinical Chemistry Parameters of Monkeys With Daprodustat-Treatment for 39 Weeks Followed by 6-Week Recovery Period

Parameters	mg/kg/day	Male		Female	
		Week 39	Week 45	Week 39	Week 45
Alkaline phosphatase (U/L)	0	690	501.5	247.7	218.5
	3	-9	--	+36	--
	10	+21	--	+19	--
	50	+71	+59	+60	+54
Total bilirubin (μ mol/L)	0	3.2	4	3	4
	3	+3	--	-23	--
	10	+41	--	+77	--
	50	+203	+88	+183	+63
Creatinine (μ mol/L)	0	62	71	59	57.5
	3	-8	--	-7	--
	10	10	--	0	--
	50	18	-18	12	-14
Glucose (mmol/L)	0	3.25	3	3.35	3.25
	3	+2	--	+1	--
	10	-10	--	-4	--
	50	-23	-17	-14	0
Total iron (ng/dl)	0	149.8	160	141.7	144.5

Parameter	Major Findings				
	3	+4	--	+38	--
	10	+17	--	+21	--
	50	-14	+39	+25	+82
Total iron binding capacity (ng/dl)	0	361.2	370	337.3	327.5
	3	+1	--	+6	--
	10	+15	--	+21	--
	50	+4	-37	+54	NC
Unsaturated iron binding capacity (ng/dl)	0	211.3	210	195.7	183
	3	-1	--	-16	--
	10	+14	--	+21	--
	50	+23	-93	+75	NC

Source: Reviewer's analysis
+/- increase/decrease
-- not available
Abbreviations: NC, not calculable

Gross pathology Dark area and foci in stomach at $\geq$ MD and dark discoloration and pale foci in liver at HD were observed, which are likely daprodustat-related and correlated microscopically with mucosal hemorrhage. Dark areas were present in the lungs of 2 HD males, correlating histologically with minimal hemorrhage. These findings resolved at the end of recovery period.

Table 144. Gross Pathology Findings in Monkeys After the Daprodustat-Treatment for 39 Weeks

End of Dosing		Daprodustat (mg/kg/day)							
		Male (n=4)				Female (n=4)			
Organs	Findings	0	3	15	50	0	3	15	50
Liver	Discoloration dark	--	--	--	--	--	--	--	1
	Foci pale	--	--	--	1	--	--	--	1
Stomach	Area dark	--	--	1	2	--	--	1	--
	Foci dark	--	--	--	--	--	--	--	1

Source: Reviewer's analysis
-- no findings observed

Organ weights Daprodustat-related changes in organ weights (% relative to body weight) were noted. Increased organ weights of heart up to 21% and 19%, kidney up to 17% and 15%, liver up to 20-24% were noted at HD in males and females, respectively. The affected organs are associated with daprodustat-related hematopoiesis. Recovery or trend of recovery were noted at the end of recovery period.

Histopathology Adequate battery: Yes Daprodustat-related findings were noted in multiple tissues/organs including brain, bone marrow, kidneys, stomach, and the whole body. Majority of findings are likely related to pharmacological effect of daprodustat increasing hematocrit. Increased hypercellularity of bone marrow and iron deposition in kidney are associated with direct pharmacological effect of daprodustat. Findings in the brain (mononuclear cell infiltration in the choroid plexus and subacute inflammation, edema, and hemorrhage in the superficial brainstem) and stomach (hemorrhage and ulceration of glandular mucosa) are considered daprodustat-related adverse findings which showed trend of recovery but persisted after the recovery period.

Table 145. Histopathology Findings in Monkeys After Daprodustat-Treatment for 39 Weeks Followed by 6-Week Recovery Period

End of Dosing (N=4)		Male				Female			
Daprodustat	Daily Dose (mg/kg/day)	0	3	10	50	0	3	10	50
Bone marrow: Hypercellularity:	Erythroid								
	Minimal	0	1	1	0	0	0	2	3

Parameter	Major Findings								
	Slight	0	0	2	0	0	0	0	0
	Moderate	0	0	0	4	0	0	0	1
	Brain: Inflammation: Subacute								
	Slight	0	0	0	0	0	0	0	1
	Infiltration: Choroid plexus								
	Minimal	0	0	1	3	0	0	0	4
	Slight	0	0	0	1	0	0	0	0
	Mineralization								
	Slight	0	0	0	0	0	0	0	1
	Kidney: Deposits: Pigment								
	Minimal	0	0	0	3	0	0	1	1
	Moderate	0	0	0	1	0	0	0	0
	Hypertrophy/hyperplasia: Arteriolar								
	Slight	0	0	0	1	0	0	0	0
	Kidney Prussian Blue#								
	Positive								
	Minimal	0	-	-	0	0	-	-	1
	Marked	0	-	-	1	0	-	-	0
	Stomach: Hemorrhage								
	Minimal	0	0	1	1	0	0	1	2
	Slight	0	0	1	1	0	0	0	0
	Erosion/ulceration: Glandular mucosa								
	Minimal	0	0	0	2	0	0	0	0
	Infiltration: mononuclear cell								
	Minimal	0	0	0	0	0	0	0	2
	Whole Body: Congestion								
	Minimal	0	0	3	2	0	0	2	2
	Slight	0	0	0	2	0	0	1	2
<i>End of Recovery (N=2)</i>									
	Daprodustat					Male		Female	
	Daily Dose (mg/kg/day)					0	50	0	50
	Brain								
	Infiltration: Choroid plexus								
	Minimal	0		1		0		0	
	Kidney								
	Deposits: pigment								
	Minimal	0		1		0		1	
	Slight	0		1		0		0	
	Spleen								
	Hematopoiesis/extramedullary: Increased								
	Moderate	0		1		0		0	
	Deposits: Pigment								
	Minimal	0		1		0		0	
	Stomach								
	Hemorrhage								
	Minimal	1		1		0		0	
	Hyperplasia								
	Minimal	0		0		0		1	
	Whole body								
	Congestion								
	Minimal	0		1		0		1	

Parameter	Major Findings

Source: Reviewer's analysis
#=#number of animals with positive findings

Toxicokinetics The systemic exposure of daprodustat based on the C_{max} and AUC increased with dose increment with less than dose-proportional manner. The sex difference was less than 2-fold between male and female and the t_{max} ranged from 0.5 to 8 hours. The metabolites of daprodustat [GSK2391220 (M2), GSK2531403 (M3), GSK2499166 (M4), GSK2531399 (M5), GSK2531398 (M6) and GSK2531400 (M13)] were detected in plasma in all drug-treated groups with increased exposure with dose increment and the metabolite to parent ratios exposures (AUC) were less than 0.09. The maximum plasma concentrations for the metabolites were generally observed between 1 and 8 hours after dosing during Week 39.

Table 146. Toxicokinetic Parameters in Monkeys After Treatment of Daprodustat for 39 Weeks

Parameter ^a	Period	Dose of GSK1278863 (mg/kg/day)		
		3 (n = 8)	10 (n = 8)	50 (n = 12)
AUC ₀₋₄ (μg.h/mL)	Day 1	2.92 [1.40 – 4.86]	11.0 [4.51 – 21.3]	47.1 [18.3 – 75.7]
	Week 4	3.63 [2.06 – 7.28]	14.1 [10.8 – 17.7]	51.8 [22.6 – 100]
	Week 26	4.32 [1.01 – 12.8]	9.74 [4.19 – 14.1]	42.7 [13.6 – 82.3]
	Week 39	3.49 ^b [1.67 – 5.83]	9.46 [4.74 – 18.3]	29.8 [20.3 – 51.3]
C _{max} (μg/mL)	Day 1	0.866 [0.365 – 1.49]	2.14 [1.25 – 3.73]	5.34 [2.38 – 9.43]
	Week 4	1.35 [0.602 – 2.70]	2.24 [1.40 – 4.68]	4.53 [2.72 – 7.99]
	Week 26	0.799 [0.395 – 2.03]	1.57 [0.720 – 2.50]	2.81 [1.73 – 6.26]
	Week 39	0.655 ^b [0.282 – 1.02]	1.30 [0.856 – 2.01]	2.58 [1.32 – 4.20]

Source: Applicant's submission

^a Results are reported as mean and [range].

^b n = 7; male animal 204 given 3 mg/kg/day was euthanized during Week 38 (Day 262) due to deteriorating clinical conditions.

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration

Source: Reviewer's analysis

Abbreviations: ALP, alkaline phosphatase ; APTT, activated partial thromboplastin time; AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; HD, high dose; LD, low dose; MD, mid dose; PT, prothrombin time; RBC, red blood cell; TK, toxicokinetic

13.2.4. Reproductive Toxicology

13.2.4.1. Fertility and Early Embryonic Development

Title/ Study Number: GSK1278863A: Oral (Gavage) Once Daily Male Fertility Study in the CrI:CD(SD) Rat/ CD2009/00389/00

Key Study Findings

- Daprodustat-related death occurred in 20 mg/kg/day HD males (2/25).
- Daprodustat-related noteworthy findings were mostly at HD, which include dark red discoloration of kidney, liver, and stomach.

- Daprodustat had no effect on reproductive potential in male Sprague Dawley rats up to 20 mg/kg/day.

GLP Compliance: Yes

Title/ Study Number: GSK1278863A: Oral Female Fertility and Early Embryonic Development Study in Rats/
CD2009/00236/00

Key Study Findings

- Maternal toxicity at HD of 100 mg/kg/day includes significant decrease in body weight gain (BWG) during the premating period of Day 0-14 (80%) and up to gestation Day 7 (28%) with corresponding food consumption decrease up to 15% compared to controls.
- In HD females, statistically significant decrease of mean uterine weight (19%), number of corpora lutea (14%), number of implantations (15%), number of live fetuses (21%), and a marked increase of total postimplantation loss were observed.
- There were no effects on estrous cycles, days needed for mating, and mating and fertility indices up to 100 mg/kg/day.
- NOAEL for maternal toxicity, female fertility, and embryo-fetal development in rats is 7 mg/kg/day based on the GSK1278863A-related adverse findings in HD.

Table 147. Methods of FEED Study in Female Rats/ CD2009/00236/00

Study Feature	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	0, 2, 7, or 100 mg/kg/day Females: Once Daily, 2 weeks prior to mating, during mating and up to GD 6, inclusive. Mated females and their litters were terminated on GD 20. Males: No treatment
Route of administration:	Oral (gavage)
Formulation/vehicle:	Suspension/1% (w/v) Methylcellulose
Species/strain:	Rat/Crl:CD(SD)
Number/sex/group:	25/female/group
Satellite groups:	No satellite groups
Study design:	Standard FEED study design based on ICH S5(R3) included PD and ADA assessments.
Deviation from study protocol affecting interpretation of results:	None of the deviations impacted the overall integrity of the study or interpretation of study results

Source: Reviewer's analysis

Abbreviations: ADA, anti-drug antibody; FEED, Fertility and Early Embryonic Development; GD, gestation day; GLP, good laboratory practice; ICH, International Conference on Harmonization; PD, pharmacodynamic; SD, standard deviation

Table 148. Observations and Results of FEED Study in Female Rats/ CD2009/00236/00

Parameter	Major Findings
Mortality	One female at 100 mg/kg/day was prematurely terminated on Day 17 due to 24% body weight loss compared to Day 0. Red material around the mouth was observed approximately 1 hour postdose for 5 days prior to euthanasia. This female had microscopic findings of dark red discoloration of the ovaries.

Parameter	Major Findings
Clinical signs	Red and/or clear material around mouth and nose were observed within 15 min or 1 hour following dose administration beginning on study Day 8 and continuing until GD 6 in multiple females at 100 mg/kg/day
Body weights	Premating BWG, Day 0-14: Dose-dependent reduced mean BWG (13, 20, and 80% vs. control at 2, 7, or 100 mg/kg/day, respectively, with statistical significance only at the HD). Gestation BWG presented statistically significant ($p < 0.05$) changes at 100 mg/kg/day, 28% decrease during GD 0 to 7, and 32% increase during GD 7 to 14 vs. controls. Corresponding decrease in food consumption (12- to %) were observed during pre-mating period and GD 0-7. Decreases in BWG and food consumption were reversible when dosing stopped.
Necropsy findings Cesarean section data	Decreased mean gravid uterine weight (19%), mean number of corpora lutea (14%), number of implantation sites (15%), and number of live fetuses (21%) were observed with statistical significance at 100 mg/kg/day. Mean litter proportion of total postimplantation loss (primarily early resorptions) increased by >200% at 100 mg/kg/day.
Necropsy findings Offspring	There were no treatment-related fetal body weight changes or external fetal malformations/developmental variations observed:

Source: Reviewer's analysis

Abbreviations: BWG, body weight gain; FEED, Fertility and Early Embryonic Development; GD, gestation day; HD, high dose

13.2.4.2. Embryo-Fetal Development

Title/ Study Number: GSK1278863A: Oral (Gavage) Once Daily Embryo Fetal Development Study in the Crl:CD(SD) Rat/ CD2009/00388/01 (aka Study No. AFA00832)

Key Study Findings

- Maternal toxicity at the HD of 60 mg/kg/day (significant decreases in body weight gain (BWG; -21% overall) and food consumption (FC) compared to controls during the dosing period).
- There was a 7% decrease in gravid uterine weights, 6.5-fold increase in post-implantation loss, 15% decrease in mean litter size, and 4.4-fold increase in the percentage of fetuses dead or resorbed in litters with one or more live fetuses in HD females compared to controls.
- Skeletal variations of slight-delayed ossification were observed, which often are related to maternal toxicity.
- NOAEL for both maternal toxicity and embryo-fetal development is defined at 7 mg/kg/day based on decreased BWG and uterine weight of dams, and increased post-implantation loss and fetuses death/resorption associated with maternal toxicity.

Table 149. Methods for Oral Embryo-Fetal Developmental Study in Rat/ CD2009/00388/01 (aka Study No. AFA00832)

Study Feature	Method Details
GLP compliance	Yes

Study Feature	Method Details
Dose and frequency of dosing:	0, 0.5 (LD), 7 (MD), 60 (HD) mg/kg/day Once daily on GD 6-17. Mated females and their litters were euthanized on GD 21.
Route of administration:	Oral (gavage)
Formulation/vehicle:	Suspension/1% (w/v) Methylcellulose (400 cps) in ultra pure water
Species/strain:	Rat/ Crl: CD (SD)
Number/sex/group:	22/females/group
Satellite groups:	No satellite groups
Study design:	Standard EFD study design based on ICH S5(R3)
Deviation from study protocol affecting interpretation of results:	None of the deviations impacted the overall integrity of the study or interpretation of study results.

Source: Reviewer's analysis

Abbreviations: EFD, embryo-fetal developmental; GD, gestation day; GLP, good laboratory practice; HD, high dose; ICH, International Conference on Harmonization; LD, low dose; MD, mid dose; SD, standard deviation

Table 150. Observations and Results for the Oral (Gavage) Once Daily Embryo Fetal Development Study in the Crl:CD(SD) Rat/ CD2009/00388/01 (aka Study No. AFA00832)

Parameters	Major Findings
Mortality	None
Clinical signs	Slight excess salivation (45% of animals) on at least one of three occasions between Days 9 and 17 and sparse hair on limbs (14% of animals) were the most common clinical signs. These findings were observed with higher incidence at the HD. Red perivaginal substance and/or mild dehydration, hyperpnea, ptosis, tachypnea, and scant feces were observed in 2 HD females. One of these females had a litter that consisted of all early resorptions.
Body weights and Food consumption	Mean BWG decreases were 22% during dosing period and 19% after the dosing stopped (with trend for reversibility) in the HD animals compared to controls. Food consumption decreases corresponding to the BWG decreases in the HD was reversible after the dosing cessation.
Necropsy findings	No drug-related findings.
Cesarean section data	Most cesarean section data fell within the range of historical control data except for decreased litter size, increased incidence of resorptions/litter, and the percentage of fetuses dead or resorbed in litters with one or more live fetuses in the HD group (see Table 151). However, the mean percentage of early resorptions did not fall outside of the historical control range.

Table 151. Summary of Cesarean Section Data in Rats Treated With GSK1278863A

Group	1	2	3	4	Historical Control	
					2007-2009	
Dose (mg/kg/day)	0	0.5	7	60	Mean	Range
Rats tested, N	22	22	22	22	1581	
Pregnant, n(%)	20 (90.9)	19 (86.4)	17 (77.3)	20 (90.9)	1458 (92.7)	72.0-100
Cesarean, N	20	19	16	20		
Litter sizes	14.4	14.4	14.1	12.3*	14.4	13.0-16.0
Live fetuses						
N	287	274	226	245		
Mean	14.4	14.4	14.1	12.2		
Dead fetuses						
N	0	0	0	1		
Mean	0.0	0.0	0.0	0.0	0	--
Resorptions						
Mean	0.3	0.4	0.5	2.0 *	0.7	0.2-1.5
Early resorptions						

Parameters	Major Findings					
N fetuses (N litters)	6 (6)	8 (7)	8 (5)	28 (9)		
Mean	0.3	0.4	0.5	1.4	0.7	0.2-1.5
Late resorptions						
N fetuses (N litters)	0 (0)	0 (0)	0 (0)	11 (2)		
Mean	0.0	0.0	0.0	0.6*	0	0-0.1
Post-implantation loss %						
Mean	2.1	2.8	3.3	13.7		
Dams with litters with one or more live fetuses, N	20	19	16	19		
Live fetuses						
N	287	274	226	245		
Mean	14.4	14.4	14.1	12.9		
% Dead or resorbed						
Mean	2.1	2.8	3.3	9.2*	4.6	1.3-8.4

Source: Reviewer's analysis

* number, mean, or percentage is higher than historical control data

Necropsy findings
Offspring

All daprodustat-treated groups displayed increases in the litter and fetal percentages of skeletal variations when compared to historical and concurrent controls. These findings are not considered adverse as they represent developmental delays due to maternal toxicity and would reverse.

Other noteworthy findings not included in the labeling based on low incidence (1 fetus in 1 litter/dose level) were:

- One fetus with a cleft in the palate and snout in the HD group. A cervical rib was present at the 7th cervical vertebrae in two fetuses, one each in the 7 and 60 mg/kg/day groups.
- One fetus had a misaligned arch in a sacral vertebra and misaligned ileum in the HD group.

Table 152. Skeletal Variations Observed in Litters From Dams Treated With GSK1278863A

Findings With Number and (%) of Affected Litters (L) and Fetuses (F)	Group 1	Group 2	Group 3	Group 4	Historical Control 2007-2009	
	Control	0.5 mg/kg/day	7 mg/kg/day	60 mg/kg/day	N (%)	Range %
Number of litters	20	19	16	19	998	998
Number of fetuses examined	146	142	117	128	7330	7330
Skull: Frontal, incompletely ossified	L 0 (0)	L 0 (0)	L 0 (0)	L 2 (10.5)a	L 1 (0.10)	L 0-4.5
Cervical vertebrae: arch, incompletely ossified	L 0 (0)	L 1 (5.3)a	L 1 (6.2)a	L 2 (10.5)a	L 2 (0.20)	L 0-4.5
Ribs: Short (13 th)	L 0 (0)	L 1 (5.3)	L 1 (6.2)	L 4 (21.0)a	L 20 (2.00)	L 0-12.0
Thoracic vertebrae: ossification sites	13.16	13.09	13.02a	13.00b	13.08	13.03-13.18
Lumbar vertebrae: ossification sites	5.83	5.88	5.98a	5.97a	5.91	5.81-5.96
Ribs (pairs): ossification sites	13.11	13.07	13.02	13.00b	13.07	13.02-13.90

Source: Reviewer's analysis

L=litter incidence with number and (%) of affected litters

^a value outside of the historical control (HC) data range; not statistically significant (SS)

^b value outside of HC range and SS (p ≤ 0.05) compared to vehicle control

Source: Reviewer's analysis

Abbreviations: BWG, body weight gain; HD, high dose

Title/ Study Number: GSK1278863A: Oral (Stomach Tube) Once Daily Embryo Fetal Development Study in the Dutch Belted Rabbit/ CD2009/00390/00

Key Study Findings

- Maternal toxicity was observed with mortality, lower body weight gain up to 42%, and abortion at HD of 60 mg/kg/day.
- There were no fetal malformations associated with feed restriction. Other malformations including irregularly shaped anterior fontanelle and manubrium, fused sternal centra, small eye and socket, small lung, incompletely ossified sternal centra, and body edema were noted at 60 mg/kg/day outside the historical control data range.
- NOAELs were 30 mg/kg/day for maternal toxicity and for embryo-fetal toxicity based on maternal lower body weight gain and fetal malformations at 60 mg/kg.

Table 153. Methods of Oral Embryo-Fetal Developmental Study in Rabbits

Study Feature	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	4, 30, or 60 mg/kg/day, 5 mL/kg, once daily between GDs 7-19. Mated females were euthanized on GD 29. Doses were defined based on a dose range study where doses $\geq$ 125 mg/kg/day resulted in mortality due to body weight loss and reduced food consumption. At $\geq$ 30 mg/kg/day in the dose range study, pharmacologically-mediated increases in erythropoiesis (hemoglobin, as well as hematocrit and RBCs, up $\sim$ 13% compared to control mean) were observed.
Route of administration:	Oral (stomach tube)
Formulation/vehicle:	Suspension/1% (w/v) Methylcellulose (400 cps) in ultra pure water
Species/strain:	Rabbit/Dutch belted [Haz(DB)SPF]
Number/sex/group:	22/sex/group
Satellite groups:	Toxicokinetics (9/group), blood collection on GD 11
Study design:	Standard EFD study design based on ICH S5(R3)
Deviation from study protocol affecting interpretation of results:	None of the deviations impacted the overall integrity of the study or interpretation of study results.

Source: Reviewer's analysis

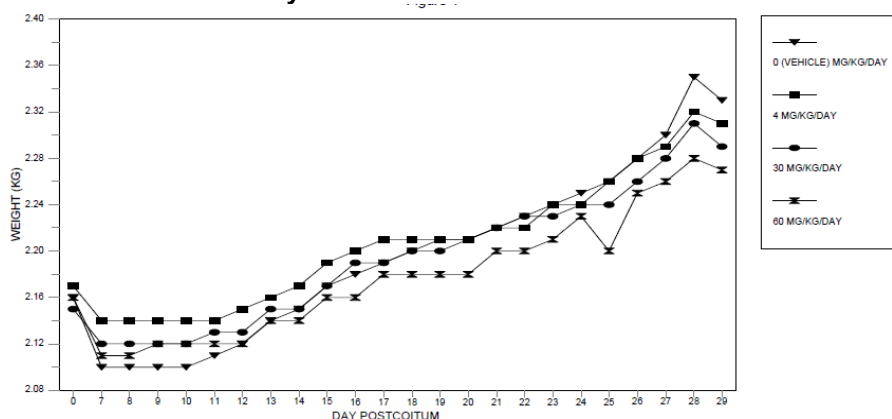
Abbreviations: EFD, embryo-fetal developmental; GD, gestation day; GLP, good laboratory practice; ICH, International Conference on Harmonization; RBC, red blood cell

Table 154. Observations and Results of the Oral (Stomach Tube) Once Daily Embryo Fetal Development Study in the Dutch Belted Rabbit/ CD2009/00390/0

Parameter	Major Findings
Mortality	One rabbit at HD of 60 mg/kg/day, which presented body weight loss and low food consumption, aborted on GD 25 and was euthanized. No feces were noted on Gestation Days 3, 15-17, and 24. Another HD dam lost weight (BW on GD 20 was 10% lower than GD 7), had severely reduced food consumption from GD 13 through GD 20, had scant/no feces on GD 5 and GD 17 through GD 20, red substance in the cage pan on GD 19 and was euthanized on GD 20. Findings in this female were considered test article-related.
Clinical signs	Unremarkable

Parameter	Major Findings
Body weights and food consumption	Maternal toxicity Reduced body weight gain was observed at 4, 30, and 60 mg/kg/day of 33%, 25%, 42%, respectively, compared to controls at the end of the dosing period GDs 7-20. During the postdose period of GDs 20-29, reduced BWG was 33% lower with statistical significance at ≥ 30 mg/kg/day suggesting reversibility at the HD and progression at the MD. Overall BWG combining dosing and postdosing period of GDs 7-29, were lower by 17%, 26%, and 30% in 4, 30, and 60 mg/kg/day groups, respectively, vs. control. There were no test article related food consumption changes observed other than the HD females with early mortality.

Figure 45. Body Weight in Pregnant Rabbits Treated With GSK1277863A Between Gestation Days 7-20



Source: Applicant's submission

Necropsy findings	No daprodustat-related macroscopic findings in dams were observed.
Cesarean section data	Litter size and the mean number of live fetuses were decreased with dose dependency and statistical significance in daprodustat-treated groups, but the finding was within the historical control data range. Preimplantation and postimplantation loss were increased in daprodustat-treated groups without a clear dose response and statistical significance, and the incidence was within the historical control data range. The upper limit of the individual % postimplantation loss in control group was high at 28.6%.

Table 155. Summary of Cesarean Section Data in Rabbits Treated With GSK1277863A

Group	1	2	3	4	Historical Control	
					2004-2010	
Dose (mg/kg/day)	0	4	30	60	Mean	Range
Rabbits tested, N	22	22	22	22	--	--
Pregnant, n(%)	21 (95.4)	22 (100)	21 (95.4)	22 (100)	95.6	86.7-100
Cesarean (litters), N	20	21	21	19	--	--
Aborted/Naturally delivered (litters), N	0	0	0	2	--	--
Litter size						
Mean	7.2	6.0 ^a	5 ^a	5.7 ^a	--	--
Live fetuses						
N	145	127	123	109	--	--
Mean	7.2	6.0 ^a	5.8 ^a	5.7 ^a	6.5	5.5-7.4
Dead fetuses						
N	0	0	1	0	--	--
Mean	0	0	0 ^d	0	0.12 ^c	0-1.85 ^c

Parameter	Major Findings					
Resorptions						
Mean	0.5	0.5	1.5 ^a	0.7	0.6	0.3-0.9
Early resorptions						
N	10	10	29	14	--	--
Mean	0.5	0.5	1.4	0.7	0.4	0.1-0.8
Late resorptions						
N	0	1	2	0	--	--
Mean	0	0	0.1	0	0.2	0-0.7
% Pre-implantation loss						
Mean	1.7	8.9	9.9	12.7	10.3	1.7-18.2
% Post-implantation loss						
Mean	6.8	7.6	19.5 ^b	10.6	7.8	3.6-13.4
Dams with litters with one or more live fetuses, N	20	21	21	19	95.3	84.6-100
Implantations, N	7.8	6.6 ^a	7.4	6.5 ^a	7.1	6.0-7.8
Live fetuses						
N	145	127	123	109		
Mean	7.2	6.0 ^a	5.8 ^a	5.7 ^a	6.5	5.5-7.4
% Dead or resorbed						
Mean	6.8	7.6	19.5 ^b	10.6	6.4	3.0-8.1
Source: Reviewer's analysis						
Statistically significant vs. control						
^a p≤0.05						
^b p≤0.01						
^c historical control data from reference article provided by the Applicant (Spence, 2003)						
^d Mean±SD is 0.0±0.2						
-- Information not available						
N= total number, Mean= number per litter						

Parameter	Major Findings
Necropsy findings Offspring	Noteworthy findings include irregularly shaped anterior fontanelle and manubrium, fused sternal centra, small eye and socket, small lung, incompletely ossified sternal centra, and body edema were noted at 60 mg/kg/day outside the historical control data range.

Table 156. Skeletal Variations Observed in Litters From Dams Treated With GSK1278863A

Findings With Number and (%) of Affected Litters (L)	Group 1	Group 2	Group 3	Group 4	Historical Controls#	
	Control	0.5 mg/kg/day	7 mg/kg/day	60 mg/kg/day	N (%)	Range %
Number of Litters	20	21	16	19	998	998
Number of Fetuses Examined	145	127	124	109	7330	7330
Malformations # Litters with a finding (% of litters with a finding)						
Skull: anterior fontanelle, irregularly shaped	1 (5)	1 (4.8)	1 (4.8)	3 (15.8)	0.42 ^a	0-5.88 ^a
Manubrium, irregularly shaped	0 (0)	0 (0)	0 (0)	2 (10.5)	0.42 ^b	0-6.67 ^b
Sternal centra, fused	1 (5)	1 (4.8)	2 (9.5)	3 (15.8)	0.42 ^b	0-6.67 ^b
Eye: bulge depressed	0 (0)	1 (4.8)	0 (0)	2 (10.5)	0.42 ^e	0-7.14 ^e
Eye: small	0 (0)	1 (4.8)	0 (0)	2 (10.5)	0.42 ^e	0-7.14 ^e
Lungs: small	0 (0)	0 (0)	0 (0)	2 (10.5)	0.32	0-6.25
Variations # Litters with a finding (% of litters with a finding)						
Sternal centra, incompletely ossified	0 (0)	0 (0)	0 (0)	4 (21)	5.08 ^c	0-14.29 ^c
Body: edema	0 (0)	0 (0)	0 (0)	2 (10.5)	0.42 ^d	0-6.67 ^d

Source: Reviewer's analysis

L=litter incidence with number and (%) or litters affected

#=historical control data (1999-2002) from reference article provided by the Applicant (Spence, 2003)

^a Historical control data of skull bone fusion

^b Historical control data of sternebral malformation

^c Historical control data of incomplete ossification of sternebra

^d Historical control data of local edema

^e Historical control data of no eye bulge

Toxicokinetic	Systemic exposure to daprodustat increased with dose increments in less than a dose proportional manner observed on GD 11.

Table 157. Toxicokinetic Parameters in Pregnant Female Rabbits

Dose Group	Dose Level (mg/kg/day)	AUC _(0-t) (µg.h/mL)	C _{max} (µg/mL)	t _{max} (h)
2	4	22.8	1.49	4
3	30	133	9.24	4
4	60	197	11.8	8

Source: Applicant's submission

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; t_{max}, time of peak plasma concentration

Source: Reviewer's analysis

Abbreviations: BW, body weight; BWG, body weight gain; GD, gestation day; HD, high dose; MD, mid dose

Study Number/ Title: GSK1278863A: Oral Study to Determine Concentrations of GSK1278863 and its Metabolites in Rabbit Plasma (GlaxoSmithKline Study No. G12109)/ 2012N140611/00

Key Study Findings

- The systemic exposure to daprodustat and its metabolites (M2, M3, M4, M5, M6, or M13) were detected in pregnant rabbit plasma after oral dosing of 60 mg/kg/day once daily between Gestation Days 7 to 11, which reflects that the fetuses are exposed to daprodustat and its major metabolites during the organogenesis period.
- Daprodustat exposure was minimum 16-fold higher compared to metabolites exposure in plasma.

Table 158. Methods for the Study to Determine Concentrations of GSK1278863 and its Metabolites in Rabbit Plasma (GlaxoSmithKline Study No. G12109)/ 2012N140611/00

Study Feature	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	0 or 60 mg/kg/day, once daily on Gestation Days 7-11
Route of administration:	Oral (gavage)
Formulation/vehicle:	Suspension/1% (w/v) Methylcellulose (400 cps @2%)
Species/strain:	Rabbit/Dutch Belted (Haz:[DB]SPF)
Number/sex/group:	2 dams for control, 4 dams for 60 mg/kg/day group
Satellite groups:	None
Study design:	Study designed for toxicokinetic analysis of the daprodustat and its metabolites of M2, M3, M4, M5, M6, or M13.
Deviation from study protocol affecting interpretation of results:	No deviations reported.

Source: Reviewer's analysis

Abbreviations: GLP, good laboratory practice

Table 159. Observations and Results of the Oral Study to Determine Concentrations of GSK1278863 and its Metabolites in Rabbit Plasma (GlaxoSmithKline Study No. G12109)/ 2012N140611/00

Parameter	Major findings
Mortality	There were no unscheduled mortalities.
Pregnancy	Females were pregnant; no caesarean-section was performed.
Toxicokinetics	Systemic exposure (AUC and C _{max}) to daprodustat was detected in the treated group and minimum 16-fold higher compared to metabolites of M2, M3, M4, M5, M6, or M13.

Table 160. Systemic Exposure to Daprodustat and the Major Metabolites after Administration of Daprodustat in Pregnant Rabbits

Parameter ^a (n=4)	Analyte						
	GSK 1278863	GSK 2391220 (M2)	GSK 2531403 (M3)	GSK 2499166 (M4)	GSK 2531399 ^b (M5)	GSK 2531398 (M6)	GSK 2531400 (M13)
AUC _{0-t} (µg.h/mL)	188 [171 – 207]	11.7 [11.2 – 12.0]	2.69 [1.35 – 4.46]	1.37 [1.04 – 2.07]	0.594 [0.466 – 0.723]	0.210 [0.0300 – 0.492]	0.194 [0.149 – 0.263]
C _{max} (µg/mL)	17.7 [15.7 – 20.5]	1.02 [0.918 – 1.20]	0.222 [0.126 – 0.332]	0.118 [0.0900 – 0.157]	0.0432 [0.0307 – 0.0556]	0.0167 [0.00436 – 0.0363]	0.0158 [0.0118 – 0.0180]
Median T _{max} (h)	3 [0.5 – 4]	3 [2 – 4]	3 [2 – 4]	3 [2 – 4]	2.3 [0.5, 4.0]	3 [2 – 4]	3 [2 – 4]

a. Results are reported as mean unless stated otherwise and [range].

b. n=2, GSK2531399 plasma concentrations for animals 201 and 205 were not reportable.

Source: Applicant's submission

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, time of peak plasma concentration

Source: Reviewer's analysis

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration

13.2.4.3. Prenatal and Postnatal Development (PPND)

Title/ Study Number: GSK1278863A + metabolites [GSK2391220A (M2), GSK2506104A (M3) and GSK2531401A (M13)]: Oral Gavage and Subcutaneous Injection Tolerability, Toxicokinetics and Pre- and Postnatal Developmental Dose Range Study in Pregnant Rats/ 2016N305715/00

Pregnant Sprague Dawley rats were treated with daprodustat at 0 (1% methylcellulose) or 60 mg/kg/day with (Group 2, 3) or without (Group 1) metabolite cocktail (M2, M3, and M13) SC injection twice daily (6 hours between doses). A metabolite cocktail was prepared at 2 different sets of doses, 1X or 3X: 2.5, 3.2, or 1.8 mg/kg/day or 7.5, 9.6, or 5.4 mg/kg/day of M2, M3, or M13, for Group 2 and 3, respectively. Main study animals were dosed from GD 6 to LD 6 and TK study animals were dosed from GD 6 to GD 10. The extended duration study (Group 4-7) with a decreased dosing regimen of 0, 10 or 40 mg/kg/day daprodustat was conducted with or without the metabolite cocktail (2.5, 3.2, or 1.8 mg/kg/day, M2, M3, and M13, respectively).

Table 161. Study Design of Dose Range Study For Tolerability, Toxicokinetics, and Pre- and Postnatal Development in Pregnant Rats

Group No.	GSK1278863A Metabolite Cocktail	
	(mg/kg/day) ^a	(mg/kg/day) ^b
1	0	0
2	60	M2 2.5

Group No.	GSK1278863A Metabolite Cocktail		
	(mg/kg/day) ^a	(mg/kg/day) ^b	
3	60	M3	3.2
		M13	1.8
		M2	7.5
4	0	M3	9.6
		M13	5.4
		0	0
5	10	M2	2.5
		M3	3.2
		M13	1.8
6	40	0	0
7	40	M2	2.5
		M3	3.2
		M13	1.8

Source: Reviewer's analysis

^a Oral administration, once a day

^b Subcutaneous injection, twice a day

Abbreviations: M2, GSK2391220; M3, GSK2506104; M13, GSK2531401

Key Study Findings

There were 2 mortalities including one in control group and one in 60 mg/kg/day group with 3X metabolite cocktail (Group 3). The cause of death was not determined at the necropsy for the control animal or the female in the 60 mg/kg/day + 3X metabolite group, however this female lost 12 g of body weight from GD 20 to 21. Another 60 mg/kg/day + 3X metabolite GSK12788763A-treated animal was prematurely terminated due to cannibalism of the F1 litter (7/13).

Daprodustat-related effects were observed at 60 mg/kg/day with decreased mean maternal body weight gain during late gestation of 44% or 60%, Group 2 and 3, respectively. Decreases of 30% and 40% were also observed at 40 mg/kg/day without (Group 6) and with (Group 7) 1X metabolite cocktail. Decreases in body weight compared to controls for 40 and 60 mg/kg/day corresponded with decreased mean food consumption. Mean body weight gain/body weight loss persisted at 60 mg/kg/day with decreased food consumption during the lactation period (53% or 90% compared to controls, Group 2 and 3, respectively) but the effects on body weight or food consumption at 40 mg/kg/day resolved during LD 1 to 7.

In addition, there were increases in the duration of parturition of 16.8 and 20 min/pup, Group 2 and 3, respectively, at 60 mg/kg/day compared to controls mean of 6 minutes/pup. The increase of stillborn pups and found dead/missing (presumed cannibalized) pups decreased mean pup survival and litter sizes by LD 7 (22% decrease compared to control) at 60 mg/kg/day, and decreased pup body weight up to ~30% from birth to PND 7 were also observed at 60 mg/kg/day. The findings at 40 mg/kg/day were similar to that of 60 mg/kg/day with slightly less severity and trend of reversibility.

The NOAEL was defined at 10 mg/kg/day with C_{max} 72.4 µg/mL and AUC_{0-t} µg.h/mL, based on maternal toxicity and reduced embryo-fetal survival at the next highest dose of 40 mg/kg/day, suggesting metabolite levels may impact maternal toxicity and associated embryo-fetal toxicity.

Title/ Study Number: GSK1278863A + Metabolites [GSK2391220A (M2), GSK2506104A (M3) and GSK2531401A (M13)]: Oral Gavage and Subcutaneous Injection Pre- and Postnatal Development Study in Rats/ 2018N365151/00

Key Study Findings

- There was one mortality in the F0 generation at the HD of 40 mg/kg/day that appears daprodustat-related, 2 mortalities of unclear relationship, and 1 mortality that were considered unrelated.
- Body weight gain during LD 1 to 4 was lower in HD with corresponding lower food consumption during GD 18-21 and LD 4-8 compared to controls.
- The daprodustat-related lower survival of F1 generation was statistically significant and noted based on the lower viability index on PND 1 to 4, smaller live litter size on PND 4 and 21, and increased number of dead pups on PND 2 to 4.
- Slightly longer delivery time was noted in HD (3/24) compared to controls.
- The systemic exposure to daprodustat in F0 generation rats increased as the dose increased in a less than dose proportional manner and metabolites (M2, M3, or M13) were detected in plasma at similar levels across the doses between GD 9 and LD 21.
- There was daprodustat detected in F1 generation suckling pups, which suggests the secretion of daprodustat into milk.
- The maternal body weight gain of F1 generation was lower in HD (40 mg/kg/day) females during gestation and lactation compared to controls.
- Effects (e.g., reduced litter size) at the HD on the F2 generation fell within the historical control range and were deemed not daprodustat-related.
- NOAEL is determined at 7 mg/kg/day, based on maternal toxicity and reduced embryo-fetal survival at the next highest dose of 40 mg/kg/day.

Table 162. Methods of Prenatal and Postnatal Developmental Study in Rats, Study 2018N365151/00

Study Feature	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	Daprodustat at 0.8 (LD), 7 (MD), or 40 (HD) mg/kg/day, once daily GSK2391220A (M2), GSK2506104A (M3), GSK2531401A (M13) at 1.25, 1.8 and 0.9 mg/kg/dose, respectively, BID, 1 st dose immediately following the oral dosing of daprodustat. 2 nd dose is 6 hours apart from 1 st dose. F0 females were treated with daprodustat or vehicle from GD 6 to GD 23 (did not deliver a litter), Lactation Day 20 (delivered a litter) or Lactation Day 21 (TK group).
Route of administration:	Daprodustat: oral gavage Metabolite Cocktail: subcutaneous injection
Formulation/vehicle:	Daprodustat: Suspension/1% (w/v) aqueous methylcellulose (400 cps @ 2%). Metabolite Cocktail: Solution/25mM Phosphate Buffer, pH 7.4
Species/strain:	Rat/ Sprague Dawley [CrI:CD(SD)]
Number/sex/group:	F0 generation: 24 females/groups; 6 females/group (TK group)
F0 Generation	Termination on Lactation Day 21 or 22 after weaning of their litter, or on GD 24 for those which did not deliver

Study Feature	Method Details						
F1 Generation	<u>Weaning</u> F1 offspring was weaned on PND 21. F1 generation (48/sex/group) was selected, and 2/sex/litter were assigned to 2 subsets for developmental landmark evaluation. Sexual maturation examination started on PND 27 for females or PND 39 for males. Table 163. Parameters for the Behavioral Assessment <table border="1"> <thead> <tr> <th>Subset</th> <th>Parameters</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Acoustic Startle on PND 60 to 62 Estrous Evaluation beginning on PND 64 to 73 Reproductive Capacity beginning on PND 73 to 80</td> </tr> <tr> <td>2</td> <td>Acoustic Startle on PND 27 to 29 Motor Activity on PND 54 to 61 Morris Water Maze on PND 65 to 85</td> </tr> </tbody> </table> Source: Reviewer's analysis Abbreviations: PND, postnatal day <u>Mating</u> F1 males and females (subset 1) on PND 73 to 80 were cohabited with non-littermates from the same group in a ratio of 1:1 for up to 10 days. Pregnant F1 females were allowed to deliver naturally and stayed with their F2 generation and were evaluated until PND 7 <u>Toxicokinetics</u> F1 offspring 3/sex/timepoint/group were selected for TK sampling.	Subset	Parameters	1	Acoustic Startle on PND 60 to 62 Estrous Evaluation beginning on PND 64 to 73 Reproductive Capacity beginning on PND 73 to 80	2	Acoustic Startle on PND 27 to 29 Motor Activity on PND 54 to 61 Morris Water Maze on PND 65 to 85
Subset	Parameters						
1	Acoustic Startle on PND 60 to 62 Estrous Evaluation beginning on PND 64 to 73 Reproductive Capacity beginning on PND 73 to 80						
2	Acoustic Startle on PND 27 to 29 Motor Activity on PND 54 to 61 Morris Water Maze on PND 65 to 85						
F2 Generation	F2 growth and development were observed up to PND 7						
Satellite groups:	Toxicokinetics: samples collected from F0 on GD9 and Lactation Day 21, F1 dams on PND 10						
Study design:	Standard PPND study design based on ICH S5(R3)						
Deviation from study protocol affecting interpretation of results:	The deviations did not impact the integrity of the study.						
Source: Reviewer's analysis Abbreviations: BID, twice daily; GD, gestation day; GLP, good laboratory practice; HD, high dose; ICH, International Conference on Harmonization; LD, low dose; MD, mid dose; PND, postnatal day; PPND, pre- and postnatal development; TK, toxicokinetic							

Table 164. Observations and Results of Prenatal and Postnatal Developmental Study in Rats, Study 2018N365151/00

Parameter	Major Findings
F0 Generation	
Mortality F0 Generation	Total of 4 deaths occurred: One in the control group and 3 in the HD group of 40 mg/kg/day. Control group: one female was euthanized on GD 23 due to complication during delivery evaluated at the necropsy. There were 2 daprodustat related deaths in HD (40 mg/kg/day) group One female was found dead on GD 21 due to massive body weight loss between GD 18 to 20, which is daprodustat-related. Two females were euthanized due to moribundity. At the necropsy, the causes of death were determined as parturition issue (daprodustat-related) and perforation in the esophagus, respectively.

Parameter	Major Findings
Body weights and Food consumption F0 Generation	Body weight gain during Lactation Days 1 to 4 was lower by 38% in HD of 40 mg/kg/day without statistical significance, which appears daprodustat-related. Statistically significant transient lower food consumption was noted during GD 18 to 21 (16%) and Lactation Days 4 to 8 (12%) in HD of 40 mg/kg/day group compared to controls.
Clinical signs, Natural delivery, Maternal necropsy	No daprodustat-related findings observed
Parturition	Slightly longer (20-26.7 min) delivery time was noted in 3 HD females compared to the high range of controls (19 min)
Litter observations (F1 Generation/preweaning)	Daprodustat-related effects on the survival of F1 generation were noted (see Table 165) Transient lower body weight of F1 generation (per litter) were noted in HD (40 mg/kg/day) rats until PND 7, but they reached similar body weight range on PND 21 Increased mean number of live born pups per litter with statistical significance at 0.8 or 7 mg/kg/day was due to higher stillborn per litter in controls, therefore, it is not drug-related. There were no drug-related effects on implantation.

Table 165. Summary of Cesarean Section Data in F1 Litters From Dams Treated With GSK1278863A

Dose (mg/kg/day)		Daprodustat	0	0.8	7	40	Historical Control ^d	
		Metabolite ^c M2	2.5	2.5	2.5	2.5		
		Metabolite ^c M3	3.2	3.2	3.2	3.2		
		Metabolite ^c M13	1.8	1.8	1.8	1.8	Mean	Range
F0 tested	N	24	24	24	24	24	--	--
Implantations	N/litter	13.6	13.8	13.4	13.0	13.0	14.2	12.4-16.2
F1 litters (preweaning)								
Viable litters at birth (PND 1)	N	22	23	22	23	23	--	--
Viable litters at weaning (PND 21)	N	22	23	22	23	23	--	--
Pups delivered	N	283	298	284	281	281	--	--
Unknown vital status ^f	N	1 (0.4) ^e	0	0	2 (0.7) ^e	2	--	--
Live born	N	274 (96.8) ^e	296 (99.3) ^e	283 (99.6) ^e	270 (96.1) ^e	270	289.3 (99.0) ^e	110-395 (97.2-100)
	N/litter	12.4	12.9 ^b	12.9 ^b	11.7	11.7	13.2	11.8-15.1
Stillborn	N	8 (2.8) ^e	2 (0.7 ^b) ^e	1 (0.4 ^b) ^e	9 (3.2) ^e	9	2.8 (0.9) ^e	0-9 (0.0-2.8)
	N/litter	0.4	0.1	0.0	0.4	0.4	0.1	0.0-0.4
Viability index	% Survival PND 1-4	98.2	98.3	97.5	86.6 ^b	86.6 ^b	98.7	93.9-100.0
Lactation index	% Survival PND 4-21	100	100	100	98.5 ^b	98.5 ^b	98.8	89.1-100.0
Total pups PND 2-4	N	273	294	280	259	259	296.2	218-392
Pups found dead PND 2-4	N	4	3	4	33	33	3.1	0-19
	%	1.5	1.0	1.4	12.7 ^b	12.7 ^b	0.9	0.0-5.3
Total pups PND 5-7	N	269	291	276	226	226	289.1	217-378
Pups found dead	N	0	0	0	3	3	0.7	0-3

Parameter	Major Findings							
PND 5-7	%	0.0	0.0	0.0	1.3 ^b	0.2	0.0-0.9	
Live litter size	Mean	12.2	12.6	12.5	10.3 ^b	13.1	11.8-14.8	
PND Day 4								
Live litter size	Mean	11.4	11.9	11.7	9.3 ^b	12.8	9.2-14.7	
PND Day 21								
Body weight	PND 1	7.0	6.8	7.0	6.3	6.7	6.2-7.2	
(g)/ litter	PND 4	10.2	10.0	10.2	8.9	9.6	8.8-10.7	
	PND 7	15.1	15.0	15.3	13.6 ^a	13.8	12.3-15.8	
	PND 21	41.2	41.5	42.4	40.9	41.1	35.7-48.4	

Source: Reviewer's analysis

^a Statistical significance (p≤0.05)

^b Statistical significance (p≤0.01)

^c BID 6 hours apart,

^d GSK reference No. R71242G

^e % of total number

^f Maternal cannibalization or incomplete birth precluded identification of vital status at birth.

-- Not available

Abbreviations: PND, postnatal day

Necropsy findings: No daprodustat-related findings observed.

Offspring

Toxicokinetics

F0 Generation

Table 166. Toxicokinetic Parameters of Daprodustat and its Human Metabolites GSK2391220 (M2), GSK2506104 (M3), and GSK2531401 (M13) in Pregnant F0 Generation Rats (TK Animals) on Gestation Day 9 (Excerpted From Submission)

Analyte	Parameter	Oral Dose GSK1278863 (mg/kg/day) ^a		
		0.8	7	40
GSK1278863	AUC _{0-t} (µg·h/mL)	146	855	2240
	C _{max} (µg/mL)	8.51	52.0	128
	T _{max} (h)	6.08	6.00	6.00
GSK2391220 (M2)	AUC _{0-t} (µg·h/mL)	2.65	2.50	2.51
	C _{max} (µg/mL)	1.97	1.55	1.63
	T _{max} ^b (h)	0.250	0.250	6.25
GSK2506104 (M3)	AUC _{0-t} (µg·h/mL)	2.73	2.47	2.36
	C _{max} (µg/mL)	2.19	1.72	1.75
	T _{max} ^b (h)	0.250	0.250	6.25
GSK2531401 (M13)	AUC _{0-t} (µg·h/mL)	2.16	1.94	1.88
	C _{max} (µg/mL)	1.51	1.19	1.33
	T _{max} ^b (h)	0.250	0.250	6.25

Source: Applicant's submission

Note: Composite toxicokinetic parameters were derived from mean plasma concentration data.

^a Daprodustat was administered once daily by oral gavage, followed by twice daily subcutaneous injection (approximately 6 hours apart) administration of human metabolite cocktail [GSK2391220A (M2), GSK2506104A (M3), and GSK2531401A (M13)] at nominal doses of 2.5, 3.2, and 1.8 mg/kg/day.

^b T_{max} was calculated from the first daily dose.

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, time to maximum concentration

Parameter Major Findings

Table 167. Toxicokinetic Parameters of Daprodustat and Its Human Metabolites GSK2391220 (M2), GSK2506104 (M3), and GSK2531401 (M13) in F0 Generation Rats (TK Animals) on Lactation Day 21 (Excerpted From Submission)

Analyte	Parameter	Oral Dose GSK1278863 (mg/kg/day) ^a		
		0.8	7	40
GSK1278863	AUC _{0-t} (µg·h/mL)	98.4	613	1620
	C _{max} (µg/mL)	8.12	47.6	92.6
	T _{max} (h)	4.00	4.00	6.00
GSK2391220 (M2)	AUC _{0-t} (µg·h/mL)	2.72	2.24	3.03
	C _{max} (µg/mL)	1.55	1.23	1.56
	T _{max} ^b (h)	6.25	6.25	0.250
GSK2506104 (M3)	AUC _{0-t} (µg·h/mL)	2.91	2.28	2.90
	C _{max} (µg/mL)	1.74	1.37	1.76
	T _{max} ^b (h)	6.25	6.25	0.250
GSK2531401 (M13)	AUC _{0-t} (µg·h/mL)	2.15	1.64	2.16
	C _{max} (µg/mL)	1.23	0.983	1.21
	T _{max} ^b (h)	6.25	6.25	0.250

Source: Applicant's submission

Note: Composite toxicokinetic parameters were derived from mean plasma concentration data.

^a Daprodustat was administered once daily by oral gavage, followed by twice daily subcutaneous injection (approximately 6 hours apart) administration of human metabolite cocktail [GSK2391220A (M2), GSK2506104A (M3), and GSK2531401A (M13)] at nominal doses of 2.5, 3.2, and 1.8 mg/kg/day.

^b T_{max} was calculated from the first daily dose.

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, time to maximum concentration

F1 Generation (Postweaning)

Mortality	One LD (0.8 mg/kg/day) male was found dead on PND 69.
Body weights	The body weight gains of HD rats during PND 22-29 were lower in F1 generation males (9%) and females (8%) with statistical significance compared to controls. Maternal mean body weight gain of F1 generation was lower at HD (40 mg/mg/kg) during gestation (GD 15-21) and 75% lower body weight gain on Lactation Days 1-7 was noted compared to controls with statistical significance.
Clinical signs	Daprodustat-related dehydration and whole-body cold to touch were observed (18 pups in 3 litters and 15 pups in 2 litters, respectively) in the preweaning period. HD (40 mg/kg/day) rats presented localized swelling along the surface of the abdomen starting from PND 53 (females) and PND 71 (males), and this finding was not reversed until termination.
Sexual maturation	No drug-related findings observed. Noteworthy findings include vaginal patency delayed by 0.9 day (statistically significant) and lower mean number of implantations by 9% at HD of 40 mg/kg/day compared to controls. These changes were within the historical control data range. In males, there was no daprodustat-related effect on spermatogenesis in testes based on the qualitative evaluation of spermatogenesis with normal progression of spermatogenic cycle.

Parameter	Major Findings
Behavioral assessments: Startle reflex, Locomotor activity, Water maze	No daprodustat-related effects on these parameters were noted
Necropsy findings: F1 Generation	No daprodustat-related effects on femur length were observed in F1 generation rats. At HD of 40 mg/kg/day, the following findings were observed with low incidence which is unclear for the test article-relatedness.

Table 168. Necropsy Findings Observed in Litters From Dams Treated With GSK1278863A

Group	Dosing Compound		1	2	3	4
	Daprodustat		0	0.8	7	40
			2.5,	2.5,	2.5,	2.5,
Dose (mg/kg/day)	Metabolite Cocktail (M2, M3, M13)		3.2,	3.2,	3.2,	3.2,
			1.8	1.8	1.8	1.8
F1 Generation (Postweaning)						
Organs	N	M	20	23	21	20
		F	22	23	22	20
Kidneys	Large	M	0	0	0	1
		F	0	0	0	1
	Pelvis, moderate dilation (right)	M	0	0	0	1
		F	0	0	0	0
Liver	Rough in appearance, all lobes	M	0	0	0	2
		F	1	0	0	0
	Thick	M	0	0	0	1
		F	0	0	0	0
	Large	M	0	0	0	1
		F	0	0	0	0

Source: Reviewer's analysis

Parameter	Major Findings

Toxicokinetics: F1 Generation	Table 169. Toxicokinetics Parameters of Daprodustat and Its Human Metabolites GSK2391220 (M2), GSK2506104 (M3), and GSK2531401 (M13) in F1 Generation Rats (TK Animals) on Postnatal Day 10

Plasma concentrations (ng/mL) on PND 10				
Oral Dose GSK1278863 (mg/kg/day) ^a	0	0.8	7	40
Males				
GSK1278863	NQ-2510	4590-9310	19300-31700	40000-44600
GSK2391220 (M2)	NQ-0.903	1.31-2.93	4.01-17.9	8.15-51.1
GSK2506104 (M3)	NQ	0.491-1.39	0.407-3.32	0.553-7.29
GSK2531401 (M13)	NQ	NQ-0.595	0.203-1.21	NQ-4.05
Females				
GSK1278863	NQ	3570-7830	18600-38500	36500-59500
GSK2391220 (M2)	NQ	0.754-6.07	3.51-12.7	7.38-24.1
GSK2506104 (M3)	NQ	0.324-2.94	0.480-1.81	0.434-4.59
GSK2531401 (M13)	NQ	NQ-1.30	NQ-1.17	NQ-2.63

Source: Applicant's submission

Range of individual animals reported.

^a Daprodustat was administered once daily to F0 females by oral gavage, followed by twice daily subcutaneous injection (approximately 6 hours apart) administration of human metabolite cocktail [GSK2391220A (M2), GSK2506104A (M3), and GSK2531401A (M13)] at nominal doses of 2.5, 3.2, and 1.8 mg/kg/day.

Abbreviations: NQ, not quantifiable (<0.200 ng/mL); PND, postnatal day; TK, toxicokinetic

F2 Generation

Body weights, Clinical signs	No daprodustat-related changes noted between PND1 to 7 in F2 generation rats.

Litter size and survival of F2 Generation	Decrease in the mean number of F2 generation of live born pups or the surviving pups was noted at HD of 40 mg/kg/day based on the mean number of liveborn, surviving pups/litter and litter size on PND 1 or 7. The trend was noted without statistical significance and within the historical control data range, therefore determined not drug-related.

Source: Reviewer's analysis

Abbreviations: GD, gestation day; HD, high dose; LD, low dose; PND, postnatal day

13.2.5. Juvenile Toxicology

Title/ Study Number: GSK1278863A: Oral Juvenile Toxicity Study in Rats/ 2020N436444/00

Key Study Findings

- At the MD and HD, there were daprodustat-related increases in the mean age of vaginal patency considered secondary to decreased mean body weight/body weight gain. At the HD, there was also increased estrous cycle length in females, considered secondary to the effects on growth and the early evaluation of estrous cycles (PND 57 vs. 63 to 70).
- Statistically significant increased RBC mass was observed at the HD.
- Minimal decrease in femur length was noted at HD at the end of dosing period but the decrease did not resolve in males and worsened in females at the end of recovery period. This effect corresponded with decreased body weight gain at this dose, where females had decreased mean body weight compared to controls through PND 43.

- Daprodustat-related findings of increased cellularity in the bone marrow of sternum and extramedullary hematopoiesis in the spleen were observed at HD at the end of dosing. The NOAEL is at LD of 2/0.8 mg/kg/day.

Table 170. Methods for Oral Juvenile Toxicity Study in Rats/ 2020N436444/00

Study Feature	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	0 (control), 2/0.8 (LD), 10/4 (MD), or 20/10 (HD) mg/kg/day, Dose level reduction on PND 12. Doses were based on a dose range study in which higher doses (40/20 mg/kg/day) were not tolerated (mortality due to dehydration and decreased BW). In the dose range study hemoglobin was elevated 12 to 13% and hematocrit was elevated 19 to 25% compared to controls. F0 generation dams were not dosed.
Route of administration:	Oral gavage
Formulation/vehicle:	Suspension/ 1% [w/v] aqueous methylcellulose [400 cps @ 2%]
Species/strain:	Rat/ Sprague-Dawley (CrI:CD[SD])
Number/sex/group:	F1 generation juvenile rats were dosed with 0, 2, 10, or 20 mg/kg/day daprodustat every other day between PND4 to10. On PND12, each dosing group was divided into 3 subsets and dosed with reduced daprodustat at 0, 0.8, 4, or 10 mg/kg/day. Subset 1: 10/sex/subset 1 (main study) between PND 12 to 63 Subset 2: 20/sex/subset 2 (off dose study/behavior/PND 63 TK between PND 12 to 70, with 12 -week recovery (necropsied on Days 158 and 159) Subset 3: 68/sex/subset 3 (TK study) between PND 12 to 21
Satellite groups:	N/A
Study design:	Untreated F0 generation delivered naturally and F1 generation rats were culled to 5-6 pups/sex (4 litters/group for PND 4 TK, 2-3 litters/group for PND 10 and 12 TK, all with 6/sex/group)

Table 171. Study Design of Juvenile Toxicity Study With Daprodustat

Group No.	Test Material	Dosage Level (mg/kg) ^a	Concentration (mg/mL)	Dosage Volume (mL/kg)	No. of Juvenile Rats ^b					
					Subset 1 ^c		Subset 2 ^c		Subset 3 ^c	
					M	F	M	F	M	F
1	Control Article	0	0	5	10	10	20	20	68	68
2	GSK1278863	2/0.8	0.4/0.16	5	10	10	20	19	68	68
3	GSK1278863	10/4	2/0.8	5	10	10	20	20	68	68
4	GSK1278863	20/10	4/2	5	10	10	20	20	68	68

Source: Applicant's submission

Study Feature **Method Details**
Table 172. Evaluation Plan of Developmental Parameter in Juvenile Rats

Procedure	Groups 1 to 4 (all study phases unless indicated)
Vaginal Patency (females)	PND 24 until evident (Off-dose study)
Balano-Preputial Separation (males)	PND 39 until evident (Off-dose study)
Ophthalmology	PND 57-62 (Main study)
Estrous Cycling (females)	PND 57 to 70 (Off-dose study)
Motor Activity	PND 98 ± 2 days (Off-dose study)
Acoustic Startle	PND 103 ± 2 days (Off-dose study)
Functional Observational Battery (FOB)	PND 106 ± 3 days (Off-dose study)
Morris Water Maze	PND 118-133 (Off-dose study)
Hematocrit only	PND 35 (Off-dose study)
Hematology	PND 64 (Main study) or PND 158-159 (Off-dose study)
Coagulation	PND 64 (Main study) or PND 158-159 (Off-dose study)
Clinical Chemistry	PND 64 (Main study) or PND 158-159 (Off-dose study)
Urinalysis	PND 64 (Main study) or PND 158-159 (Off-dose study)
TK	PND 4, 10, 12, 21, and 63 (TK study)
Gross Necropsy	PND 64 (Main study) or PND 158-159 (Off-dose study)
Complete Necropsy	All rats (Main study) and 10/sex/group (Off-dose study)
Organ Weights	All rats (Main study) and 10/sex/group (Off-dose study)
Tissue Collection	All rats (Main study) and 10/sex/group (Off-dose study)
Bone Marrow Smears	All rats (Main study) and 10/sex/group (Off-dose study)
Femur Length and Long Bone Collection*	Necropsy (Main and Off-dose studies; 10/sex/group that received a full necropsy)
Histology and Histopathology**	Post-necropsy. All retained tissues for identification of potential target organs from all rats in Groups 1 and 4 (Main study). Target organs identified during the main study from all rats in Groups 1 and 4 (Off-dose study), and femurs for females in Groups 1 and 4 (Off-dose study)
Spermatogenesis	Stage-dependent qualitative evaluation of spermatogenesis from control and highest dose groups (Main study)
Pathology Peer Review	Post-necropsy
* From the right hindlimb. Excess tissue was removed to expose the head and greater trochanter and the medial and lateral condyle. The length of the right femur (greater trochanter to lateral condyle) was measured and recorded.	
** Tissues listed in Section 3.6.7 (Tissue Collection and Preservation).	

Source: Applicant's submission
Abbreviations: PND, postnatal day; TK, toxicokinetic

Deviation from study protocol affecting interpretation of results: The deviations did not impact the integrity of the study.

Source: Reviewer's analysis
Abbreviations: BW, body weight; HD, high dose; LD, low dose; MD, mid dose; PND, postnatal day; TK, toxicokinetic

Table 173. Observations and Results of Oral Juvenile Toxicity Study in Rats/ 2020N436444/00

Parameter	Major Findings
Mortality	There were 13 deaths observed (found dead, euthanized due to moribundity, or presumed to be cannibalized), but only one death at MD that appeared to be drug-related (dehydration and body weight loss with no evidence of trauma or gavage error). There were no dose dependency and mortalities were even observed in controls, therefore no mortalities could be clearly attributed to daprodustat in this study.
Clinical signs	Dose-related dehydration at MD (1/30) and HD (4/30) during dosing period was observed.
Body weights/Body weight gain	Transient lower body weight gain (6-15%) was observed at the MD and HD between PND 4 and 32, and the body weights were comparable between the control and drug treatment groups by the end of dosing period. Changes in BWG was not correlated with decreased food consumption. BWG decrease persisted in MD and HD females with lower BWG (17% and 20%, respectively) during the recovery period (PND 71-155),

Parameter	Major Findings																		
Developmental hallmarks																			
Sexual maturity and Estrous cycling Assessment	The findings were not direct effect from drug but likely secondary to mean body weight effects. Dose dependent increase in mean age of vaginal patency by 1.7 and 3.1 days, at MD and HD, respectively, was observed which were within the historical control data range (29.3-34.1 days but were considered secondary to decreased mean BW/BWG. The mean estrous cycle length of 4.6 days was increased at HD by 0.4 days compared to controls. The increase was outside the historical control data range (4.2-4.3 days). Increase in number of females (4/20) in continuous (6 or more days) diestrus was noted. The increase was outside the historical control data range of 3 females/study, but within a range observed previously by the testing facility. No drug-related effects were observed in balano-preputial separation in males.																		
Neurobehavioral testing	No drug-related effects were observed in FOD, Acoustic startle habituation, Motor activity, and Morris water maze tests.																		
Ophthalmology	No drug-related effects were observed.																		
Hematology	Daprodustat-related increase in RBC mass, reticulocyte and decrease in platelet were observed: During the dosing period on PND 35, the PCV (%) increased by 20% (male) and 23% (female) at HD with statistical significance ($p \leq 0.01$). At the end of dosing period on PND 64, increases in RBC, hemoglobin, hematocrit, and reticulocyte counts and decrease in platelet were observed in HD animals. At the end of recovery period on PND 160, the change in hematology parameters resolved except the increase in RBC count (11%) in HD males and decrease in platelet count (12%) in HD females.																		
Table 174. Hematology Parameters % Changes Compared to Controls <table border="1"> <thead> <tr> <th>Daprodustat</th> <th>RBC</th> <th>HB</th> <th>HCT</th> <th>Ret</th> <th>PLT</th> </tr> </thead> <tbody> <tr> <td>HD Male</td> <td>12^{↑a}</td> <td>20^{↑a}</td> <td>21^{↑a}</td> <td>48^{↑a}</td> <td>28^{↓a}</td> </tr> <tr> <td>HD Female</td> <td>11^{↑a}</td> <td>16^{↑a}</td> <td>16^{↑a}</td> <td>57^{↑a}</td> <td>16[↓]</td> </tr> </tbody> </table> Source: Reviewer's analysis ^{↑/↓} increase/decrease ^a $p \leq 0.01$ Abbreviations: HB, Hemoglobin; HCT, Hematocrit; PLT, Platelet; RBC, Red blood cells; Ret, Reticulocyte		Daprodustat	RBC	HB	HCT	Ret	PLT	HD Male	12 ^{↑a}	20 ^{↑a}	21 ^{↑a}	48 ^{↑a}	28 ^{↓a}	HD Female	11 ^{↑a}	16 ^{↑a}	16 ^{↑a}	57 ^{↑a}	16 [↓]
Daprodustat	RBC	HB	HCT	Ret	PLT														
HD Male	12 ^{↑a}	20 ^{↑a}	21 ^{↑a}	48 ^{↑a}	28 ^{↓a}														
HD Female	11 ^{↑a}	16 ^{↑a}	16 ^{↑a}	57 ^{↑a}	16 [↓]														
Coagulation	Prolongation of APTT at HD (8% [↑] in males, 21% [↑] in females) with decrease in fibrinogen (24% [↓] in males, 25% [↓] in females) were observed at the end of dosing PND 64, which resolved at the end of recovery period on PND 160. This finding is likely daprodustat-related.																		
Clinical chemistry	Drug-related decrease in total cholesterol by 23% in HD males and 29% in HD females and increase in triglycerides 151% in HD males were observed with statistical significance ($p \leq 0.01$) at the end of dosing on PND 64. This finding resolved at the end of recovery period on PND 160.																		
Urinalysis	No daprodustat-related findings noted.																		
Organ weights	No daprodustat-related findings noted.																		
Femur length	Minimal decrease in femur length of HD males and females (0.97X and 0.98X controls, respectively) was noted at the end of dosing period (PND 64). After the recovery period (PND159), the decrease of femur length remained minimal (0.97X control) in HD males but worsened in the HD females (0.95X controls, $p \leq 0.01$).																		
Macroscopic findings	No daprodustat-related findings noted.																		

Parameter	Major Findings
Microscopic findings	Increased cellularity in the bone marrow of sternum and incidence and/or severity of extramedullary hematopoiesis in the spleen were observed at HD at the end of dosing period (PND 64). The Applicant explains that the increased cells in the bone marrow and spleen were predominately of the erythroid lineage. This finding is likely from the pharmacological effect of drug increasing hematopoiesis. Dilatation of the kidney was noted at low incidence in daprodustat-treated males at $\geq 2/0.8$ mg/kg/day. Increased hepatocellular vacuolation was observed in HD females. Minimal focal epidermal hyperplasia of the skin was noted in one HD female.
Toxicokinetic	Daprodustat was detected in plasma. The systemic exposure (AUC_{0-24hr} and C_{max}) increased with dose increment and the increase was less than dose-proportional. The daprodustat levels were maintained through PND 63 and peaked at PND 21 despite lowering the doses, although dosing was more frequent. There was no evidence of gender difference (< 2-fold) in systemic exposure to daprodustat. The T_{max} ranged between 1.5 – 12 hours, with increased T_{max} on PND 12 (dose dependent).

Table 175. Toxicokinetics Parameters in Juvenile Rats After Daprodustat-Treatment

Parameter	Period	Dose of GSK1278863 (mg/kg) ^b		
		2/0.8	10/4	20/10
AUC_{0-24a} (h* μ g/mL)	PND 4	156	691	961
	PND 10	443	981	1410
	PND 12	465	1300	1490
	PND 21	506	1630	2420
	PND 63	180	770	1430
	Mean	350	1080	1540
	SD	168	391	534
C_{max} (μ g/mL)	PND 4	9.92	38.7	51.9
	PND 10	26.4	47.6	66.4
	PND 12	23.0	59.9	67.9
	PND 21	25.5	80.3	119
	PND 63	9.84	49.7	92.4
	Mean	18.9	55.3	79.4
	SD	8.36	15.9	26.3
T_{max} (hr)	PND 4	1.5	1.5	1.5
	PND 10	1.5	1.5	1.5
	PND 12	8	8	12
	PND 21	7.6	1.5	1.5
	PND 63	1.5	1.5	1.5

Source: Applicant's submission

^a For PND 4 and 10, AUC_{0-24} was estimated by dividing AUC_{0-48} by 2. AUC_{0-24} results are presented for informational purposes (AUC comparison between PNDs 4 and 10 and PNDs 12, 21 and 63) and should be interpreted cautiously.

^b Animals were dosed 2, 10 and 20 mg/kg once every other day (on PNDs 4, 6, 8 and 10) from PNDs 4 through 10. Animals were dosed 0.8, 4 and 10 mg/kg once daily from PNDs 12 through 63.

Abbreviations: AUC , area under the concentration-time curve; C_{max} , maximum plasma concentration; PND, postnatal day; SD, standard deviation; T_{max} , time to maximum concentration

Source: Reviewer's analysis

Abbreviations: APTT, activated partial thromboplastin time; AUC , area under the concentration-time curve; BW, body weight; BWG, body weight gain; C_{max} , maximum plasma concentration; FOD, functional observational battery; HD, high dose; MD, mid dose; PCV, packed cell volume; PND, postnatal day; RBC, red blood cells

13.2.6. Genetic Toxicology

13.2.6.1. Summary of Genetic Toxicology Studies

Genotoxicity Studies With Daprodustat

Study No./ Study Title: 2012N139517/00/ GSK1278863A: Oral Liver Comet Assay in Crl:CD(SD) Rats

Key Study Findings

Male Sprague Dawley rats (n=5/group) were treated with 2 oral doses of 250, 500 or 1000 mg/kg/day daprodustat, approximately 21 to 22 hours apart. The vehicle used was 1% w/v aqueous methylcellulose.

Daprodustat was not genotoxic in vivo Comet assay in the rat liver.

GLP compliance: Yes

Study is valid.

Table 176. Results From the Oral Liver Comet Assay With Daprodustat in Rats

Test Article	Dose ¹ (mg/kg/day)	No. of Animals ²	Liver % Tail Intensity (i.e. % Tail DNA) ³
Vehicle control	0	5M	1.34 (0.43)
GSK1278863	250	5M	1.21 (0.32)
GSK1278863	500	5M	1.19 (0.48)
GSK1278863	1000	5M	1.28 (0.36)
Positive control ⁴	250	3M	13.70 (1.72)

Source: Original NDA submission

¹. All doses are expressed in terms of parent compound

². M = Male

³. Figures in parenthesis = standard deviation

⁴. Positive control = Ethyl methane sulphonate (EMS) produced a clear unequivocal positive response

Genotoxicity Studies With Metabolites of Daprodustat:

M2 (GSK2391220), M3 (GSK2531403 and GSK2506104, enantiomeric M3), M4 (GSK2487818), M5 (GSK2506102), M6 (GSK2531398) or M13 (GSK2531401) metabolites

13.2.7. In Vitro

Bacterial Mutation Assays

M2, M3, M4, M5, M6, or M13 were evaluated at concentrations up to 5000 µg/plate in the absence or presence of an in vitro metabolic activation system (S9-mix) in bacterial mutation assay (Ames Test) with *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and *Escherichia coli* WP2 *uvrA* (pKM101): **Study No.** 2011N120130, 2011N119269, 2011N119441, 2012N143001, 2012N150862, 2012N150330 and 2011N120131, for M2, M3 (GSK2506104), M3 (GSK2531403), M4, M5, M6, and M13, respectively (GLP compliant).

Key Study Findings

M2, M3, M4, M5, M6 and M13 were not mutagenic in the bacterial mutation assay.

In Vitro Mutation Assay With L5178 Mouse Lymphoma Cells at the TK Locus

M2, M3 or M13 were evaluated for 3 or 24 hours either in the presence or absence of metabolic activation system (S9-mix) in L5178U (TK^{+/-}) mouse lymphoma cells:

Study No. 2011N124122, 2011N118972, 2011N120944, and 2011N120224-01 for M2, M3 (GSK2506104), M3 (GSK2531403), and M13, respectively.

Key Study Findings

M2 and M3 were not genotoxic in the presence or absence of metabolic activation for both 3 and 24 hour treatment. Of note, the in vivo chromosome aberration assay in the bone marrow of M13 treated mice and 2-year carcinogenicity in mice with metabolite cocktail containing M13 did not demonstrate genotoxicity or carcinogenicity, respectively.

Table 177. Results From the In Vitro Mutation Assay With Metabolites of Daprodustat in L5178 Mouse Lymphoma Cells

Test Article	Concentration ¹ µg/mL	3 hr Treatment -S9-mix		3 hr Treatment +S9-mix		24 hr Treatment -S9-mix	
		Mean Relative Total Growth (%)	Mean Mutant Frequency (x10 ⁻⁶)	Mean Relative Total Growth (%)	Mean Mutant Frequency (x10 ⁻⁶)	Mean Relative Total Growth (%)	Mean Mutant Frequency (x10 ⁻⁶)
Dimethyl sulfoxide	0	99.72	135.55	99.29	112.89	100.10	89.59
GSK2531401	40	NT	NT	70.40	121.76	NT	NT
GSK2531401	60	NT	NT	63.28	134.48	NP	NP
GSK2531401	100	NP	NP	NP	NP	73.44	135.84
GSK2531401	120	NT	NT	40.57	210.44	NT	NT
GSK2531401	140	NT	NT	28.76	188.87	NT	NT
GSK2531401	160	NT	NT	11.91	265.64 ³	NT	NT
GSK2531401	200	99.92	113.39	NP	NP	NP	NP
GSK2531401	250	64.54	189.90	NT	NT	72.33	119.53
GSK2531401	300	40.75	186.13	NP	NP	NP	NP
GSK2531401	340	23.82	205.37	NT	NT	NT	NT
GSK2531401	350	NT	NT	NT	NT	33.05	193.08
GSK2531401	380	9.69	238.66	NT	NT	NT	NT
GSK2531401	425	NT	NT	NT	NT	17.38	252.89 ³
Methyl methane sulphonate	20	47.43	1010.69 ²	NT	NT	NT	NT
Methyl methane sulphonate	5	NT	NT	NT	NT	71.64	611.23 ²
Dimethyl benzanthracene	1	NT	NT	33.35	1368.52 ²	NT	NT

1. Expressed in terms of parent compound

2. Positive control induced an acceptable response

3. Mean mutant frequency exceeds the sum of the mean vehicle control mutant frequency plus the Global Evaluation Factor (126 x 10⁻⁶). Statistically significant trend (Spearman's rank correlation coefficient). Significant to the 1% level.

NT Not Tested

NP Not plated

Source: Applicant's submission

13.2.8. In Vivo

Title / Study Number: 2011N124885/00/ GSK2531401A: Intravenous Liver Comet Assay in Crl:CD1(Icr) Mice

Key Study Findings

Male Crl:CD1 (ICR) mice (n=5-6/group) were treated with 2 intravenous (IV) doses of 0 (0.9% (w/v) aqueous sodium chloride), 25, 100, 200, or 300 mg/kg GSK2531401A (Metabolite 13; M13), approximately 21 hours apart with a single harvest 3 hours after the last dose. One oral gavage dose of 30 mg/kg Ethyl Methane-Sulfonate (EMS) was used as positive control. 4 independent studies were conducted; however, due to a technical failure of the electrophoresis procedure, the Comet data for one study were invalidated and have not been reported.

Mice given 300 mg/kg M13 convulsed and died immediately following dosing. A mild increase in alanine aminotransferase activity was observed in 3/6 mice given 200 mg/kg/day. In these mice, a corresponding minimal increase in aspartate aminotransferase activity was also noted. Highest individual values in this group (no. 102) were 265 and 263 U/L for alanine aminotransferase and aspartate aminotransferase, respectively. M13 was negative at 25 or 100 mg/kg/day across 3 independent studies. Increase of % Tail DNA was evident at the maximum tolerated dose (MTD) 200 mg/kg/day GSK2531401A (M13). But the positive result was not reproducible across 3 independent studies. Therefore, the result of Comet assay is equivocal.

GLP Compliance: Yes

Study is valid: Yes

Table 178. Result of Liver Comet Assay With Metabolites of Daprodustat in Mice

% Tail DNA					Historical control data 2009-2011 (GSK)#	
Test article	mg/kg/day	Study 1	Study 3	Study 4	Mean	Range
	0	1.52	0.91	1.51		
GSK2531401A	25	--	1.05	1.18	1.23	0.85-1.88
	100	1.08	1.15	0.94		
	200	2.43*	2.36	1.17		
EMS	300	14.52	21.18	13.70		

Source: Reviewer's analysis

*Study 2 was invalidated

Historical control data at GSK based on 55 animals (45 male and 10 female)

* p<0.05

-- Not tested

Title/ Study Number: 2012N132787/00 / GSK2531401A (M13 Metabolite of GSK1278863A): Induction of Chromosome Aberrations in the Bone Marrow of Treated Mice

Key Study Findings

- Male Crl:CD1 (ICR) mice (n=5-6/group) were treated with IV (infusion) doses of 0 (vehicle), 25, 100, or 200 mg/kg GSK2531401A (M13) or 40 mg/kg of cyclophosphamide (positive control), IV (bolus injection). Samples were collected at 16 or 42 hours.
- M13-related reduction of the mitotic index (31% cytotoxicity as measured via mitotic inhibition relative to the concurrent vehicle control) in bone marrow was observed at 16 hours. Chromosome aberrations were not induced by M13 in the bone marrow cells.

GLP Compliance: Yes

Study is valid: Yes

Table 179. Results From the Chromosome Aberrations Assay With Metabolites of Daprodustat in Mice

Test article	Dose ¹ (mg/kg)	Sampling Time (hours)	Mitotic Index (MI)	Cytotoxicity (% reduction in MI compared to control value)	Aberrant Cells (excluding gaps) Mean %
Vehicle control	0	16	5.4	-	0.40
GSK2531401	25	16	4.8	11%	0.20
GSK2531401	100	16	4.5	17%	0.42
GSK2531401	200	16	3.7	31%	0.17
Cyclophosphamide	40	16	1.9	65%	20.6*

Source: Applicant's submission

Carcinogenicity Study

Title/ Study Number: GSK1278863A: A 2-Year Oral (gavage) Carcinogenicity Study in Rats/ 2014n196080

Key Study Findings

A daprodustat-related neoplastic finding was observed. The incidence of fibroadenoma in the mammary gland at HD (4 mg/kg/day) in males was statistically significant and the incidence rate was approximately 13% which exceeds the historical control data range (maximum incidence rate 6.52%). However, the incidence of the combination of adenoma and fibroadenoma in the mammary gland was not statistically significant. Regardless of the daprodustat-related fibroadenoma findings in males, the clinical relevance of fibroadenoma in the mammary gland of males is considered to be low.

Table 180. Methods for the 2-Year Carcinogenicity Study in Rats

Study Feature	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	0 (vehicle), 0.02, 0.1, 0.8, or 4 (males)/ 7 (females) mg/kg/day, QD
Route of administration:	Oral gavage
Formulation/vehicle:	Suspension/ 1% (w/v) methyl cellulose (400 cP@ 2%) with 0.2% Tween 80 (v/v) with 5mM NaOH in Ultra-Pure Water, pH 6 (±0.2)

NDA 216951
Jesduvroq (daprodustat)

Species/strain:	Rat/Sprague-Dawley
Number/sex/group:	70/Sex/Group, 3 to 9/Sex/TK Group
Satellite groups:	TK group: 3/sex/TK Groups for 0.02 and 0.8 mg/kg/day 6/Sex/TK Groups for 0 and 0.1 mg/kg/day 9/Sex/TK Group for 4 or 7 mg/kg/day for males and females
Study design:	
Deviation from study protocol affecting interpretation of results:	None of the deviations impacted the overall integrity of the study or interpretation of study results
Source: Reviewer's analysis	
Abbreviations: QD, once daily; TK, toxicokinetic	

Title/ Study Number: GSK1278863A: A 2-Year Carcinogenicity Study in Mice/ 2014n221714

Key Study Findings

There were no neoplastic changes attributed to oral (gavage) administration of GSK1278663A at doses ≤ 3 mg/kg/day with concomitant SC treatment of metabolic cocktail (M2, M3, M13) to Crl:CD1 (ICR) mice for up to 104 weeks. There was no significant or dose-related increase in the incidence of tumors.

Table 181. Methods for the 2-Year Carcinogenicity Study in Mice

Study Feature	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	Daprodustat: 0, 0.2, 0.8, 0.8 or 3 mg/kg/day, QD Daprodustat metabolite cocktail: GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13) 2, 2.5, and 1.3 mg/kg/day, respectively, QD
Route of administration:	Oral Gavage of daprodustat (QD), Subcutaneous injection of metabolite cocktail M2, M3, M13 (QD)
Formulation/vehicle:	Oral formulation (Daprodustat): 1% (w/v) methyl cellulose (400 cP@ 2%) with 0.2% Tween 80 (v/v) with 5mM NaOH in ultra pure water, pH 6 (± 0.2) Subcutaneous formulation (M2, M3 and M13; metabolite cocktail): Aqueous 25mM phosphate buffer, pH7.4 (± 0.2)
Species/strain:	Mice/Crl:CD1 (ICR)
Number/sex/group:	Main study: 30/sex/group
Satellite groups:	TK group: 12/sex/control, 51/sex/group daprodustat groups

Study design:

Table 182. Study Design of 2-Year Carcinogenicity Study in Mice

Group No.	Dose Level (mg/kg/day) #		Dose Volume (mL/kg)		Dose Concentration (mg/mL) #		Number of Animals			
	Oral	SC	Oral	SC	Oral	SC	Main Study		Toxicokinetic Study ^a	
							Males	Females	Males	Females
1/ Reference Items (oral + SC)	0	0	10	1	0	0	60	60	12	12
2/ GSK1278863(oral) + metabolites (SC) §	0.2	2.0, 2.5 and 1.3	10	1	0.02	2.0, 2.5 and 1.3	60	60	51	51
3/ GSK1278863(oral) + Reference Item (SC)	0.8	0	10	1	0.08	0	60	60	0	0
4/ GSK1278863(oral) + metabolites (SC) §	0.8	2.0, 2.5 and 1.3	10	1	0.08	2.0, 2.5 and 1.3	60	60	51	51
5/GSK1278863 (oral) + metabolites (SC) §	3	2.0, 2.5 and 1.3	10	1	0.3	2.0, 2.5 and 1.3	60	60	51	51

Source: Applicant's submission

Deviation from study
protocol affecting
interpretation of results:

None of the deviations impacted the overall integrity of the study or
interpretation of study results

Source: Reviewer's analysis

Abbreviations: QD, once daily

14. Clinical Pharmacology

14.1. In Vitro Studies

14.1.1. Plasma protein binding

Plasma Protein Binding of Daprodustat

In a study to determine the extent of the binding of daprodustat to plasma proteins from mouse, rat, rabbit, dog, monkey, and human pooled plasma (3 subjects/pooled sample), plasma samples were incubated, using equilibrium dialysis, at daprodustat concentrations of 0.2, 1, 10 and 50 µg/mL [Report CD2010/00340]. In addition, the protein binding of daprodustat to human serum albumin (HSA) (0.600mM) or α1-acid glycoprotein (AAG) (0.015 and 0.150mM) was determined using equilibrium dialysis at daprodustat concentrations of 0.2, 1, 10 and 30 µg/mL.

The binding of daprodustat to plasma protein was high in all species evaluated (generally >98%), specifically in human, 99.4% at the three low test concentrations, and 98.3% at 50 µg/mL. Binding was also high to HSA (>99.3%), while low to AAG (<20%). In general, there was no evidence of concentration-dependent protein binding of daprodustat in all species over the concentration range evaluated.

Plasma Protein Binding of Metabolites of Daprodustat

In a study to determine the extent of human plasma protein binding for the six predominant circulating human metabolites of daprodustat [M2 (GSK2391220), M3 (GSK2531403 and GSK2506104), M4 (GSK2499166), M5 (GSK2531399), M6 (GSK2531398), and M13 (GSK2531400 and GSK2531401)], pooled human plasma was obtained from three non-fasted

male donors [2012N131897]. Binding was determined by equilibrium dialysis at concentrations of 10, 100, 250 and 500 ng/mL and were incubated for 18 hours. Concentrations of each metabolite were determined by ultra-performance liquid chromatography with tandem mass spectroscopy (UPLC-MS/MS). The plasma protein binding of M2, M3 (both enantiomers), M6, and M13 (both enantiomers) was determined to be low, less than 34% for each metabolite across concentrations. There was no evidence of concentration-dependent protein binding of M2, M3, M6, and M13 over the range evaluated. The plasma protein binding of M4 and M5 was determined to be lower (68 and 74%, respectively) at the lowest test concentration (10 ng/mL) and increased with increasing concentrations up to ~98%, suggesting concentration-dependent protein binding of M4 and M5 over the concentration range evaluated.

14.1.2. Distribution in red blood cells

The in vitro blood cell association of daprodustat was determined in blood (a single human plasma sample) by adding daprodustat at concentrations of 1.0 and 10 µg/mL (n=3/concentration/species, 0.5% (v/v) DMSO) after incubation for 30 minutes. All quantitative analysis was by LC-MS/MS. There was generally minimal association of daprodustat with blood cells in vitro with blood/plasma ratios of 1.23 and 0.75 at 1.0 and 10 µg/mL, respectively.

14.1.3. Metabolism studies

Oxidative Enzymology in Human Liver Microsomes

The oxidative enzymology of [¹⁴C]daprodustat (5µM) was investigated using pooled human liver microsomes and rh-CYP enzymes (Supersomes™). Human liver microsomes from mixed-gender pools of 15 human livers were used. Inhibition of daprodustat metabolism was investigated by incubating [¹⁴C]daprodustat with human liver microsomes in the presence and absence of the selective CYP inhibitors: azamulin, sulphaphenazole, quinidine, montelukast, furafylline, and benzylnirvanol (inhibitors of CYP3A4, CYP2C9, CYP2D6, CYP2C8, CYP2C19, CYP1A2, respectively). Supersomes contained individually over-expressed rh-CYP enzymes (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) derived from baculovirus infected insect cells. [¹⁴C]daprodustat incubations with pooled human liver microsomes or recombinant CYP enzymes were conducted. The reactions were started by the addition of an NADPH regenerating system. The supernatants from [¹⁴C] GSK1278863 incubations with human liver microsomes or recombinant CYP enzymes were analyzed by radio-HPLC. [¹⁴C]daprodustat was metabolized in human liver microsomes to three metabolites, M8, M9/M22, and M10 (mono-oxidation). An additional NADPH-dependent radio-peak was detected in incubations with human liver microsomes. This peak was not detected by LC/MS and therefore was not structurally characterized, but its rate of formation was calculated. These metabolites, which constituted 95% of the total radioactivity, were inhibited by montelukast, a selective CYP2C8 inhibitor. [¹⁴C]daprodustat was metabolized to M2, M3, M4, M5, M6, M7, M8, M9/M22, M10, and M17, in human recombinant CYP2C8 incubations. Metabolites M2 through M7 (di-oxidation) were not detected in human liver microsomes. In human recombinant CYP3A4 incubations only M8 and M9/M22 were formed but turnover was low and only constituted 5% turnover. There were no metabolites detected in incubations with recombinant CYP1A2, CYP2C9, CYP2C19 or

CYP2D6. The data from human liver microsomes with selective inhibitors and scaled results from recombinant human CYP enzymes suggest that CYP2C8 is the primary CYP enzyme involved in the oxidative metabolism of daprodustat in vitro with a minor contribution by CYP3A4.

Induction of CYP Enzymes in Human Hepatocytes

The effects of daprodustat (0.1, 0.3, 1.0, 3.0, 10, 30 and 100 μ M) on the mRNA levels of CYP1A2, 2B6 and 3A4 were evaluated in cultured human hepatocytes from three donors after exposure to daprodustat for 48 hours. The mRNA level for each specific CYP was determined using quantitative real-time polymerase chain reaction technology (TaqMan™). No notable increases in the mean mRNA levels of CYP1A2, CYP2B6 or CYP3A4 were observed. Minor reductions in mRNA levels (ratio of treated over control <0.5) were apparent for CYP1A2 at 100 μ M and CYP3A4 at all concentrations tested and the decreases in mRNA level observed may not be associated with hepatocyte health because no decreases in total RNA and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were observed. Treatment with daprodustat caused no notable increases in the mean mRNA levels of CYP1A2, CYP2B6 or CYP3A4 within the concentration range examined.

Inhibition of CYP Enzymes in Human Microsomes

The potential of daprodustat to inhibit CYP1A2, 2C9, 2C19, 2D6 or 3A4 in a concentration-dependent or metabolism dependent manner was investigated in vitro with human liver microsomes and using selective probe substrates in the presence and absence of daprodustat (0.1 to 100 μ M). For concentration-dependent inhibition (half maximal inhibitory concentration [IC₅₀] determination), daprodustat (0.1 to 100 μ M) was pre-incubated with human liver microsomal protein and the respective probe substrates (at a final concentration approximating the Km) with NADPH (NADP⁺, glucose-6-phosphate and glucose 6-phosphate dehydrogenase) generating system was added to start the reactions. The selective probe substrates used in the presence and absence of daprodustat were: hydroxylation of phenacetin (1A2); 4-hydroxylation of diclofenac (2C9); 4-hydroxylation of S-mephenytoin (2C19); 1-hydroxylation of bufuralol (2D6); 1-hydroxylation of midazolam, oxidation of nifedipine and o-hydroxylation of atorvastatin (3A4). The reactions to measure metabolism-dependent inhibition were carried out in the presence and absence of NADPH.

The potential for daprodustat to inhibit the major human CYPs was low in human liver microsomes with all IC₅₀ values >100 μ M. In addition, daprodustat did not display any metabolism-dependent inhibition in human liver microsome CYP1A2, 2C9, 2C19, 2D6 or 3A4-nifedipine assays.

14.1.4. Transporter interaction studies

P-glycoprotein Transport and Membrane Permeability

P-gp Substrate Potential and Permeability

The P-gp substrate activity and intrinsic apparent permeability of daprodustat were evaluated in Madin Darby canine kidney (MCDK) cells transfected with the human multi-drug resistant 1

gene (hMDR1) [Report UH2008/00018]. Bidirectional [apical-to-basolateral (A→B) and basolateral-to-apical (B→A)] transport of daprodustat (5μM) was studied across MDCKII-hMDR1 cell monolayers in the absence and presence of GF120918 (P-gp inhibitor at 2μM). The apical efflux ratio was defined as the ratio of unidirectional transmonolayer flux of test compound across the cell monolayers from the basolateral to apical (B→A) compartment divided by that of the same compound from the A→B compartment. An apical efflux ratio >2 for a compound suggests P-gp-mediated transport. Daprodustat was not a substrate of human P-gp in vitro, as the apical efflux ratio was ~1 in the absence or presence of GF120918 in MDCKII-hMDR1 cell monolayers.

Passive Membrane Permeability

A study was conducted to determine the passive membrane permeability and the absorptive membrane permeability of daprodustat using bio-relevant buffer [fasted state simulated intestinal fluid (FaSSIF)] in MDCKII-hMDR1 cells in the presence of the potent P-gp inhibitor, GF120918 [Report CD2008/00421]. The passive membrane permeability of [¹⁴C]daprodustat (3μM) was determined at pH 7.4, and the absorptive membrane permeability of [¹⁴C]daprodustat (3μM) was determined at pH 5.5 and 7.4 to simulate conditions in the gastro-intestinal tract. Permeabilities were measured using MDCKII-hMDR1 cell monolayers in the presence of GF120918.

[¹⁴C]daprodustat was determined to have moderate passive membrane permeability of 21 nm/s at pH 7.4. The absorptive membrane permeabilities of [¹⁴C]daprodustat in the presence of FaSSIF were both high at pH 7.4 and pH 5.5 with a P7.4[abs] value of 191 nm/s and a P5.5[abs] value of 325 nm/s. The presence of biorelevant buffer, FaSSIF, increased the permeability of daprodustat. In addition, daprodustat permeability is increased at pH 5.5 ([Table 183](#)).

Table 183. Daprodustat In Vitro Passive and Absorptive Membrane Permeability

Compound	Permeability	pH	Transport medium		Px (Permeability coefficient) (nm/s)	Permeability Class
			Apical well	Basolateral well		
3 μM [¹⁴ C]GSK1278863G	Passive	7.4	DMEM	DMEM	21 ± 2	Moderate
	Absorptive	7.4	FaSSIF	DMEM + 1% HSA	191 ^a	High
	Absorptive	5.5	FaSSIF	DMEM + 1% HSA	325 ± 6.9	High
3 μM [³ H]amprenavir	Passive	7.4	DMEM	DMEM	171 ± 13.6	High

Data are the mean ± SD from three monolayers unless noted

a: Results for absorptive permeability at pH 7.4 are the mean of two wells due to sampling error of one well.

The pH of DMEM in all investigations is pH 7.4

Permeability classification was as low (<10 nm/s), moderate (10 - 100 nm/s) or high (>100 nm/s).

FaSSIF: Fasted State Simulated Intestinal Fluid. DMEM: Dulbecco's Modified Eagle Medium. HSA: Human serum albumin

Source: Report CD2008/00421

P-gp Transport Inhibition

Daprodustat was evaluated as an in vitro inhibitor of human P-gp [Report CD2008/00669]. Inhibition of P-gp-mediated transport of digoxin (0.03μM) by daprodustat (0.1 to 100μM) was investigated by determining the basolateral to apical ([B→A]) transport of [³H]digoxin by MDCKII-hMDR1 cells transfected with the human MDR1 gene, which express the P-gp protein. Daprodustat did not inhibit transport of digoxin via human P-gp.

Evaluation as Human BCRP Substrate

The bi-directional transport of daprodustat (3μM) was investigated using MDCKII-breast cancer resistance protein (BCRP) cells transfected with the human BCRP gene and heterologously

expressing human BCRP protein [Report 2015N237800]. Assessments were made in the presence and absence of the BCRP inhibitor, GF120918. After 90 minutes of incubation, samples were analyzed by liquid scintillation counting or LC-MS/MS. A compound is classified as a BCRP substrate if the apical efflux ratio in the absence of inhibitor GF120918 is ≥ 2 and this efflux collapses to ~ 1 in the presence of inhibitor, GF120918. The apical efflux ratio of daprodustat at $3\mu\text{M}$ was determined as 4.4 and 0.65 in the absence and presence of GF120918, respectively, in the MDCKII-BCRP cell line. Thus, daprodustat is a substrate of human BCRP with an efflux ratio of 4.4 at a concentration of $3\mu\text{M}$.

Table 184. In Vitro BCRP Substrate Potential of Daprodustat

Compound	Rate A→B (nmoles/h/cm ²)	Rate B→A (nmoles/h/cm ²)	Apical Efflux Ratio	BCRP Substrate	A→B Mass Balance (%)	B→A Mass Balance (%)
3 μM GSK1278863	0.032 $\pm$ 0.0018	0.14 $\pm$ 0.0073	4.4	Y	93 $\pm$ 3.1	96 $\pm$ 6.8
3 μM GSK1278863 + 2 μM GF120918A	0.042 ^a $\pm$ 0.0024	0.027 $\pm$ 0.0036	0.65	-	85 ^a $\pm$ 3.3	86 $\pm$ 1.6
3 μM [¹⁴ C]cimetidine	0.040 $\pm$ 0.0056	0.17 $\pm$ 0.0028	4.2	Y	99 $\pm$ 0.81	99 $\pm$ 0.19
3 μM [¹⁴ C]cimetidine + 2 μM GF120918A	0.051 $\pm$ 0.0097	0.030 $\pm$ 0.0064	0.59	-	100 $\pm$ 1.8	100 $\pm$ 0.36

Data presented are the mean $\pm$ SD of 4 replicates, unless otherwise indicated

a: Mean of 3 replicates, one replicate failed mass balance criteria.

Source: Report 2015N237800

Abbreviations: BCRP, Breast Cancer Resistance protein; SD, standard deviation

OATP1B1 and OATP1B3 Substrate Potential

To evaluate the substrate potential of daprodustat and its six predominant circulating human metabolites; M2 (GSK2391220), M3 (GSK2506104), M4 (GSK2487818), M5 (GSK2506102), M6 (GSK2531398) and M13 (GSK2531401), the time-dependent transport of daprodustat and metabolites (0.01 to $10\mu\text{M}$) in OATP1B1 and OATP1B3 transporter expressing Human Embryonic Kidney (HEK-293) cells was measured by LC-MS/MS [Report 2015N255319].

Daprodustat was a substrate of OATP1B1 but was not a substrate of OATP1B3. M13 was not a substrate of OATP1B1 but was a substrate of OATP1B3. M2, M4, M5, and M6 were substrates of the OATP1B1 and OATP1B3 transporters. M3 was not a substrate of OATP1B1 nor OATP1B3.

Inhibition of OATP1B1 and OATP1B3

In a study to determine if daprodustat is an inhibitor of the human OATP1B1 and OATP1B3, the inhibition of uptake of the probe substrate [³H]estradiol 17 β -D-glucuronide ([³H]EG) by daprodustat (0.1 to $100\mu\text{M}$, n=3/concentration) was investigated using OATP1B1 (heterologously expressed in Chinese Hamster Ovary (CHO) cells) and OATP1B3 (expressed in human embryonic kidney MSRII (HEK-MSRII) cells after transduction of a BacMam baculovirus coding for OATP1B3) [Report CD2008/00231]. Cells were incubated for 5 minutes for OATP1B1 and 10 minutes for OATP1B3. For analysis of total radioactivity, the contents of each well were determined by liquid scintillation counting in glass vials containing scintillant.

Daprodustat inhibited OATP1B1 and OATP1B3 with calculated IC₅₀ values of $6\mu\text{M}$ and $11\mu\text{M}$, respectively.

Table 185. In Vitro OATP1B1 and OATP1B3 Inhibition by Daprodustat

Test Compound	Concentration (µM)	OATP1B1		OATP1B3	
		Uptake Rate of [3H]EG (fmol/cm ² /min)	Uptake Rate of [3H]EG (% control)	Uptake Rate of [3H]EG (fmol/cm ² /min)	Uptake Rate of [3H]EG (% control)
[3H]EG only	None	12 ^a ± 0.083	100 ± 0.68	6.3 ^c ± 0.28	100 ± 4.5
[3H]EG + rifamycin	10	0.49 ± 0.014	4.0 ^b ± 0.12	0.27 ^c ± 0.033	4.2 ^d ± 0.53
[3H]EG + GSK1278863	0.1	13 ± 0.051	110 ± 0.42	5.6 ± 0.11	90 ± 1.8
	1	12 ± 0.21	96 ± 1.7	5.8 ± 0.20	92 ± 3.2
	3	9.3 ± 0.38	75 ± 3.1	5.4 ± 0.080	86 ± 1.3
	10	4.8 ± 0.28	39 ± 2.3	3.3 ± 0.18	52 ± 2.8
	30	0.94 ± 0.037	7.6 ± 0.30	0.62 ± 0.060	9.9 ± 0.95
	100	0.49 ± 0.065	4.0 ± 0.53	0.17 ± 0.008	2.7 ± 0.13
IC50 (µM) of GSK1278863		6.0		11	

Key:

Data is the mean ± SD from three wells after background correction.

a: Acceptance criteria ≥6 fmol/cm²/min

b: Acceptance criteria ≤15% of control [3H]EG value

c: Acceptance criteria ≥3-fold signal to noise (average probe substrate uptake rate in uninhibited control wells/average uptake in rifamycin inhibited wells)

d: Acceptance criteria ≤33% of control [3H]EG value

Source: Report CD2008/00231

Abbreviations: [3H]EG, [3H] estradiol glucuronide; IC₅₀, half maximal inhibitory concentration

In a study to determine if the six predominant circulating human metabolites of daprodustat [M2 (GSK2391220), M3 (GSK2531403), M4 (GSK2487818), M5 (GSK2506102), M6 (GSK2531398) and M13 (GSK2531401)] are inhibitors of the human OATP1B1, transport inhibition of fluorescein labelled methotrexate via the OATP1B1 (expressed in HEK-MSR2 cells after transduction of a BacMam baculovirus coding for OATP1B1) was evaluated after the incubation with each metabolite (0.085 to 50µM, n=8) [Report 2013N166580]. Metabolites M2, M3, M4, M5, M6 and M13 did not inhibit human OATP1B1 and OATP1B3 at concentrations ≤50µM.

Substrate Potential With OAT1, OAT3, OCT2, MATE1 and MATE2-K

In a study to evaluate the potential of daprodustat and six predominant circulating human metabolites of daprodustat [M2 (GSK2391220), M3 (GSK2506104), M4 (GSK2487818), M5 (GSK2506102), M6 (GSK2531398) and M13 (GSK2531401)] to act as substrates of OAT1, OAT3, OCT2, MATE1 and MATE2-K, the substrate potential of each test article was determined by measuring the time-dependent uptake of the test article in transporter expressing and control cells (S2 cells for OAT1 and OAT3; HEK-293 cell for OCT2, MATE1 and MATE2-K) [Report 2015N249257]. After incubating each test article with the various transporter expressing cells, the cells were washed and quantity of each test article associated with the cells was measured by LC-MS/MS. Daprodustat, M4, M5 and M6 were not substrates of the transporters OAT1, OAT3, OCT2, MATE1 and MATE2-K. M3 and M13 were substrates of OAT3, but not of OAT1, OCT2, MATE1 and MATE2-K. M2 was a weak substrate of OAT1 and OAT3, but not of OCT2, MATE1 and MATE2-K.

Inhibition of OAT1, OAT3, OCT2, MATE1 and MATE2-K

In a study to evaluate daprodustat and six predominant circulating human metabolites of daprodustat [M2 (GSK2391220), M3 (GSK2506104), M4 (GSK2487818), M5 (GSK2506102), M6 (GSK2531398) and M13 (GSK2531401)] for inhibition of the OAT1 and OAT3, OCT2 and MATE1 and MATE2-K (expressed in solute carrier (SLC) transporter expressing cells (S2) (OAT1 or OAT3) or HEK-293 cells (OCT2, MATE1 or MATE2-K)) activity of each transporter was measured by liquid scintillation counting (LSC) using respective radiolabeled probe

substrates in the absence or presence of an inhibitor cocktail at a single concentration of each (daprodustat, 1.4 μ M; M2, 20 μ M; M3, 20 μ M; M4, 10 μ M; M5, 5 μ M; M6, 10 μ M and M13, 10 μ M) [Report 2015N242070]. Daprodustat and its six predominant circulating human metabolites (M2, M3, M4, M5, M6 and M13) did not inhibit OAT1, OAT3, OCT2, MATE1, and MATE2-K-mediated transport of each respective probe substrate.

Daprodustat Uptake by Human Hepatocytes

In a study to investigate the transport processes involved in the in vitro hepatic uptake of daprodustat (0.5 and 0.3 μ M) using cryopreserved human hepatocytes from 3 different donors, uptake experiments were conducted in the presence and absence of an inhibitor cocktail [Report 2015N232657]. Incubations performed in the presence of inhibitor cocktail were used to determine the passive uptake of daprodustat into the human hepatocytes. The inhibitor cocktail included the rifamycin SV and cyclosporine A (inhibitors of OATP1B1 and OATP1B3), montelukast (inhibitor of OATP2B1) and imipramine (inhibitor of OCT1). [3H]estradiol glucuronide ([3H]EG) (0.02 μ M) was used as positive control substrate in a separate set of incubations under the conditions stated. Daprodustat uptake in cryopreserved human hepatocytes from 3 different donors, at 0.5 or 0.3 μ M, was not inhibited by the inhibitor cocktail, suggesting a primarily passive process is involved. Uptake of [3H]EG was inhibited by the inhibitor cocktail, with a signal-to-noise ratio between 3.4 and 7.9, demonstrating functionality of the cell system. There was no notable difference (≥ 2 -fold) in daprodustat transport into cryopreserved human hepatocytes in the presence of prototypical inhibitors (cyclosporine A, rifamycin, montelukast, and imipramine) of OATP1B1, OATP1B3, OATP2B1 or OCT1 transporters, suggesting that daprodustat is not a substrate of these transporters.

M4 Uptake by Human Hepatocytes

In a study to investigate the time dependent, active uptake in the in vitro hepatic uptake of daprodustat metabolite M4 (GSK2487818) (0.2, 2 and 15 μ M) using pooled cryopreserved human hepatocytes, uptake experiments were conducted in the presence and absence of an inhibitor cocktail [Report 2015N259558]. Incubations performed in the presence of inhibitor cocktail were used to determine the passive uptake of M4 into the human hepatocytes. The inhibitor cocktail included the rifamycin SV and imipramine. [3H]estradiol glucuronide ([3H]EG) (0.02 μ M) was used as positive control substrate in a separate set of incubations under the conditions stated. M4 uptake in cryopreserved pooled human hepatocytes at 15 μ M was not inhibited by the inhibitor cocktail, suggesting a primarily passive process is involved. Uptake of [3H]EG was inhibited by the inhibitor cocktail, with a signal to noise ratio of 3.8 to 4.2, demonstrating functionality of the cell system. There was no notable difference (≥ 2 -fold) in M4 transport into pooled cryopreserved human hepatocytes in the presence of prototypical inhibitors (rifamycin, and imipramine) of OATP1B1, OATP1B3, OATP2B1 or OCT1 transporters, suggesting that M4 is not a substrate of these transporters in a qualified physiologically relevant system.

14.2. In Vivo Studies

Single Dose in Renal Impairment Subjects (Study PHI112843)

Title: A Phase I, single-dose, randomized and single-blind (Part 1) fixed sequence and open-label (Part 2), study to evaluate the safety, pharmacokinetics, and pharmacodynamics of GSK1278863A in subjects with renal impairment and matched healthy volunteers.

Study Design: This was a single-center, Phase I, 2-part study. Part 1 was conducted in subjects with moderate (Stage 3; eGFR 30 to 59 mL/min/1.73 m²) to severe (Stage 4; eGFR 15 to 29 mL/min/1.73 m²) renal impairment (Cohort 1) and subjects with normal renal function (eGFR $\geq$ 90 mL/min/1.73 m²; Cohort 2) and employed a single-dose, 2-period crossover, randomized sequence, with daprodustat dose levels of 50 and 150 mg. Part 2 was conducted in subjects with hemodialysis-dependent renal impairment (Stage 5; eGFR <15 mL/min/1.73 m²; Cohort 3) and employed a single-dose, 2-period, fixed sequence, open-label design. During Part 2, study drug was administered before dialysis in Period 1 and post-dialysis in Period 2, with a single daprodustat dose of 150 mg in both periods. Blood samples from the arterial and venous side of the circulation were compared at fixed timepoints following dosing during an active dialysis session (Period 1) to evaluate the effect of dialysis on daprodustat plasma concentrations.

Treatment periods for both Part 1 and Part 2 were separated by a 7 to 10-day washout period. Biomarkers associated with erythropoiesis, namely EPO, reticulocyte count, hemoglobin (Hb), Hct, RBC, and hepcidin as well as markers related iron metabolism and utilization, including serum ferritin, TIBC, and UIBC, were also assessed. VEGF was also measured in this study. Pharmacodynamic (PD) assessments occurred out to 48 hours post-dose.

Results and Conclusions

Pharmacokinetics

Daprodustat pharmacokinetic (PK) parameters in subjects with Stages 3 to 4 (moderate to severe) renal impairment and their matched healthy subjects are similar. Daprodustat exposure [AUC(0-inf)] increased in a dose-proportional manner for the 50 and 150 mg doses. There were no marked differences in daprodustat PK between subjects with moderate to severe renal impairment (Cohort 1) and matched healthy subjects (Cohort 2). Daprodustat PK was similar in hemodialysis subjects (Cohort 3) on a non-dialysis day and subjects with moderate to severe renal impairment (Cohort 1). In HDD subjects (Cohort 3), plasma daprodustat exposures tended to be higher during an active dialysis session compared to those observed on the day without dialysis, indicating a reduction in the clearance of daprodustat during the dialysis procedure. There were no consistent differences in daprodustat concentrations between the arterial and venous samples, suggesting daprodustat was not significantly cleared by hemodialysis.

Table 186. Daprodustat Pharmacokinetic Parameters Following Single-Dose Administration (Study PHI12843)

Parameter	Study period	Geometric mean (CV%)									
		Moderate to Severe Renal Impairment, Non-dialysis (Cohort 1)				Normal, Healthy Subjects (Cohort 2)				Severe Renal Impairment, Hemodialysis (Cohort 3)	
		N=8		N=4		N=4		N=4			
	n	50 mg	n	150 mg	n	50 mg	n	150 mg	n	150 mg	
C _{max} (ng/mL)	Period 1	4	442.643 (70.25)	4	2001.681 (26.15)	1	634.670	3	1350.562 (50.67)	4	2178.434 (50.88)
	Period 2	4	892.094 (34.93)	4	1560.810 (61.26)	3	563.246 (24.24)	1	1786.722	4	1574.335 (36.60)
	TOTAL	8	628.394 (66.00)	8	1767.553 (44.76)	4	580.310 (20.62)	4	1448.439 (43.31)	8	1851.914 (44.77)
T _{max} * (hr)	Period 1	4	1.000 (1.00-1.00)	4	1.500 (1.00-4.00)	1	1.000 (1.00-1.00)	3	2.000 (1.00-3.00)	4	1.750 (1.00-2.50)
	Period 2	4	2.000 (1.00-3.00)	4	1.500 (1.00-3.02)	3	3.000 (2.00-3.00)	1	1.000 (1.00-1.00)	4	1.000 (1.00-1.00)
	TOTAL	8	1.000 (1.00-3.00)	8	1.500 (1.00-4.00)	4	2.500 (1.00-3.00)	4	1.500 (1.00-3.00)	8	1.000 (1.00-2.50)
AUC(0-24) (hr*ng/mL)	Period 1	--	--	--	--	1	1489.112	3	3836.708 (38.29)	4	6236.685 (57.23)
	Period 2	4	1726.414 (63.78)	4	3618.040 (35.49)	3	1371.529 (35.30)	1	3080.310	4	3552.857 (44.16)
	TOTAL	4	1726.414 (63.78)	4	3618.040 (35.49)	4	1400.024 (28.85)	4	3631.764 (32.99)	8	4707.234 (57.80)
AUC(0-inf) (hr*ng/mL)	Period 1	4	1113.597 (36.64)	4	4948.861 (31.08)	1	1489.235	3	3842.137 (38.18)	4	6252.600 (57.55)
	Period 2	4	1726.615 (63.78)	4	3620.741 (35.52)	3	1371.822 (35.31)	1	3081.059	4	3554.539 (44.18)
	TOTAL	8	1386.634 (53.92)	8	4233.030 (35.44)	4	1400.278 (28.86)	4	3635.839 (32.92)	8	4714.352 (58.02)
t _{1/2} (hr)	Period 1	4	1.350 (17.50)	4	1.645 (39.86)	1	1.829	3	2.096 (77.90)	4	4.105 (85.32)
	Period 2	4	1.500 (29.18)	4	2.200 (59.02)	3	1.933 (10.91)	1	1.385	4	2.097 (36.83)
	TOTAL	8	1.423 (22.90)	8	1.902 (49.04)	4	1.906 (9.33)	4	1.890 (65.73)	8	2.934 (72.01)

Source: Study PHI12483 Report, Table 18

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CV, coefficient of variation; T_{1/2}, half-life; T_{max}, time to maximum concentration

Pharmacodynamics

Rapid, dose-dependent increases were observed in mean EPO concentrations in all cohorts following dosing in Period 1, with approximately 3- to 10-fold greater increases observed in subjects with renal impairment (Cohorts 1 and 3) as compared to normal healthy subjects. In Cohort 3, EPO concentrations were higher in Period 1 as compared to Period 2, which may be function of the higher plasma concentrations observed in these subjects during the dialysis period as compared to the period without dialysis. At 48 hours post-dose, EPO concentrations in the healthy subjects (Cohort 2) had returned to near-baseline levels, but remained elevated above baseline concentrations in subjects with renal impairment and hemodialysis subjects.

Mean total RBC counts were similar between the 3 cohorts at baseline and increased slightly in all cohorts at 24 and 48 hours post-dose during Period 1. Total RBC in healthy subjects

(Cohort 2) and hemodialysis subjects (Cohort 3) returned to baseline levels at the beginning of Period 2 but remained elevated in subjects with renal impairment (Cohort 1). Mean hemoglobin concentrations in subjects with renal impairment (Cohort 1) increased gradually over time. In healthy subjects (Cohort 2), mean Hb concentrations increased following dosing in both periods, but no obvious dose-related trends were observed. In hemodialysis subjects (Cohort 3), small increases in mean Hb concentrations were noted at 24 hours following dosing in Period 1, but levels returned to baseline at the follow-up assessment.

Repeat Dose (14 Days) in Renal Impairment Subjects (Study PHI115573)

Study Title: A repeat-dose, open-label, parallel-group study to assess the pharmacokinetics of daprodustat and metabolites in normal subjects and subjects with impaired renal function.

Study Design: This was a repeat-dose, parallel-group study in subjects with normal renal function, non-hemodialysis subjects with renal impairment (Stages 3/4/5 CKD), and hemodialysis subjects with Stage 5 CKD. All subjects received a 5 mg daprodustat dose once daily for 14 days (normal renal function and non-hemodialysis subjects) or 15 days (hemodialysis subjects). Blood samples were collected on Days 1 and 14 for all subjects and additionally on Day 15 for hemodialysis subjects to allow for collection on dialysis and non-dialysis days. EPO and hepcidin were also assessed.

Results and Conclusions

Pharmacokinetics

Daprodustat exposure ($AUC_{0-\infty}$ and C_{max}) following single and/or repeat doses was not clinically different in subjects with normal renal function (190 ng.h/mL and 93 ng/mL, respectively), subjects with Stage 3/4 non-dialysis (ND) CKD (176 ng.h/mL and 75 ng/mL), or subjects with Stage 5 hemodialysis CKD on a dialysis day [213 ng.h/mL and 74 ng/mL] and on a non-dialysis day [188 ng.h/mL and 76 ng/mL]. The steady-state C_{max} of daprodustat was 18 to 21% lower in all subjects with renal impairment. Overall, the PK of daprodustat was not meaningfully affected by hemodialysis.

The major circulating metabolites, defined as $\geq 10\%$ drug-related material (DRM; equal to AUC of metabolite divided by the sum of AUC of all metabolites and parent) in plasma, were M2, M3, and M13 in all populations assessed.

Daprodustat was not substantially eliminated by the kidney or dialysis, while elimination of its metabolites, being more polar than parent drug, was more susceptible to changes in renal function and dialysis. Dialysis clearance (CL_d) values for daprodustat were 7.61 and 7.00 mL/min at 3 and 4 h post-dose, respectively, with negligible extraction ratios, while metabolite CL_d values at 3 and 4 h post-dose ranged from 140 to 167 mL/min with extraction ratios >0.42 .

Daprodustat did not accumulate in subjects with normal renal function, subjects with Stage 3/4 ND, or hemodialysis Stage 5 CKD subjects. At steady state, daprodustat was highly bound to plasma protein (percent protein bound $>99\%$) in all subject groups. The plasma protein binding of M4 and M5 was lower (~ 31 to 41% at the 3-hour time point) in subjects

with renal impairment compared to healthy subjects (~45 and 55%). This may be due to loss of protein in urine, which is common with CKD.

For subjects with Stage 3/4 ND and Stage 5 hemodialysis CKD (dialysis day), exposure parameter values (AUC and $C_{\max}$) of all daprodustat predominant metabolites were higher than in subjects with normal renal function ([Table 187](#)). The exception to this was for metabolites M4, M6 and M13 where a slightly lower $C_{\max}$ was observed for subjects with Stage 5 hemodialysis CKD (dialysis day). Subjects with Stage 5 hemodialysis CKD on a non-dialysis day had the highest metabolite exposure. Lower AUC and $C_{\max}$ values for the predominant metabolites were observed on a dialysis day compared to a non-dialysis day; however, the apparent $t_{1/2}$ were comparable between the 2 cohorts (except for metabolite M13, which was longer on a dialysis day), indicative of dialysis clearance of metabolites.

For subjects with Stage 3/4 ND and Stage 5 hemodialysis CKD (dialysis day), the higher exposure and reduced elimination of metabolites results in accumulation for most metabolites (accumulation ratios [Rac] up to 1.4-fold in subjects with Stage 3/4 ND; for Stage 5 CKD, accumulation ratios were calculated based on half-life estimates to be up to 1.8-fold and 1.4-fold for dialysis day and non-dialysis day, respectively, except for M13, for which Rac values were 2.6-fold and 1.5-fold, respectively). No apparent time dependencies from single-dose to multiple-dose administration were observed for any predominant metabolite.

Table 187. Daprodustat and Metabolite Exposure in Subjects With Varying Degrees of Renal Impairment Following Administration of Daprodustat on Day 14/15 (Study PHI115573)

Analyte	Parameter	Ratio of GLS Means [90% CI]				
		Stage 3/4 vs. Normal – Day 1	Stage 3/4 vs. Normal – Day 14	Stage 5 HD vs. Normal – Day 14	Stage 5 HD Day 15 vs. Normal Day 14	Stage 5 HD Day 14 vs. Stage 5 HD Day 15
Daprodustat	AUC ^a	0.97 [0.66, 1.42]	0.93 [0.62, 1.40]	1.12 [0.77, 1.64]	0.99 [0.68, 1.45]	1.13 [0.99, 1.29]
	C _{max}	0.59 [0.38, 0.91]	0.80 [0.52, 1.24]	0.79 [0.53, 1.19]	0.82 [0.54, 1.22]	0.97 [0.76, 1.23]
M2	AUC ^a	2.21 [1.63, 3.00]	2.18 [1.51, 3.15]	2.22 [1.58, 3.13]	4.78 [3.40, 6.73]	0.46 [0.43, 0.50]
	C _{max}	1.07 [0.74, 1.54]	1.23 [0.85, 1.77]	1.13 [0.81, 1.59]	1.74 [1.24, 2.44]	0.65 [0.55, 0.77]
M3	AUC ^a	2.61 [1.88, 3.62]	2.60 [1.81, 3.74]	2.74 [1.96, 3.83]	5.92 [4.24, 8.27]	0.46 [0.43, 0.50]
	C _{max}	1.10 [0.78, 1.54]	1.31 [0.94, 1.84]	1.28 [0.94, 1.75]	1.87 [1.37, 2.56]	0.69 [0.59, 0.80]
M4	AUC ^a	1.63 [1.21, 2.19]	1.60 [1.10, 2.33]	1.57 [1.11, 2.22]	2.69 [1.90, 3.80]	0.58 [0.54, 0.64]
	C _{max}	1.01 [0.72, 1.41]	1.14 [0.82, 1.60]	0.99 [0.72, 1.35]	1.52 [1.11, 2.07]	0.65 [0.54, 0.79]
M5	AUC ^a	2.73 [1.91, 3.90]	2.84 [1.96, 4.13]	2.94 [2.08, 4.16]	6.24 [4.42, 8.82]	0.47 [0.42, 0.52]
	C _{max}	1.07 [0.77, 1.50]	1.32 [0.95, 1.84]	1.29 [0.95, 1.76]	1.84 [1.35, 2.50]	0.70 [0.60, 0.82]
M6	AUC ^a	2.08 [1.53, 2.84]	2.08 [1.44, 3.01]	1.90 [1.35, 2.67]	3.92 [2.79, 5.51]	0.48 [0.45, 0.52]
	C _{max}	1.07 [0.77, 1.48]	1.20 [0.87, 1.66]	0.92 [0.68, 1.24]	1.58 [1.17, 2.13]	0.58 [0.50, 0.68]
M13	AUC ^a	2.78 [1.89, 4.09]	2.75 [1.86, 4.06]	2.01 [1.40, 2.90]	4.50 [3.14, 6.46]	0.45 [0.39, 0.51]
	C _{max}	1.00 [0.72, 1.40]	1.25 [0.89, 1.74]	0.90 [0.66, 1.23]	1.27 [0.93, 1.73]	0.71 [0.60, 0.83]

Source: Study PHI115573 CSR Table 19

^a AUC(0-inf) for Day 1 and AUC(0-t) for Days 14 and 15

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CI, confidence interval; GLS, geometric least squares; HD, hemodialysis

Pharmacodynamics

In Stage 3/4 ND CKD subjects and Stage 5 hemodialysis CKD subjects, mean EPO concentrations increased from baseline and reached a maximum at 8 to 12 h post-dose and returned approximately to baseline levels at 24 h post-dose. In Stage 3/4 CKD subjects, the maximum observed increase in EPO on Day 14 occurred at 8 hours post-dose and was ~2.4-fold the pre-dose value on Day 14. In Stage 5 CKD subjects, the maximum observed increases on Days 14 and 15 also occurred at 8 hours post-dose and were ~4.7-fold (Day 14) and ~6.2-fold (Day 15) the pre-dose value on those days, respectively. There was no evidence of EPO accumulation in either population.

Repeat Dose (14 days) in End-Stage Renal Disease Subjects on Peritoneal Dialysis (Study 200942)

Study Title: A repeat-dose, open-label study to assess the pharmacokinetics of daprodustat and metabolites in subjects with End-Stage Renal Disease undergoing peritoneal dialysis.

Study Design: This was a repeat dose, single arm study in End-Stage Renal Disease (ESRD) subjects undergoing peritoneal dialysis. Subjects received oral daprodustat at a dose of 5 mg once per day for 14 days. Serial blood samples were collected on Days 1 and 14 for PK and PD assessments, with additional pre-dose samples collected during the study. Dialysate PK samples were collected on Days 1, 14, and 15. Assessed PD markers included EPO and hepcidin.

Daprodustat was readily absorbed with a median time to maximum concentration (T_{max}) at 1- and 2-hours post-dose following single and repeat daprodustat doses, respectively. Daprodustat was rapidly eliminated from systemic circulation in ESRD subjects undergoing peritoneal dialysis with a $t_{1/2}$ of approximately 2 hours following single and repeat doses. Daprodustat did not accumulate in plasma (geometric least squares [GLS] mean ratio: 0.896) and exhibited time-independent PK following repeat daprodustat doses in ESRD subjects.

Daprodustat metabolites appeared slowly in plasma with the median t_{max} ranging from 3 to 9 hours post-dose, and the $t_{1/2}$ of daprodustat metabolites ranged from 3 to 27 hours following repeat daprodustat doses. All daprodustat metabolites were at steady-state concentrations following 14 days of oral daprodustat doses in ESRD subjects. Following 14 days of daprodustat doses, all the daprodustat metabolites appeared to accumulate in plasma, except for M4 (GLS mean ratio range: 1.02 [M4] to 1.95 [M13]). M2, M3, and M13 were the major circulating metabolites of daprodustat in the ESRD subjects, as defined by >10% DRM in plasma. The results of the study showed no changes in protein binding at pre-dose, 3 hours, and 12 hours post-dosing for daprodustat, M4, and M5. Daprodustat was >99% protein bound, and the 2 metabolites measured (M4 and M5) were approximately 69 to 76% protein bound in subjects undergoing peritoneal dialysis.

Table 188. Daprodustat and Metabolite Exposure in Subjects With End-Stage Renal Disease Following Administration of Daprodustat on Day 14 (Study 200942)

Analyte	Parameter	Geometric Mean [%CI]		
		CAPD n=1	APD n=7	Overall N=8
Daprodustat	AUC _(0-t) (ng.h/mL)	163 (NC)	131 (78.8)	137 (67.1)
	C _{max} (ng/mL)	57.8 (NC)	30.3 (104)	34.5 (94.3)
M2	AUC _(0-t) (ng.h/mL)	258 (NC)	162 (50.7)	178 (49.0)
	C _{max} (ng/mL)	20.5 (NC)	11.0 (33.1)	12.4 (41.1)
M3	AUC _(0-t) (ng.h/mL)	360 (NC)	229 (69.4)	250 (63.2)
	C _{max} (ng/mL)	23.4 (NC)	13.3 (48.2)	14.9 (49.7)
M4	AUC _(0-t) (ng.h/mL)	114 (NC)	59.7 (51.5)	67.9 (54.6)
	C _{max} (ng/mL)	18.4 (NC)	7.26 (40.4)	8.75 (57.6)
M5	AUC _(0-t) (ng.h/mL)	83.0 (NC)	65.2 (63.0)	68.4 (54.7)
	C _{max} (ng/mL)	5.05 (NC)	3.59 (46.5)	3.84 (43.1)
M6	AUC _(0-t) (ng.h/mL)	97.7 (NC)	66.0 (53.5)	71.4 (49.6)
	C _{max} (ng/mL)	9.22 (NC)	4.86 (35.6)	5.52 (43.2)
M13	AUC _(0-t) (ng.h/mL)	242 (NC)	206 (20.1)	213 (18.9)
	C _{max} (ng/mL)	13.3 (NC)	10.4 (17.4)	10.9 (18.7)

Source: Study 200942 CSR Table 11

Abbreviations: APD, automated peritoneal dialysis; AUC, area under the concentration-time curve; CAPD, continuous ambulatory peritoneal dialysis; C_{max}, maximum plasma concentration; NC,

Pharmacodynamics

Baseline EPO concentrations were similar between continuous ambulatory peritoneal dialysis (CAPD; 13.9 IU/L) and automated peritoneal dialysis (APD; 17.0 IU/L) subjects. EPO concentrations increased post-dose, reaching maximum levels at 8 to 12 hours post-dose, and then declined to pre-dose values by 24 hours. Maximal concentrations were 32.2 and 44.8 IU/L for the CAPD and APD subjects, respectively. For the ESRD subjects, EPO levels tended to be lower on Day 14 as compared to Day 1, and high variability in EPO concentrations was observed. Similar patterns were observed for CAPD and APD subjects.

Food Effect in Healthy Subjects and Drug Interaction With a Strong CYP2C8 Inhibitor Gemfibrozil (Study PHI113634)

Study Title: Open-label study to assess the pharmacokinetics of daprodustat co-administered with a high-fat meal or an inhibitor of CYP2C8 (gemfibrozil).

Study Design: This was a single-center, open-label, single-sequence study in healthy subjects to assess the PK of oral daprodustat administered alone or co-administered with gemfibrozil (strong CYP2C8 inhibitor; oral). Subjects were administered 3 treatments: daprodustat 100 mg (fasted), daprodustat 100 mg (high-fat meal), and daprodustat 100 mg plus gemfibrozil 600 mg at steady state.

Table 189. Study Design for PHI113634

Treatment Assignment	Day of Study
Daprodustat single-dose 100 mg, Fasted	1
Daprodustat single-dose 100 mg + High-Fat Meal Food ^a	3
Gemfibrozil 600 mg BID for 3 days	5-7
Gemfibrozil 600 mg BID + Daprodustat single-dose 100 mg	8
Gemfibrozil 600 mg BID	9

Source: Table 7 Summary of clinical pharmacology
Abbreviations: BID, twice daily

Results and Conclusions

Twenty-three subjects were enrolled, and 21 subjects completed the study. Co-administration of a single 100 mg daprodustat dose with a high-fat, high-calorie meal resulted in an 11 and 31% decrease in daprodustat $AUC_{(0-inf)}$ and C_{max} , respectively, and a 1 h mean delay of t_{max} relative to fasted administration. Similar $t_{1/2}$ values were observed for the two prandial states. The 90% confidence interval (CI) of the adjusted geometric mean ratio was 0.84-1.01 for $AUC_{(0-inf)}$ and 0.61-0.82 for C_{max} , concluding that a high-fat meal did not have a clinically significant effect on the AUC and C_{max} of daprodustat. For daprodustat metabolites, slight increases in exposure ($AUC_{(0-inf)}$) were observed when daprodustat was co-administered with food. The 90% CIs of $AUC_{(0-inf)}$ GLSM ratios for daprodustat metabolites ranged from 0.99 to 1.21.

Table 190. Effect of High-Fat, High-Calorie Meal on Daprodustat and Metabolite PK (Study PHI113634)

Analyte	Fed:Fasted Comparison ^a GLSM Ratio [90% CI]			
	t _{max} ^b	C _{max}	AUC(0-t)	AUC(0-inf)
Daprodustat	1.00 [0.50, 1.50]	0.71 [0.61, 0.82]	0.92 [0.84, 1.01]	0.92 [0.84, 1.01]
M2	0.50 [0.00, 1.00]	1.04 [0.95, 1.12]	1.10 [1.03, 1.18]	1.10 [1.03, 1.18]
M3	0.50 [0.00, 1.00]	1.04 [0.97, 1.12]	1.11 [1.04, 1.19]	1.11 [1.04, 1.19]
M4	0.50 [0.00, 1.00]	0.97 [0.89, 1.06]	1.05 [0.99, 1.12]	1.05 [0.99, 1.12]
M6	0.50 [0.00, 1.00]	1.02 [0.95, 1.10]	1.08 [1.01, 1.15]	1.08 [1.02, 1.15]
M5	0.50 [0.00, 1.00]	1.03 [0.96, 1.10]	1.11 [1.04, 1.19]	1.13 [1.06, 1.21]
M13	1.00 [0.00, 1.50]	0.98 [0.90, 1.08]	1.08 [1.00, 1.17]	1.08 [1.00, 1.16]

Source: Study PHI113634 CSR Table 14

^a Daprodustat 100 mg Fed: Daprodustat 100 mg Fasted

^b Presented as estimated median difference fed:fasted [90%CI]

Abbreviations: AUC, area under the concentration-time curve; CI, confidence interval; C_{max}, maximum plasma concentration; GLSM, geometric least square mean; PK, pharmacokinetic; T_{max}, time to maximum concentration

Co-administration of a single 100 mg dose of daprodustat with a strong CYP2C8 inhibitor (gemfibrozil) resulted in a 3.9- and 18.6-fold increase in daprodustat C_{max} and AUC_(0-inf), respectively. This significant increase in both rate and extent of systemic exposure of daprodustat when co-administered with gemfibrozil is consistent with daprodustat being primarily metabolized by CYP2C8. The decrease in CYP2C8 metabolism of daprodustat is reflected by its ~4-fold increase in t_{1/2}, 2.51 h to 9.26 h when co-administered with gemfibrozil. T_{max} was also delayed from 1.00 to 4.00 h. The appearance of daprodustat metabolites was delayed by ~9-10 h as t_{max} shifted from 3-4 h for each metabolite to ~12 h when daprodustat was co-administered with gemfibrozil; the C_{max} and AUC_(0-inf) of metabolites were reduced by at least 90% and 67%, respectively.

Table 191. Summary of Selected Plasma Daprodustat Pharmacokinetic Parameters Following a Single Dose Administration of Daprodustat

Treatment	n	C _{max} (ng/mL)	t _{max} ² (h)	AUC(0-t) (ng.h/mL)	AUC(0-∞) (ng.h/mL)	T _{1/2} (hr)
GSK1278863A 100mg fasted (A)	23	2110 (31.5)	1.00 (1.00-4.00)	5235 (33.3)	5243 (33.3)	2.51 (33.6)
GSK1278863A 100mg + food (B)	21	1466 (45.6)	2.00 (1.00-6.00)	4647 (19.1)	4655 (19.1)	2.23 (30.9)
GSK1278863A 100mg + gemfibrozil (C2)	21	8161 (19.3)	4.00 (2.00-4.00)	90894 (21.9)	93655 (22.5)	9.26 (23.9)

Source: Study PHI113634 Table 11.2 and Table 11.3

¹geometric mean (CV%)

²median (range)

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CV, coefficient of variation; T_{1/2}, half-life; T_{max}, time to maximum concentration

Table 192. Statistical Comparisons for Effect of Gemfibrozil on Plasma Daprodustat and Metabolite Pharmacokinetics (Study PHI113634)

Analyte	Daprodustat + Gemfibrozil: Daprodustat Alone Treatment ^a Comparison GLS Mean Ratio [90% CI]		
	C _{max} (ng/mL)	AUC(0-t) (ng.h/mL)	AUC(0-inf) (ng.h/mL)
Daprodustat	3.92 [3.50, 4.38]	18.0 [16.6, 19.6]	18.6 [17.0, 20.2]
M2	0.10 [0.09, 0.12]	0.33 [0.30, 0.37]	0.38 [0.34, 0.43]
M3	0.10 [0.09, 0.11]	0.33 [0.29, 0.37]	ND
M4	0.09 [0.07, 0.10]	0.31 [0.26, 0.36]	ND
M5	0.07 [0.06, 0.08]	0.16 [0.13, 0.18]	ND
M6	0.09 [0.08, 0.11]	0.28 [0.23, 0.34]	ND
M13	0.02 [0.01, 0.03]	ND	ND

Source: Study PHI113634 CSR Table 15

^a Daprodustat + Gemfibrozil: Daprodustat 100 mg + Gemfibrozil 600 mg steady state; Daprodustat Alone: Daprodustat 100 mg
Abbreviations: AUC, area under the concentration-time curve; CI, confidence interval; C_{max}, maximum plasma concentration; GLS, geometric least square; ND, not determined

Food Effect (CKD Meal) in Healthy Subjects (Study 207727 Part 2)

Study Title: A single center, single dose, open-label, randomized, 2-way crossover study in healthy Japanese male subjects to evaluate the bioequivalence of daprodustat tablets (2 mg tablet vs. 4 mg tablet) (Part 1) and the food effect on the pharmacokinetics of daprodustat (Part 2).

Study Design: The purpose of Part 2 of this study was to assess the effect of food on the single-dose PK of daprodustat in healthy Japanese male subjects administered the commercial tablet formulation.

Part 2 was a single-center, single dose, fed/fasted, open-label, randomized, 2-way crossover study in healthy Japanese male subjects. Subjects received a daprodustat 4 mg tablet (commercial formulation made by Process 1) 30 minutes after the end of a standard CKD meal (500-700 kcal, comprised of 12-16 g protein, 1 to 2 g salt, and up to 500 mg potassium) or in the fasted state. Following a washout period of 5 days, subjects crossed over to receive treatment in the opposite prandial state. Blood samples for PK analysis were collected prior to dosing and until 24 hours post-dose following each dose.

Results and Conclusions: Twelve subjects enrolled in Part 2 (food effect) of this study and all subjects completed the study. Following administration of daprodustat under fed and fasted conditions, similar daprodustat peak concentrations were observed though T_{max} was reached 1 hour later in the fed state. There were no apparent differences in t_{1/2} between fed and fasted states. Metabolites were not analyzed in this study.

Table 193. Effect of CKD Meal on Daprodustat PK Parameters (Study 207727 Part 2)

Parameter	Adjusted Geometric Mean		Adjusted Geometric Mean Ratio [90% CI] (Fed / Fasted)
	Fed (Test) n=12	Fasted (Reference) n=12	
C _{max} (ng/mL)	67.82	76.19	0.89 [0.73, 1.08]
AUC(0-t) (ng*h/mL)	142.96	156.42	0.91 [0.82, 1.01]
AUC(0-inf) (ng*h/mL)	143.12	156.55	0.91 [0.82, 1.01]

Source: Study 207727 CSR Table 16

Abbreviations: AUC, area under the concentration-time curve; CI, confidence interval, CKD, chronic kidney disease; C_{max}, maximum plasma concentration; PK, pharmacokinetic

Single Ascending Dose Study in Healthy Adults (Study PHX111427)

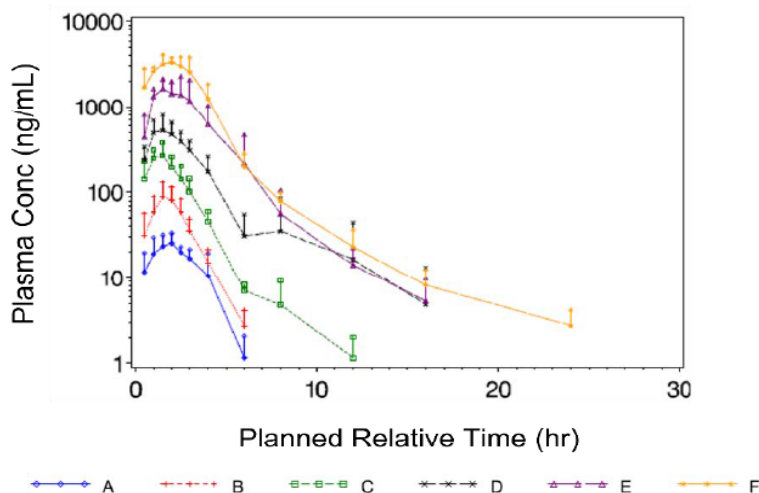
Study Title: A Phase I, randomized, single-blind, placebo-controlled, dose-escalation study to evaluate the safety, pharmacokinetics, and pharmacodynamics of single oral doses of GSK1278863A in healthy subjects.

Study Design: Study PHX111427 was a single-blind, randomized, placebo-controlled, dose-rising, single dose, sequential parallel group study to investigate the safety, tolerability, PK and PD of GSK1278863A in healthy adult subjects. Subjects were dosed sequentially in six ascending dosing cohorts corresponding to six dose levels ranging from 2 mg to 300 mg of GSK1278863A. Within each cohort, a total of six subjects were randomized to receive a single, oral dose of daprodustat or placebo in a ratio of 2:1 (four active: two placebo). Thirty-six subjects were enrolled and completed the study.

Results and Conclusion

Daprodustat was readily absorbed following single dose administration with the median maximum concentration achieved between 1.25-2.00 hours post dose across different dose levels. Daprodustat was eliminated from systemic circulation rapidly with short terminal half-lives ranging from 0.8-4.0 hours across the dose range of 2 mg-300 mg.

Figure 46. Mean (SD) Plasma Daprodustat PK Concentration-Time Plot Following Single-Dose Administration (Semi-Log; Study PHX111427)



Source: Study PHX111427 CSR Figure 11.2
Abbreviations: PK, pharmacokinetic; SD, standard deviation

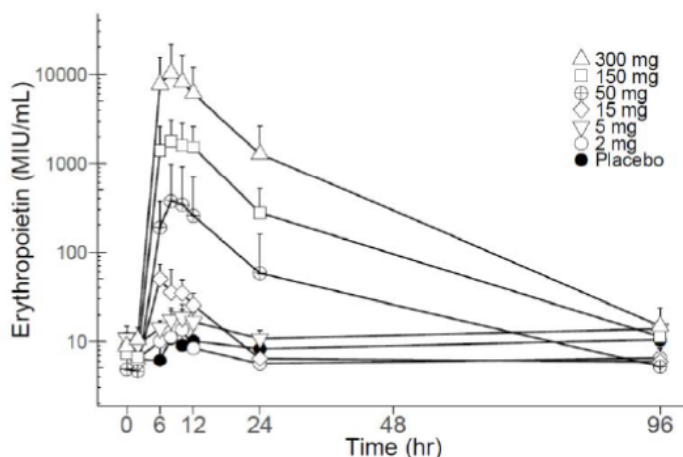
Table 194. Daprodustat Pharmacokinetic Parameters Following Single-Dose Administration (Study PHX111427)

Dose (mg)	N	C _{max} (ng/mL)	t _{max} (h)	AUC _{0-t} (ng.h/mL)	AUC _{0-inf} (ng.h/mL)	t _{1/2} (h)
2	4	29.4 (15.3)	2.00 (1.00-4.00)	74.3 (11.6)	78.0 (14.1)	0.79 (11.2)
5	4	93.8 (31.5)	1.58 (1.00-2.00)	198 (32.0)	200 (31.7)	0.83 (9.8)
15	4	286 (30.1)	1.50 (0.50-2.00)	647 (18.4)	650 (18.1)	1.40 (44.9)
50	4	521 (42.4)	1.25 (1.00-3.00)	1685 (30.7)	1694 (30.8)	2.39 (22.0)
150	4	1849 (37.9)	2.00 (1.50-2.50)	5185 (33.7)	5193 (33.6)	2.73 (26.1)
300	4	3678 (21.7)	1.75 (1.50-3.00)	10793 (24.7)	10809 (24.7)	3.99 (10.9)

Source: Table 1 Summary of clinical pharmacology
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CV, coefficient of variation; T_{1/2}, half-life; T_{max}, time to maximum concentration

Daprodustat exhibited linear pharmacokinetics with dose proportional increases in plasma exposure as the dose increased from 2 mg to 300 mg following single dose administration. Urinary excretion of GSK1278863 was very low with only 0.01% of the dose excreted in urine unchanged at the 300 mg dose level. Increase in circulating plasma EPO was observed at the 50 mg, 150 mg, and 300 mg dose levels of daprodustat, as compared to placebo.

Figure 47. Mean (+SD) EPO Concentration-Time Profile (Study PHX111427)



Source: Study PHX11427 CSR Table 12.4
Abbreviations: EPO, erythropoietin; SD, standard deviation

Dose-related increases in EPO, as compared to placebo were observed. Peak increases in EPO from placebo were observed at 6 h post-dose, with least square mean differences of 42.0 IU/L (95%CI: 15.2, 116) at 50 mg, 199.5 IU/L at 150 mg (95%CI: 85.2, 467.5), and 889.9 IU/L (95%CI: 381.3, 2076.8) at 300 mg.

Multiple Ascending Dose Study in Healthy Adults (Study PHI112842)

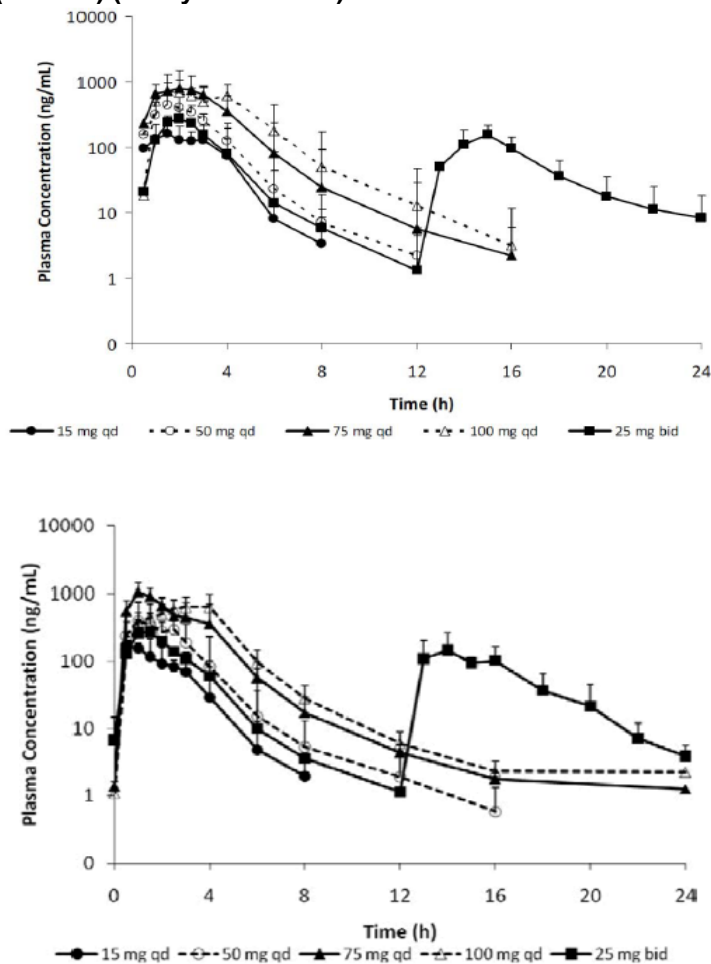
Study Title: A Phase I, randomized, single-blind, placebo-controlled, dose-escalation study to evaluate the safety, pharmacokinetics, and pharmacodynamics of repeat oral doses of GSK1278863A in healthy subjects.

Study Design: Subjects were dosed in 5 dosing cohorts corresponding to 4 once-daily regimens (15 to 100 mg) and one twice daily (BID) regimen (25 mg in the morning and evening). Dosing cohorts consisted of six subjects. Within each cohort, subjects were randomly assigned to receive either GSK1278863A or placebo in a 2:1 ratio (4 active:2 placebo). Each subject was dosed daily for up to 14 days.

Results and Conclusion

A total of 30 subjects were randomized to treatment (Figure 48). Of these, 29 completed the study, and one subject in Regimen D (25 mg BID) discontinued due to an adverse event. Daprodustat was absorbed with a median T_{max} range 1 to 4 hours and was eliminated from systemic circulation with $t_{1/2}$ values ranging from 1.0 to 2.3 h. Daprodustat exposure (C_{max} , AUC) increased dose proportionally following daily doses up to 100 mg. Daprodustat did not significantly accumulate in plasma over 14 days of dosing, and steady-state plasma concentrations were achieved by Day 2.

Figure 48. Mean (SD) Plasma Daprodustat Concentrations Versus Time on Days 1 (Top) and 14 (Bottom) (Study PHI112842)



Source: Study PHI112842 CSR Figure 2 and Figure 3
Top Panel: Day 1; Bottom Panel: Day 14
Abbreviations: bid, twice daily; QD, once daily; SD, standard deviation

Table 195. Summary of Daprodustat PK Parameters Following Repeat Oral Doses of Daprodustat in Healthy Subjects (Study PHI112842)

Parameter	Visit	Statistics	15 mg daily (N=4)	50 mg daily (N=4)	75 mg daily (N=4)	100 mg daily (N=4)	25 mg bid	
							AM (N=4)	PM (N=3)
C _{max} (ng/mL)	Day 1	Geometric mean (CV%)	237 (31)	448 (35)	939 (14)	946 (59)	306 (13)	175 (14)
	Day 14	Geometric mean (CV%)	198 (7.0)	462 (32)	1061 (31)	856 (8.0)	332 (13)	199 (13)
t _{max} (h)	Day 1	Median (min, max)	1.50 (1.00, 3.00)	1.75 (1.00, 3.02)	2.25 (1.50, 3.00)	3.50 (1.50, 4.00)	2.00 (1.50, 2.50)	14.5 (14.0, 15.0)
	Day 14	Median (min, max)	1.00 (1.00, 3.00)	1.25 (1.00, 2.50)	1.26 (1.02, 4.00)	3.25 (1.00, 4.00)	1.00 (0.98, 1.50)	14.0 (14.0, 16.0)
AUC(0-tau) (ng.h/mL)	Day 1	Geometric mean (CV%)	516 (18)	1189 (29)	2708 (16)	2852 (28)	682 (20) ^a	561 (11) ^b
	Day 14	Geometric mean (CV%)	384 (11)	1020 (36)	2754 (20)	2592 (12)	683 (10) ^a	616 (8.2) ^b
AUC(0-inf) (ng.h/mL)	Day 1	Geometric mean (CV%)	516 (18)	1189 (29)	2709 (16)	2855 (28)	683 (20)	-
t _{1/2} (h)	Day 1	Geometric mean (CV%)	1.07 (28)	1.69 (44)	2.25 (37)	2.33 (29)	1.05 (23)	-
Day14/Day1 Accumulation Ratio (Ro) ^c	-	Ratio 90% CI	0.744 (0.666, 0.832)	0.858 (0.745, 0.988)	1.02 (0.762, 1.36)	0.909 (0.720, 1.15)	1.05 (0.895, 1.24)	

Source: PHI112842 CSR Table 17

^a AUC(0-tau) is AUC₍₀₋₁₂₎ for the 25 mg BID AM group

^b AUC(0-tau) is AUC₍₁₂₋₂₄₎ for the 25 mg BID PM group

^c Ro=AUC(0-tau) day 14/AUC(0-tau) day 1

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CI, confidence interval; CV, coefficient of variation; PK, pharmacokinetic; T_{1/2}, half-life; T_{max}, time to maximum concentration

Dose-dependent increases in EPO concentrations were observed on Days 1 and 14. Mean EPO concentrations were elevated at 6 h post-dose, reached a maximum at 6 to 10 h post-dose (peaking earlier with lower doses), and returned to approximately baseline levels at 24 h post-dose. Mean change from baseline (CFB) on Day 1 was 28.8 IU/L (15 mg) and 718.1 IU/L (100 mg) and on Day 14 was 9.7 IU/L (15 mg) and 406.6 IU/L (100 mg). Mean CFB for placebo was 1.0 IU/L (Day 1) and 4.5 IU/L (Day 14).

14.3. Bioanalytical Method Validation and Performance

Multiple validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays were used for measurement of daprodustat concentrations in human plasma and urine. The bioanalytical methods are acceptable. The bioanalytical method validation is acceptable, and the parameters are summarized below.

Table 196. Summary of Method and Validation Parameters

Summary of Method and Validation Parameters	
Validation Report No	Clinical Study No.
Daprodustat in Acidified human plasma	PHI112844 PHX111427
Daprodustat and internal standard [¹³ C ₅ ¹⁵ N]-GSK1278863 were extracted from 50 μ L of acidified human plasma by means of protein precipitation. Extracts were analyzed using HPLC-MS/MS using an electrospray interface operated in negative ion mode.	
LLQ	1 ng/mL
Validated Range	1 to 1000 ng/mL
Calibration Curve Precision (%CV)	1.0 to 4.8%
Calibration Curve Accuracy (%Bias)	-5.8 to 4.2%
Within-run Precision (%CV)	0.9 to 6.1%
Between-run Precision (%CV)	1.5 to 5.3%
Within-run Accuracy (%Bias)	-2.0 to 9.4%
Between-run Accuracy (%Bias):	0.2 to 7.2%
Stability	Processed Sample stability: 96 hours at room temperature Freeze-thaw matrix stability: 3 cycles at from -20°C to room temperature. Room temperature matrix stability: 24 hours at room temperature. Long-term stability: NR
Report no: RM2009/00585/01 Study no: PHI112842, PHI112843 Daprodustat in in Acidified Human Plasma Daprodustat and internal standard were extracted from 50 μ L of acidified human plasma by means of protein precipitation. Extracts were analyzed using HPLC-MS/MS using an electrospray interface operated in negative ion mode.	
LLQ	1.002 ng/mL
Validated Range	1.002 to 1002.128 ng/mL
Calibration Curve Precision (%CV)	0.6 to 2.8%
Calibration Curve Accuracy (%Bias)	-6.1 to 6.4%
Within-run Precision (%CV)	0.5 to 7.2%
Between-run Precision (%CV)	4.0 to 10.3%
Within-run Accuracy (%Bias)	-14.8 to 14.0%
Between-run Accuracy (%Bias)	0.7 to 6.4%
Stability	Processed Sample stability: 100 hours at room temperature and 152 hours at 2-8°C Freeze-thaw matrix stability: 3 cycles at from -20°C and -70°C to room temperature;

Report No: 2010N109680_00 Study No: PHI113634 Daprodustat in human plasma Daprodustat and internal standard [¹³C₅¹⁵N]-daprodustat were extracted from 50 µL of human plasma by means of protein precipitation. Extracts were analyzed using HPLC-MS/MS using an electrospray interface operated in negative ion mode.	
LLQ	1 ng/mL
Validated Range	1 to 1000 ng/mL
Calibration Curve Precision (%CV)	1.0 to 7.5%
Calibration Curve Accuracy (%Bias)	-3.7 to 1.4%
Within-run Precision (%CV)	1.0 to 8.1%
Between-run Precision (%CV)	0.3 to 7.6 %
Within-run Accuracy (%Bias)	-12.2 to 2.9%
Between-run Accuracy (%Bias)	-4.6 to 2.1%
Stability	Processed Sample stability: 96 hours at ambient temperature Freeze-thaw matrix stability: 3 cycles at from -20°C to ambient temperature. Room temperature matrix stability: 24 hours at ambient temperature Whole blood stability: 4 hours at 37°C Long-term stability: NR
Validation report no: Study no: 2011N130002_00 PHI115573 Daprodustat and metabolites M2, M3, M4, M5, M13, M6 and internal standards were extracted from 100 µL human plasma by means of polymeric SPE (Biotage Evolute ABN). Extracts were analyzed using HPLC-MS/MS using an electrospray interface operated in negative ion mode.	
LLQ	0.100 ng/mL
Validated Range	0.100 to 100 ng/mL
Calibration Curve Precision (%CV)	2.11 to 6.65%
Calibration Curve Accuracy (%Bias)	-4.05 to 2.18%
Within-run Precision (%CV)	0.612 to 7.19%
Between-run Precision (%CV)	2.77 to 6.17%
Within-run Accuracy (%Bias)	-0.948 to 6.26%
Between-run Accuracy (%Bias)	-0.647 to 3.52%
Stability	Freeze-thaw matrix stability: 5 cycles at -20°C and -70°C Room temperature matrix stability: 29.25 hours at room temperature Post-preparative extract stability: 204 hours for parent and 278 hours for metabolites stored at 5°C Long-term frozen matrix stability: 91 days at -20°C, 59 days at -70°C Whole blood stability: 4 hours at 37°C.

Source: Reviewer's analysis

14.4. Immunogenicity Assessment—Impact of PK/PD, Efficacy, and Safety

Not applicable.

14.5. Pharmacometrics Assessment

14.5.1. Summary of Applicant's Population PK Analysis

Population pharmacokinetic (PopPK) analysis was conducted using 3523 evaluable daprodustat concentrations and 573 below the limit of quantitation (BLQ) data in a total of 707 subjects, including five studies; one Phase 2B study (205665) and four Phase 3 studies (204837, 201410, 200807 and 200808). A brief overview of the studies is provided in [Table 197](#). The dose used in the analysis included 1, 2, 4, 6, 8, 10, 12, 16, 20, 24 mg once daily (QD) and 2, 4, 6, 8, 10, 12, 16, 20, 24, 32 and 48 mg TIW. Summary statistics of the continuous and categorical covariates that were evaluated in the population PK analysis are shown in [Table 198](#).

Table 197. Summary of the Studies Included in the Analysis

Study	Population	Type of study	Dosing and Frequency	Planned expected patients for analysis	Study Duration
205665	Hemodialysis-dependent (HDD) CKD patients with anemia, switched from stable dose of ESA	Phase 2B, open-label, placebo-controlled study evaluating daprodustat and its effect on blood pressure	p.o. dosing of daprodustat: Acute challenge of 24 mg on Day 1 and 57. Hgb-based dose-individualisation with QD dosing target Hgb of 10.0-11.0 g/dL daprodustat between Day 1-57	25 subjects in daprodustat arm	8-week treatment (Serial PK visit on Day 1 and Day 57)
204837	HDD CKD patients with anemia that are currently treated with rhEPO or its analogs	Phase 3 double-blind, active-controlled study comparing TIW daprodustat against epoetin alfa	daprodustat: TIW p.o. with Hgb-based dose individualisation to target Hgb of 10.0-11.0 g/dL. Doses: 2/4/8/12/16/20/24/32/48 mg	267 subjects in daprodustat arm	52-week treatment (Single sparse PK visit between Week 8 and Week 52)
201410	CKD patients with anemia that are initiating HD or PeD, and not receiving ESA	Phase 3, sponsor-blind, randomised, active-controlled study evaluating daprodustat in patients with anemia	daprodustat: QD p.o. Hgb-based dose individualisation to target Hgb of 10.0-11.0 g/dL. Doses: 1/2/4/6/8/10/12/16/24 mg	150 subjects in daprodustat arm	52-week treatment (Single sparse PK visit on Week 4, Week 8 or Week 12)
200807	HDD or PeD dependent (PDD) CKD patients with anemia receiving ESA	Phase 3, sponsor-blind, randomised, active-controlled study evaluating daprodustat in patients with anemia	daprodustat: QD p.o. Hgb-based dose individualisation to target Hgb of 10.0-11.0 g/dL. Doses: 1/2/4/6/8/10/12/16/24 mg	204 patients in PK substudy cohort	event-driven (CVOT). Single sparse PK visit between Week 8 and Week 52.
200808	Non-dialysis dependent (NDD) CKD patients with anemia, of which a subset (40%) receives ESA	Phase 3, sponsor-blind, randomised, active-controlled study evaluating daprodustat in patients with anemia	daprodustat: QD p.o. Hgb-based dose individualisation to target Hgb of 10.0-11.0 g/dL. Doses: 1/2/4/6/8/10/12/16/24 mg	128 patients in PK substudy cohort	event-driven (CVOT). Single sparse PK visit between Week 8 and Week 52

CVOT = cardiovascular outcome trial

Source: Applicant's Population PK report TM-14152615, Table 2.3.0.

Abbreviations: CKD, chronic kidney disease; Hgb, hemoglobin; PK, pharmacokinetic; QD, once daily; TIW, three times a week

Table 198. Summary Statistics for the Continuous Covariates in the Population PK Analysis

Study Identifier	variable	N	Mean	Median	SD	Min	Max
200807	Baseline Age (y)	203	54.93	55.00	14.05	22.00	85.00
	Baseline Subject Weight (kg)	203	77.31	74.60	19.66	41.20	140.40
	Baseline Subject Height (cm)	203	166.65	167.60	9.40	143.50	194.00
	Baseline Body Surface Area (m ²)	203	1.88	1.86	0.26	1.30	2.60
	Baseline Body Mass Index (kg/m ²)	203	27.76	26.89	6.57	16.58	56.60
	Baseline GFR (mL/min)	203	-	-	-	-	-
	Baseline C-Reactive Protein (mg/L)	203	8.40	3.90	16.15	0.10	185.40
200808	Baseline Age (y)	136	66.07	67.00	13.19	27.00	93.00
	Baseline Subject Weight (kg)	136	77.76	73.35	19.40	40.50	135.00
	Baseline Subject Height (cm)	136	163.64	163.00	9.74	144.00	186.00
	Baseline Body Surface Area (m ²)	136	1.87	1.84	0.26	1.30	2.53
	Baseline Body Mass Index (kg/m ²)	136	28.96	28.00	6.55	18.00	48.41
	Baseline GFR (mL/min)	136	21.57	18.50	12.03	6.00	63.00
	Baseline C-Reactive Protein (mg/L)	136	5.70	2.90	8.62	0.10	59.80
201410	Baseline Age (y)	89	54.96	54.00	14.40	25.00	84.00
	Baseline Subject Weight (kg)	89	78.46	76.00	20.12	48.60	136.80
	Baseline Subject Height (cm)	89	168.43	168.00	11.04	124.40	192.00
	Baseline Body Surface Area (m ²)	89	1.90	1.88	0.27	1.45	2.56
	Baseline Body Mass Index (kg/m ²)	89	27.74	26.29	7.52	18.50	65.91
	Baseline GFR (mL/min)	89	8.97	8.00	3.72	3.00	23.00
	Baseline C-Reactive Protein (mg/L)	89	9.67	2.80	23.06	0.10	173.90
204837	Baseline Age (y)	235	59.66	60.00	14.12	22.00	90.00
	Baseline Subject Weight (kg)	235	76.10	74.00	18.86	36.10	138.30
	Baseline Subject Height (cm)	235	165.99	166.00	9.83	139.00	193.00
	Baseline Body Surface Area (m ²)	235	1.86	1.85	0.25	1.18	2.58
	Baseline Body Mass Index (kg/m ²)	235	27.61	26.49	6.56	16.95	51.35
	Baseline GFR (mL/min)	235	-	-	-	-	-
	Baseline C-Reactive Protein (mg/L)	235	8.59	4.30	14.06	0.20	119.60
205665	Baseline Age (y)	44	60.98	58.00	11.18	44.00	84.00
	Baseline Subject Weight (kg)	44	82.96	79.05	18.84	49.90	134.20
	Baseline Subject Height (cm)	44	166.02	167.00	10.22	136.00	185.00
	Baseline Body Surface Area (m ²)	44	1.95	1.91	0.24	1.52	2.55
	Baseline Body Mass Index (kg/m ²)	44	30.21	29.64	6.56	17.89	49.29
	Baseline GFR (mL/min)	44	-	-	-	-	-
	Baseline C-Reactive Protein (mg/L)	44	-	-	-	-	-
Overall	Baseline Age (y)	707	59.03	59.00	14.36	22.00	93.00
	Baseline Subject Weight (kg)	707	77.49	74.60	19.37	36.10	140.40
	Baseline Subject Height (cm)	707	166.04	166.95	9.95	124.40	194.00
	Baseline Body Surface Area (m ²)	707	1.88	1.86	0.26	1.18	2.60
	Baseline Body Mass Index (kg/m ²)	707	28.09	26.95	6.71	16.58	65.91
	Baseline GFR (mL/min)	707	16.58	14.00	11.44	3.00	63.00
	Baseline C-Reactive Protein (mg/L)	707	8.08	3.50	15.39	0.10	185.40

Dialysis Population	variable	N	Mean	Median	SD	Min	Max
Hemodialysis	Baseline Age (y)	568	57.33	57.00	14.14	22.00	90.00
	Baseline Subject Weight (kg)	568	77.45	74.90	19.40	36.10	140.40
	Baseline Subject Height (cm)	568	166.61	167.00	9.91	124.40	194.00
	Baseline Body Surface Area (m ²)	568	1.88	1.87	0.26	1.18	2.60
	Baseline Body Mass Index (kg/m ²)	568	27.89	26.77	6.75	16.58	65.91
	Baseline GFR (mL/min)	568	9.00	8.00	3.73	3.00	23.00
	Baseline C-Reactive Protein (mg/L)	568	8.73	3.70	16.69	0.10	185.40
Non-dialysis	Baseline Age (y)	136	66.07	67.00	13.19	27.00	93.00
	Baseline Subject Weight (kg)	136	77.76	73.35	19.40	40.50	135.00
	Baseline Subject Height (cm)	136	163.64	163.00	9.74	144.00	186.00
	Baseline Body Surface Area (m ²)	136	1.87	1.84	0.26	1.30	2.53
	Baseline Body Mass Index (kg/m ²)	136	28.96	28.00	6.55	18.00	48.41
	Baseline GFR (mL/min)	136	21.57	18.50	12.03	6.00	63.00
	Baseline C-Reactive Protein (mg/L)	136	5.70	2.90	8.62	0.10	59.80
Peritoneal dialysis	Baseline Age (y)	3	60.00	62.00	11.14	48.00	70.00
	Baseline Subject Weight (kg)	3	73.73	67.60	16.10	61.60	92.00
	Baseline Subject Height (cm)	3	166.33	169.00	15.18	150.00	180.00
	Baseline Body Surface Area (m ²)	3	1.84	1.70	0.26	1.68	2.14
	Baseline Body Mass Index (kg/m ²)	3	26.67	28.39	4.49	21.57	30.04
	Baseline GFR (mL/min)	3	6.00	6.00	-	6.00	6.00
	Baseline C-Reactive Protein (mg/L)	3	3.87	4.70	2.17	1.40	5.50

Clopidogrel Use	variable	N	Mean	Median	SD	Min	Max
No	Baseline Age (y)	637	58.53	59.00	14.57	22.00	93.00
	Baseline Subject Weight (kg)	637	77.53	74.60	19.54	36.10	140.40
	Baseline Subject Height (cm)	637	166.24	167.00	9.90	124.40	194.00
	Baseline Body Surface Area (m ²)	637	1.88	1.86	0.26	1.18	2.60
	Baseline Body Mass Index (kg/m ²)	637	28.03	26.85	6.73	16.58	65.91
	Baseline GFR (mL/min)	637	16.61	13.00	11.31	3.00	59.00
	Baseline C-Reactive Protein (mg/L)	637	8.21	3.40	15.92	0.10	185.40
Yes	Baseline Age (y)	70	63.56	64.00	11.38	39.00	88.00
	Baseline Subject Weight (kg)	70	77.13	73.80	17.82	42.90	136.80
	Baseline Subject Height (cm)	70	164.22	165.00	10.30	136.00	189.00
	Baseline Body Surface Area (m ²)	70	1.87	1.84	0.23	1.29	2.49
	Baseline Body Mass Index (kg/m ²)	70	28.67	26.99	6.52	18.25	51.74
	Baseline GFR (mL/min)	70	16.25	14.50	12.99	5.00	63.00
	Baseline C-Reactive Protein (mg/L)	70	6.83	4.00	8.17	0.20	39.40

Race/Country	variable	N	Mean	Median	SD	Min	Max
Black or African American	Baseline Age (y)	125	57.19	57.00	11.89	29.00	84.00

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Race/Country	variable	N	Mean	Median	SD	Min	Max
	Baseline Subject Weight (kg)	125	85.62	81.80	21.41	43.90	138.10
	Baseline Subject Height (cm)	125	170.24	170.00	10.23	150.00	194.00
	Baseline Body Surface Area (m ²)	125	2.00	1.96	0.28	1.37	2.60
	Baseline Body Mass Index (kg/m ²)	125	29.56	27.91	7.17	17.89	51.35
	Baseline GFR (mL/min)	125	16.18	13.50	10.70	6.00	52.00
	Baseline C-Reactive Protein (mg/L)	125	10.26	3.95	22.25	0.40	173.90
Korean	Baseline Age (y)	27	59.89	60.00	7.86	46.00	73.00
	Baseline Subject Weight (kg)	27	60.70	57.00	15.55	44.20	118.20
	Baseline Subject Height (cm)	27	160.05	157.70	8.86	147.00	182.00
	Baseline Body Surface Area (m ²)	27	1.63	1.59	0.23	1.35	2.35
	Baseline Body Mass Index (kg/m ²)	27	23.52	22.44	4.70	17.93	41.88
	Baseline GFR (mL/min)	27	19.20	10.00	18.69	7.00	51.00
	Baseline C-Reactive Protein (mg/L)	27	1.62	0.70	2.05	0.20	9.10
North East Asia	Baseline Age (y)	34	61.50	63.00	9.05	46.00	84.00
	Baseline Subject Weight (kg)	34	62.91	59.55	16.60	44.20	118.20
	Baseline Subject Height (cm)	34	160.95	160.00	8.19	147.00	182.00
	Baseline Body Surface Area (m ²)	34	1.67	1.63	0.24	1.35	2.35
	Baseline Body Mass Index (kg/m ²)	34	24.07	22.78	4.99	17.93	41.88
	Baseline GFR (mL/min)	34	19.00	15.00	13.56	7.00	51.00
	Baseline C-Reactive Protein (mg/L)	34	1.65	0.65	2.04	0.20	9.10
White	Baseline Age (y)	523	59.54	61.00	15.15	22.00	93.00
	Baseline Subject Weight (kg)	523	76.23	74.20	17.74	36.10	136.80
	Baseline Subject Height (cm)	523	165.41	165.90	9.77	124.40	192.00
	Baseline Body Surface Area (m ²)	523	1.86	1.85	0.24	1.18	2.56
	Baseline Body Mass Index (kg/m ²)	523	27.90	26.91	6.43	16.58	65.91
	Baseline GFR (mL/min)	523	16.78	14.00	11.50	3.00	63.00
	Baseline C-Reactive Protein (mg/L)	523	8.15	3.75	14.31	0.10	185.40

Note that 'Korean' is defined by the 'Country' variable, while the other three categories are defined by the 'Analysis Race' variable. Due to the selection of categories included in this table, not all subjects are included in this table, and some subjects may be included in summary statistics of both 'Korean' and one of the other categories.

Source: Applicant's Population PK report TM-14152615, Table 11.1.11, Table 11.1.12, Table 11.1.13, Table 11.1.14
Abbreviations: GFR, glomerular filtration rate; PK, pharmacokinetic; SD, standard deviation

Table 199. Summary Statistics for the Categorical Covariates in the Population PK Analysis

Study Identifier	variable	value	Frequency (perc)
200807	Concomitant Oral Iron	No	192 (94.58)
		Yes	11 (5.42)
	Concomitant IV Iron	No	163 (80.30)
		Yes	40 (19.70)
	Concomitant Iron	No	155 (76.35)
		Yes	48 (23.65)
	Concomitant CYP3A4 Inducers	No	194 (95.57)
		Yes	9 (4.43)
	Concomitant CYP3A4 Inhibitors	No	99 (48.77)
		Yes	104 (51.23)
	Concomitant CYP2C8 Inducers	No	203 (100.00)
	Concomitant CYP2C8 Inhibitors	No	203 (100.00)
	Concomitant Clopidogrel Medication	No	189 (93.10)
		Yes	14 (6.90)
	Concomitant Phosphate Binders	No	46 (22.66)
		Yes	157 (77.34)
	Phosphate Binders-Sevelamer	No	137 (67.49)
		Yes	66 (32.51)
	Concomitant BCRP Inhibitors	No	203 (100.00)
	Concomitant OATP Substrates	No	118 (58.13)
		Yes	85 (41.87)
	Proton Pump Inhibitors	No	129 (63.55)
		Yes	74 (36.45)
	Histamine-2 Antagonists	No	196 (96.55)
		Yes	7 (3.45)
	Anti-Acids	No	128 (63.05)
		Yes	75 (36.95)
	Sex	M	116 (57.14)
		F	87 (42.86)
	Ethnicity	HISPANIC OR LATINO	59 (29.06)
		NOT HISPANIC OR LATINO	144 (70.94)
	Country	ARG	21 (10.34)
		AUS	1 (0.49)
BRA		29 (14.29)	
CZE		8 (3.94)	
DEU		5 (2.46)	
GRC		2 (0.99)	
KOR		8 (3.94)	
POL		8 (3.94)	
RUS		59 (29.06)	
UKR		7 (3.45)	
USA		55 (27.09)	
Analysis Race		BLACK OR AFRICAN AMERICAN	37 (18.23)
		AMERICAN INDIAN OR ALASKAN NATIVE	2 (0.99)
	ASIAN - CENTRAL/SOUTH ASIAN HERITAGE	1 (0.49)	
	ASIAN - EAST ASIAN HERITAGE	8 (3.94)	

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Study Identifier	variable	value	Frequency (perc)
		ASIAN - JAPANESE HERITAGE	1 (0.49)
		ASIAN - SOUTH EAST ASIAN HERITAGE	1 (0.49)
		NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER	2 (0.99)
		WHITE - WHITE/CAUCASIAN/EUROPEAN HERITAGE	148 (72.91)
		MULTIPLE or MIXED RACE	3 (1.48)
	Region Code	USA	55 (27.09)
		EUROPE	89 (43.84)
		OTHER	59 (29.06)
	Hepatic Impairment	NON-HEPATIC IMPAIRED	201 (99.01)
		HEPATIC IMPAIRED	2 (0.99)
	Stage of Renal Impairment	Dialysis Dependent	203 (100.00)
	Dialysis Population	Hemodialysis	201 (99.01)
		Peritoneal dialysis	2 (0.99)
	Hyporesponder Flag	No	161 (79.31)
		Yes	42 (20.69)
	Covid-19 Diagnose	No	190 (93.60)
		Yes	6 (2.96)
		Suspected or Probable	7 (3.45)
200808	Concomitant Oral Iron	No	86 (63.24)
		Yes	50 (36.76)
	Concomitant IV Iron	No	123 (90.44)
		Yes	13 (9.56)
	Concomitant Iron	No	78 (57.35)
		Yes	58 (42.65)
	Concomitant CYP3A4 Inducers	No	122 (89.71)
		Yes	14 (10.29)
	Concomitant CYP3A4 Inhibitors	No	50 (36.76)
		Yes	86 (63.24)
	Concomitant CYP2C8 Inducers	No	136 (100.00)
	Concomitant CYP2C8 Inhibitors	No	135 (99.26)
		Yes	1 (0.74)
	Concomitant Clopidogrel Medication	No	125 (91.91)
		Yes	11 (8.09)
	Concomitant Phosphate Binders	No	111 (81.62)
		Yes	25 (18.38)
	Phosphate Binders-Sevelamer	No	132 (97.06)
		Yes	4 (2.94)
	Concomitant BCRP Inhibitors	No	134 (98.53)
		Yes	2 (1.47)
	Concomitant OATP Substrates	No	49 (36.03)
		Yes	87 (63.97)
	Proton Pump Inhibitors	No	89 (65.44)
		Yes	47 (34.56)
	Histamine-2 Antagonists	No	130 (95.59)
		Yes	6 (4.41)

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Study Identifier	variable	value	Frequency (perc)
	Anti-Acids	No	91 (66.91)
		Yes	45 (33.09)
	Sex	M	54 (39.71)
		F	82 (60.29)
	Ethnicity	HISPANIC OR LATINO	43 (31.62)
		NOT HISPANIC OR LATINO	93 (68.38)
	Country	ARG	7 (5.15)
		AUS	9 (6.62)
		BRA	27 (19.85)
		TWN	3 (2.21)
		DEU	1 (0.74)
		GRC	2 (1.47)
		HUN	12 (8.82)
		KOR	3 (2.21)
		POL	5 (3.68)
		ROU	9 (6.62)
		RUS	4 (2.94)
		UKR	12 (8.82)
		USA	42 (30.88)
	Analysis Race	BLACK OR AFRICAN AMERICAN	16 (11.76)
		ASIAN - EAST ASIAN HERITAGE	6 (4.41)
		ASIAN - JAPANESE HERITAGE	2 (1.47)
		WHITE - WHITE/CAUCASIAN/EURO- PEAN HERITAGE	111 (81.62)
		MULTIPLE or MIXED RACE	1 (0.74)
	Region Code	USA	42 (30.88)
		EUROPE	45 (33.09)
		OTHER	49 (36.03)
	Hepatic Impairment	NON-HEPATIC IMPAIRED	134 (98.53)
		HEPATIC IMPAIRED	2 (1.47)
	Stage of Renal Impairment	GFR 60-89	1 (0.74)
GFR 30-59		24 (17.65)	
GFR 15-29		72 (52.94)	
GFR less than 15 and non-Dialysis Study		39 (28.68)	
Dialysis Population	Non-dialysis	136 (100.00)	
Hypo-responder Flag	Missing	136 (100.00)	
Covid-19 Diagnose	No	114 (83.82)	
	Yes	13 (9.56)	
	Suspected or Probable	9 (6.62)	
201410	Concomitant Oral Iron	No	80 (89.89)
		Yes	9 (10.11)
	Concomitant IV Iron	No	57 (64.04)
		Yes	32 (35.96)
	Concomitant Iron	No	49 (55.06)
		Yes	40 (44.94)
	Concomitant CYP3A4 Inducers	No	86 (96.63)
		Yes	3 (3.37)

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Study Identifier	variable	value	Frequency (perc)	
	Concomitant CYP3A4 Inhibitors	No	35 (39.33)	
		Yes	54 (60.67)	
	Concomitant CYP2C8 Inducers	No	89 (100.00)	
	Concomitant CYP2C8 Inhibitors	No	89 (100.00)	
	Concomitant Clopidogrel Medication	No	80 (89.89)	
		Yes	9 (10.11)	
	Concomitant Phosphate Binders	No	48 (53.93)	
		Yes	41 (46.07)	
	Phosphate Binders-Sevelamer	No	81 (91.01)	
		Yes	8 (8.99)	
	Concomitant BCRP Inhibitors	No	89 (100.00)	
	Concomitant OATP Substrates	No	46 (51.69)	
		Yes	43 (48.31)	
	Proton Pump Inhibitors	No	60 (67.42)	
		Yes	29 (32.58)	
	Histamine-2 Antagonists	No	87 (97.75)	
		Yes	2 (2.25)	
	Anti-Acids	No	57 (64.04)	
		Yes	32 (35.96)	
	Sex	M	54 (60.67)	
		F	35 (39.33)	
	Ethnicity	HISPANIC OR LATINO	27 (30.34)	
		NOT HISPANIC OR LATINO	62 (69.66)	
	Country	ARG	15 (16.85)	
		AUS	1 (1.12)	
		CAN	2 (2.25)	
		DEU	2 (2.25)	
		IND	2 (2.25)	
		KOR	2 (2.25)	
		MYS	1 (1.12)	
		MEX	2 (2.25)	
		POL	7 (7.87)	
		RUS	31 (34.83)	
		ESP	4 (4.49)	
		GBR	1 (1.12)	
		USA	19 (21.35)	
		Analysis Race	BLACK OR AFRICAN AMERICAN	12 (13.48)
			AMERICAN INDIAN OR ALASKAN NATIVE	2 (2.25)
	ASIAN - CENTRAL/SOUTH ASIAN HERITAGE		3 (3.37)	
	ASIAN - EAST ASIAN HERITAGE		2 (2.25)	
	ASIAN - SOUTH EAST ASIAN HERITAGE		1 (1.12)	
	WHITE - WHITE/CAUCASIAN/EUROPEAN HERITAGE		69 (77.53)	
	Region Code	USA	19 (21.35)	
		EUROPE	45 (50.56)	

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Study Identifier	variable	value	Frequency (perc)
		OTHER	25 (28.09)
	Hepatic Impairment	NON-HEPATIC IMPAIRED	89 (100.00)
	Stage of Renal Impairment	Dialysis Dependent	89 (100.00)
	Dialysis Population	Hemodialysis	88 (98.88)
		Peritoneal dialysis	1 (1.12)
	Hyporesponder Flag	Missing	89 (100.00)
	Covid-19 Diagnose	No	88 (98.88)
		Yes	1 (1.12)
204837	Concomitant Oral Iron	No	221 (94.04)
		Yes	14 (5.96)
	Concomitant IV Iron	No	178 (75.74)
		Yes	57 (24.26)
	Concomitant Iron	No	166 (70.64)
		Yes	69 (29.36)
	Concomitant CYP3A4 Inducers	No	218 (92.77)
		Yes	17 (7.23)
	Concomitant CYP3A4 Inhibitors	No	125 (53.19)
		Yes	110 (46.81)
	Concomitant CYP2C8 Inducers	No	235 (100.00)
	Concomitant CYP2C8 Inhibitors	No	234 (99.57)
		Yes	1 (0.43)
	Concomitant Clopidogrel Medication	No	210 (89.36)
		Yes	25 (10.64)
	Concomitant Phosphate Binders	No	79 (33.62)
		Yes	156 (66.38)
	Phosphate Binders-Sevelamer	No	178 (75.74)
		Yes	57 (24.26)
	Concomitant BCRP Inhibitors	No	234 (99.57)
		Yes	1 (0.43)
	Concomitant OATP Substrates	No	141 (60.00)
		Yes	94 (40.00)
	Proton Pump Inhibitors	No	125 (53.19)
		Yes	110 (46.81)
	Histamine-2 Antagonists	No	219 (93.19)
		Yes	16 (6.81)
	Anti-Acids	No	161 (68.51)
		Yes	74 (31.49)
	Sex	M	129 (54.89)
		F	106 (45.11)
	Ethnicity	HISPANIC OR LATINO	62 (26.38)
		NOT HISPANIC OR LATINO	170 (72.34)
		Missing	3 (1.28)
	Country	ARG	30 (12.77)
		AUS	6 (2.55)
		BRA	18 (7.66)
		CAN	2 (0.85)
		FRA	5 (2.13)

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Jesduvroq (daprodustat)

Study Identifier	variable	value	Frequency (perc)
		ITA	8 (3.40)
		KOR	14 (5.96)
		POL	15 (6.38)
		ROU	4 (1.70)
		RUS	56 (23.83)
		ESP	17 (7.23)
		GBR	4 (1.70)
		USA	56 (23.83)
	Analysis Race	Missing	3 (1.28)
		BLACK OR AFRICAN AMERICAN	39 (16.60)
		AMERICAN INDIAN OR ALASKAN NATIVE	1 (0.43)
		ASIAN - CENTRAL/SOUTH ASIAN HERITAGE	1 (0.43)
		ASIAN - EAST ASIAN HERITAGE	15 (6.38)
		ASIAN - SOUTH EAST ASIAN HERITAGE	2 (0.85)
		NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER	1 (0.43)
		WHITE - ARABIC/NORTH AFRICAN HERITAGE	1 (0.43)
		WHITE - WHITE/CAUCASIAN/EUROPEAN HERITAGE	170 (72.34)
		MIXED WHITE RACE	1 (0.43)
		MULTIPLE or MIXED RACE	1 (0.43)
	Region Code	USA	56 (23.83)
		EUROPE	109 (46.38)
		OTHER	70 (29.79)
	Hepatic Impairment	NON-HEPATIC IMPAIRED	234 (99.57)
		HEPATIC IMPAIRED	1 (0.43)
	Stage of Renal Impairment	Dialysis Dependent	235 (100.00)
	Dialysis Population	Hemodialysis	235 (100.00)
	Hyporesponder Flag	No	171 (72.77)
		Yes	64 (27.23)
	Covid-19 Diagnose	No	234 (99.57)
		Yes	1 (0.43)
205665	Concomitant Oral Iron	No	44 (100.00)
	Concomitant IV Iron	No	44 (100.00)
	Concomitant Iron	No	44 (100.00)
	Concomitant CYP3A4 Inducers	No	43 (97.73)
		Yes	1 (2.27)
	Concomitant CYP3A4 Inhibitors	No	17 (38.64)
		Yes	27 (61.36)
	Concomitant CYP2C8 Inducers	No	44 (100.00)
	Concomitant CYP2C8 Inhibitors	No	43 (97.73)
		Yes	1 (2.27)
	Concomitant Clopidogrel Medication	No	33 (75.00)
		Yes	11 (25.00)

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Study Identifier	variable	value	Frequency (perc)	
	Concomitant Phosphate Binders	No	8 (18.18)	
		Yes	36 (81.82)	
	Phosphate Binders-Sevelamer	No	22 (50.00)	
		Yes	22 (50.00)	
	Concomitant BCRP Inhibitors	No	44 (100.00)	
	Concomitant OATP Substrates	No	18 (40.91)	
		Yes	26 (59.09)	
	Proton Pump Inhibitors	No	27 (61.36)	
		Yes	17 (38.64)	
	Histamine-2 Antagonists	No	40 (90.91)	
		Yes	4 (9.09)	
	Anti-Acids	No	41 (93.18)	
		Yes	3 (6.82)	
	Sex	M	25 (56.82)	
		F	19 (43.18)	
	Ethnicity	HISPANIC OR LATINO	22 (50.00)	
		NOT HISPANIC OR LATINO	22 (50.00)	
	Country	USA	44 (100.00)	
	Analysis Race	BLACK OR AFRICAN AMERICAN	21 (47.73)	
		WHITE - ARABIC/NORTH AFRICAN HERITAGE	1 (2.27)	
		WHITE - WHITE/CAUCASIAN/EUROPEAN HERITAGE	22 (50.00)	
	Region Code	USA	44 (100.00)	
	Hepatic Impairment	NON-HEPATIC IMPAIRED	44 (100.00)	
	Stage of Renal Impairment	Dialysis Dependent	44 (100.00)	
	Dialysis Population	Hemodialysis	44 (100.00)	
	Hyporesponder Flag	Missing	44 (100.00)	
	Covid-19 Diagnose	No	44 (100.00)	
	Overall	Concomitant Oral Iron	No	623 (88.12)
			Yes	84 (11.88)
		Concomitant IV Iron	No	565 (79.92)
			Yes	142 (20.08)
		Concomitant Iron	No	492 (69.59)
			Yes	215 (30.41)
		Concomitant CYP3A4 Inducers	No	663 (93.78)
			Yes	44 (6.22)
		Concomitant CYP3A4 Inhibitors	No	326 (46.11)
			Yes	381 (53.89)
		Concomitant CYP2C8 Inducers	No	707 (100.00)
		Concomitant CYP2C8 Inhibitors	No	704 (99.58)
			Yes	3 (0.42)
		Concomitant Clopidogrel Medication	No	637 (90.10)
			Yes	70 (9.90)
		Concomitant Phosphate Binders	No	292 (41.30)
			Yes	415 (58.70)
Phosphate Binders-Sevelamer		No	550 (77.79)	

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Study Identifier	variable	value	Frequency (perc)
		Yes	157 (22.21)
	Concomitant BCRP Inhibitors	No	704 (99.58)
		Yes	3 (0.42)
	Concomitant OATP Substrates	No	372 (52.62)
		Yes	335 (47.38)
	Proton Pump Inhibitors	No	430 (60.82)
		Yes	277 (39.18)
	Histamine-2 Antagonists	No	672 (95.05)
		Yes	35 (4.95)
	Anti-Acids	No	478 (67.61)
		Yes	229 (32.39)
	Sex	M	378 (53.47)
		F	329 (46.53)
	Ethnicity	HISPANIC OR LATINO	213 (30.13)
		NOT HISPANIC OR LATINO	491 (69.45)
		Missing	3 (0.42)
	Country	ARG	73 (10.33)
		AUS	17 (2.40)
		BRA	74 (10.47)
		CAN	4 (0.57)
		TWN	3 (0.42)
		CZE	8 (1.13)
		FRA	5 (0.71)
		DEU	8 (1.13)
		GRC	4 (0.57)
		HUN	12 (1.70)
		IND	2 (0.28)
		ITA	8 (1.13)
		KOR	27 (3.82)
		MYS	1 (0.14)
		MEX	2 (0.28)
		POL	35 (4.95)
		ROU	13 (1.84)
		RUS	150 (21.22)
		ESP	21 (2.97)
		UKR	19 (2.69)
		GBR	5 (0.71)
		USA	216 (30.55)
	Analysis Race	Missing	3 (0.42)
		BLACK OR AFRICAN AMERICAN	125 (17.68)
		AMERICAN INDIAN OR ALASKAN NATIVE	5 (0.71)
		ASIAN - CENTRAL/SOUTH ASIAN HERITAGE	5 (0.71)
		ASIAN - EAST ASIAN HERITAGE	31 (4.38)
		ASIAN - JAPANESE HERITAGE	3 (0.42)
		ASIAN - SOUTH EAST ASIAN HER- ITAGE	4 (0.57)

Study Identifier	variable	value	Frequency (perc)
		NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER	3 (0.42)
		WHITE - ARABIC/NORTH AFRICAN HERITAGE	2 (0.28)
		WHITE - WHITE/CAUCASIAN/EUROPEAN HERITAGE	520 (73.55)
		MIXED WHITE RACE	1 (0.14)
		MULTIPLE or MIXED RACE	5 (0.71)
	Region Code	USA	216 (30.55)
		EUROPE	288 (40.74)
		OTHER	203 (28.71)
	Hepatic Impairment	NON-HEPATIC IMPAIRED	702 (99.29)
		HEPATIC IMPAIRED	5 (0.71)
	Stage of Renal Impairment	GFR 60-89	1 (0.14)
		GFR 30-59	24 (3.39)
		GFR 15-29	72 (10.18)
		GFR less than 15 and non-Dialysis Study	39 (5.52)
		Dialysis Dependent	571 (80.76)
	Dialysis Population	Non-dialysis	136 (19.24)
		Hemodialysis	568 (80.34)
		Peritoneal dialysis	3 (0.42)
	Hyporesponder Flag	No	332 (46.96)
		Yes	106 (14.99)
		Missing	269 (38.05)
	Covid-19 Diagnose	No	670 (94.77)
		Yes	21 (2.97)
		Suspected or Probable	16 (2.26)

Source: Applicant's Population PK report TM-14152615, Table 11.1.15

Abbreviations: BCRP, breast cancer resistance protein; GFR, glomerular filtration rate; IV, intravenous; OATP, organic-anion-transporting polypeptides; PK, pharmacokinetic

The Applicant claimed that a 3-compartment PopPK model with first-order elimination from the central compartment together with 5 absorption transit compartments, best described daprodustat PK. Allometric scaling was applied to all drug clearance (CL) and volume terms using fixed exponents (0.75 and 1.00 respectively) to account for PK variability related to body weight.

The final daprodustat PK model parameter estimates and the corresponding 95% confidence intervals (CIs) from Bootstrap are presented in [Table 200](#). The goodness-of-fit plots for the final daprodustat PK model are presented in [Figure 49](#). The analysis plan included the Goodness-of-Fit (GoF) plots that use the normalized predicted distribution error (NPDE) instead of conditional weighted residuals (CWRES), as CWRES is not defined for BLQ observations, NPDE take the likelihood of the BLQ data into account and are, therefore, more appropriate for the M3 method that was used to model the PK data. Due to the use of the M3 method to incorporate the BLQ data, the prediction-corrected visual predictive check (VPC) could not be generated. The VPC based on all data from five studies ([Figure 50](#)) illustrated the prediction percentiles and corresponding 95% CI of simulated concentrations overlaid on the observed daprodustat concentrations and the corresponding 5th and 95th percentiles.

Table 200. Population PK Parameters of Daprodustat From the Applicant's Final PK Model

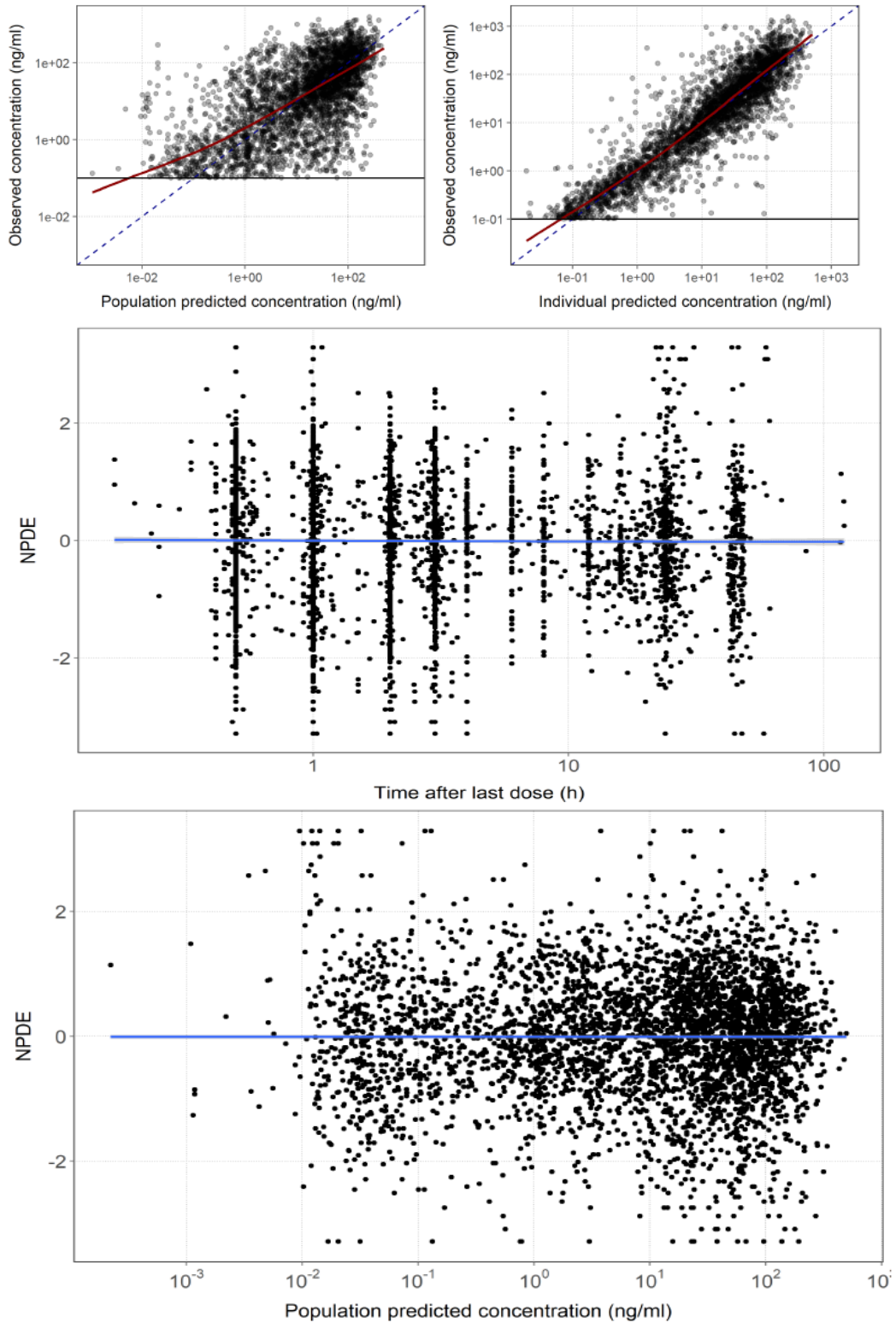
Parameter Name	Estimate	SE	RSE (%)	95% CI
CL (L/h)	24.6	[fixed]	2.76*	(23.2, 25.9)*
V2 (L)	26.2	[fixed]	4.61*	(23.8, 28.5)*
Q (L/h)	0.917	[fixed]	8.62*	(0.763, 1.07)*
V3 (L)	3.26	[fixed]	4.49*	(2.97, 3.54)*
ka (1/h)	4.96	[fixed]	9.59*	(4.03, 5.90)*
F1 (-)	1.00	[fixed]	-	-
Q2 (L/h)	0.359	[fixed]	14.4*	(0.257, 0.461)*
V6 (L)	27.1	[fixed]	37.1*	(7.38, 46.9)*
PH3ka (-)	-0.330	0.0350	10.6	(-0.399, -0.261)
PH3V3/PH3V2 (-)	0.432	0.0370	8.55	(0.360, 0.505)
PH3Q2 (-)	-1.42	0.173	12.2	(-1.76, -1.08)
Clopidogrel effect on CL (-)	-0.424	0.0603	14.2	(-0.542, -0.306)
Non-dialysis dependence effect on ka (-)	0.456	0.120	26.3	(0.220, 0.691)
Study 205665 effect on Q2 (-)	2.44	0.289	11.8	(1.88, 3.01)
Study 205665 effect on F1 (-)	-0.956	0.117	12.2	(-1.19, -0.727)
Study 205665 effect on ka (-)	0.623	0.177	28.4	(0.276, 0.970)
Inter-individual Variability	Estimate	SE	RSE (%)	%CV
ω^2 - CL	0.446	0.0169	3.78	75.0
ω^2 - ka	0.657	0.0435	6.61	96.4
Residual Error	Estimate	SE	RSE (%)	95% CI
σ - Additive error (log-scale) for all studies except Study 205665	0.979	0.00896	0.915	(0.961, 0.997)
σ - Additive error (log-scale) for Study 205665	1.63	0.0203	1.24	(1.59, 1.67)

RSE (%) is calculated as SE/Estimate*100; 95% CI is calculated as Estimate +/- 1.96*SE; %CV is calculated as $\sqrt{\exp(\omega^2)-1}$ *100. Shrinkage of Empirical Bayes Estimates for CL and ka are 14.6% and 7.6%, respectively. PH3ka, PH3V3, PH3V2 and PH3Q2 are factors that reflect the differences of these parameters in the current analysis relative to the Phase 1 estimate (parameter estimated as difference on a log-scale). *Showing RSE, 95% CI and %CV from Phase 1 PK model (L.dap) for illustrative purposes.

Source: Applicant's Population PK report TM-14152615, Table 7.3.7

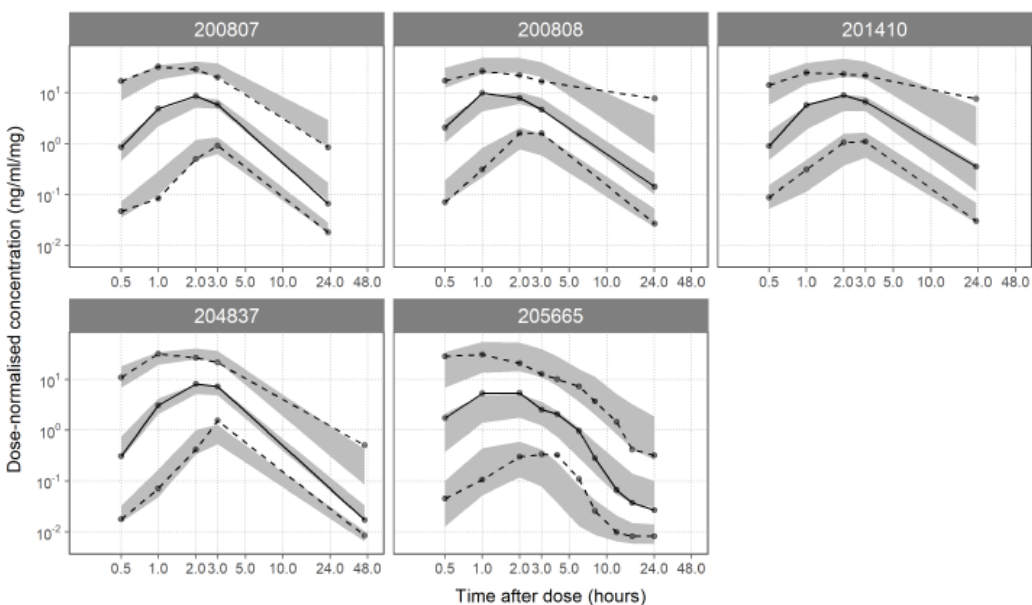
Abbreviations: CI, confidence interval; PK, pharmacokinetic; RSE, relative standard errors; SE, standard errors

Figure 49. Goodness-of-Fit Plots for the Applicant's Final Population PK Model for Daprodustat



Source: Applicant's Population PK report TM-14152615, Figure 11.1.23, 11.1.19 and 11.1.20.
Abbreviations: NPDE, normalised predicted distribution error; PK, pharmacokinetic

Figure 50. Visual Predictive Check for the Applicant's Final Daprodustat Model

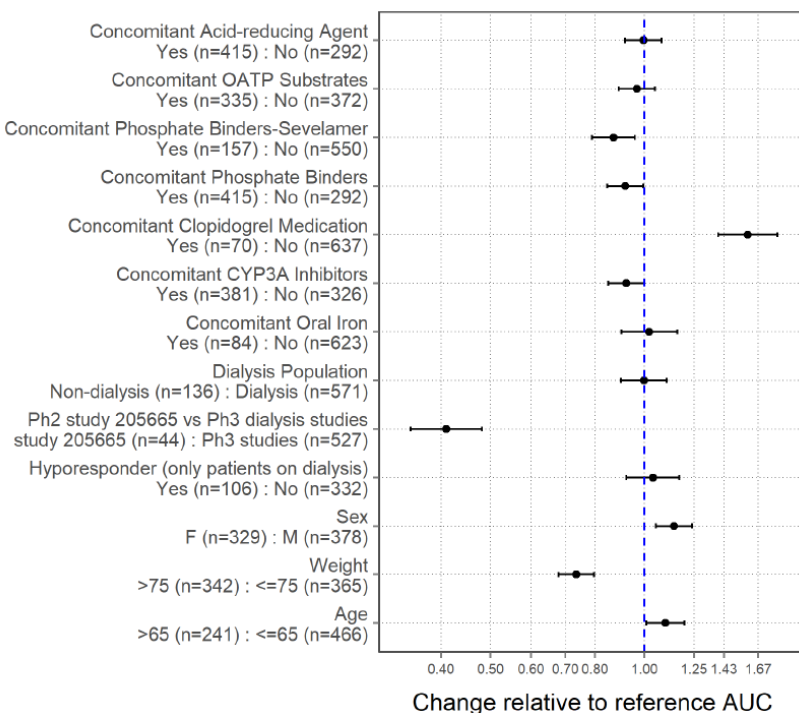


Source: Applicant's Population PK report TM-14152615, Figure 7.3.5

Covariate analysis showed only two significant covariates were identified: concomitant clopidogrel use reduced daprodustat clearance by 42.4%, and subjects that were non-dialysis dependent had a 45.6% higher k_a compared to dialysis dependent subjects. There are no significant covariate effects of age, body weight, sex, or hypo-responder status. Body weight, as a covariate, was implemented in the PopPK model as allometric exponents applied to CL and V model parameters. The simulated covariate effects on C_{max} and AUC from body weight, dialysis and Study 205665, and concomitant drug use relative to a reference subject (non-dialysis dependent, not using clopidogrel, body weight of 74.6 kg) are shown in [Figure 51](#), [Figure 52](#), and [Table 201](#). Body weight, as a covariate, was implemented in the PopPK model as allometric exponents applied to CL and V model parameters. The AUC ratio for subjects weighing >75 kg to ≤ 75 kg (estimate: 0.74, 90% CI: 0.68 – 0.80) and corresponding C_{max} ratio (estimate 0.68, 90% CI: 0.64 – 0.73) suggests these changes are unlikely to be clinically relevant given the individualized treatment modality of daprodustat in CKD.

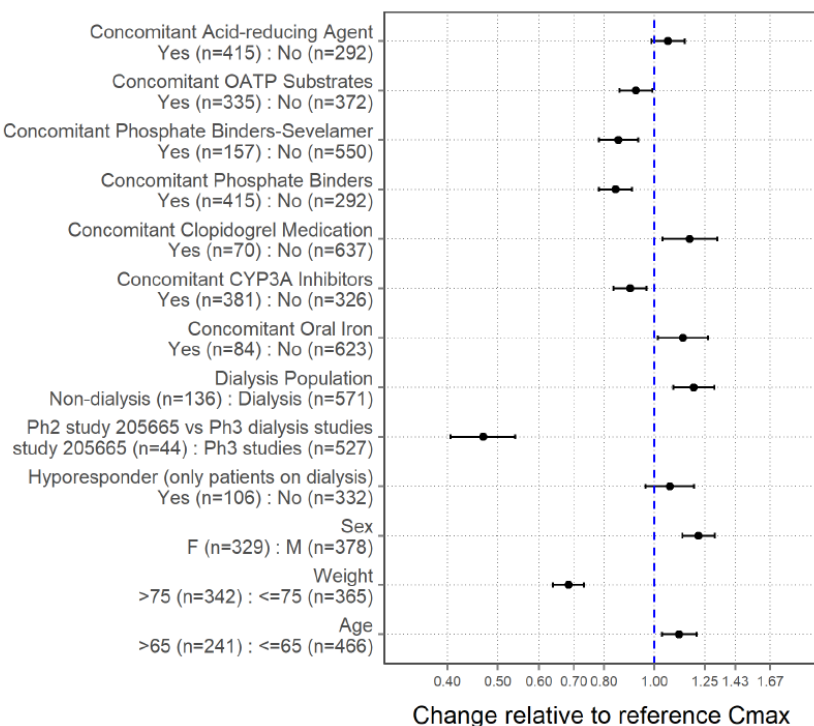
Of note, the maximum daprodustat dose level in the current analysis dataset was 24 mg for the QD dose studies and 48 mg for the TIW dose study 204837. Additional simulations were performed with the final POPK model to calculate the exposure metrics for a typical dialysis patient (without concomitant clopidogrel use and 74.6 kg body weight) at the maximum daprodustat dose level and is shown in [Table 202](#).

Figure 51. Forest Plot for Model-Predicted Dose-Normalized AUC at Steady-State



Source: Applicant's Population PK report TM-14152615, Figure 2.4.1.
Abbreviations: AUC, area under the concentration-time curve; OATP, organic-anion-transporting polypeptides

Figure 52. Forest Plot for Model-Predicted Dose-Normalised C_{max} at Steady-State



Source: Applicant's Population PK report TM-14152615, Figure 2.4.2.
Abbreviations: C_{max}, maximum plasma concentration; OATP, organic-anion-transporting polypeptides

Table 201. Simulated Covariate Effect Sizes in Typical Subjects From Final PPK Model

Covariate	Fold-change AUC	Fold-change C_{max}
Bodyweight = 50.1 kg (5 th percentile)	1.35	1.44
Bodyweight = 114.2 kg (95 th percentile)	0.73	0.68
Concomitant clopidogrel use	1.74	1.18
Dialysis (Phase 3 studies)	1.00	0.86
Dialysis (Phase 2B Study 205665)	0.38	0.41

Fold-changes of exposure metrics AUC and C_{max} are calculated relative to the following reference subject: non-dialysis dependent subject, without concomitant clopidogrel use and 74.6 kg bodyweight

Source: Applicant's Population PK report TM-14152615, Table 2.4.1.

Abbreviations: AUC, area under the concentration-time curve; C_{max} , maximum plasma concentration; PPK, population pharmacokinetic

Table 202. Simulated Exposure Estimates for Typical Dialysis Patient at Maximum Dose Levels (24 mg QD or 48 mg TIW)

Exposure estimates	24 mg QD	48 mg TIW
C_{max} (ng/ml)	282.3	564.1
Dose-normalised C_{max} (ng/ml/mg)	11.8	11.8
AUC _{0-24h} (ng.h/ml)	930.1	1853.8
Dose-normalised AUC _{0-24h} (ng.h/ml/mg)	38.8	38.6
AUC _{0-168h} (ng.h/ml)	6510.9	5580.8

QD = once daily dosing, TIW = three times weekly dosing, C_{max} = Maximum daprodustat concentration at steady state, AUC_{0-24h} = Area under the concentration curve in the first 24 hours after last dose at steady state, AUC_{0-168h} = Area under the concentration curve during one week at steady state.

Source: Applicant's Population PK report TM-14152615, Table 7.4.9.

Reviewer's Comments

The Applicant's final population PK model as described above is adequate to describe the daprodustat serum concentration-time profiles of patients with CKD in studies 205665, 204837, 201410, 200807 and 20080, following the administration of daprodustat ranging from 1 mg to 48 mg QD. The shrinkage value was 14.6% for IIV on CL/F, 7.6% for IIV on k_a , and condition number was 12.8, indicating there was no obvious bias in the parameter estimates. Therefore, the PK model is acceptable for simulating post-hoc exposure metrics, e.g., C_{max} and C_{tau} of daprodustat for exposure-response (E-R) analyses.

Covariate analysis identified 2 statistically significant covariates: concomitant clopidogrel use reduced clearance by 42.4%, and subjects that were non-dialysis dependent had 45.6% higher k_a compared to dialysis dependent subjects. There are no significant covariate effects of age, body weight, sex, or hyporesponder status. The impact of the following intrinsic and extrinsic factors on the population pharmacokinetics of daprodustat in patients with CKD were quantified using simulations of typical subjects with the final model: dialysis (no change in AUC and -14% in C_{max}), and concomitant clopidogrel use (+74% AUC and +18% C_{max}).

However, a PopPK-based DDI evaluation for concomitant clopidogrel use can be limited due to the following reasons: inadequacy of sample size, lack of clear medication history prior to study entry, lack of dosing records for concomitant medications (e.g., dose level, dose frequency, formulation, route of administration, start and stop date and clock time, duration, dose modification, presence of other concomitant medications were not recorded), missing food, and/or other potential confounding factors, and inadequacy of PK sampling timepoints relative to concomitant medication dosing. In addition, the Applicant only used a binary covariate for concomitant clopidogrel use rather than a time varying covariate model.

In summary, the Applicant's current PopPK based DDI assessment is not adequate, which may significantly underestimate the true DDI effect due to the following reasons:

1. The binary covariate analysis was performed in the Applicant's PopPK modeling without clear dosing history.
2. Lack of adequate daprodustat PK samples during the elimination phase in clinical trials.
3. Lack of medication history prior to study entry (that may have prolonged effects on drug absorption, distribution, metabolism, and elimination) and dosing records for concomitant medications: e.g., dose level, dose frequency, formulation, route of administration, start and stop date and clock time, duration, dose modification, in PopPK dataset.

The Applicant's justification based on efficacy/safety for minimum PK effect of clopidogrel and phosphate binders, iron, or acid reducing agents coadministration (use/no use) are also not acceptable. These can be confounded by dose titration to maintain target Hb range of 10 to 11.5 g/dL. Therefore, the Applicant's current PopPK based DDI assessments are not adequate.

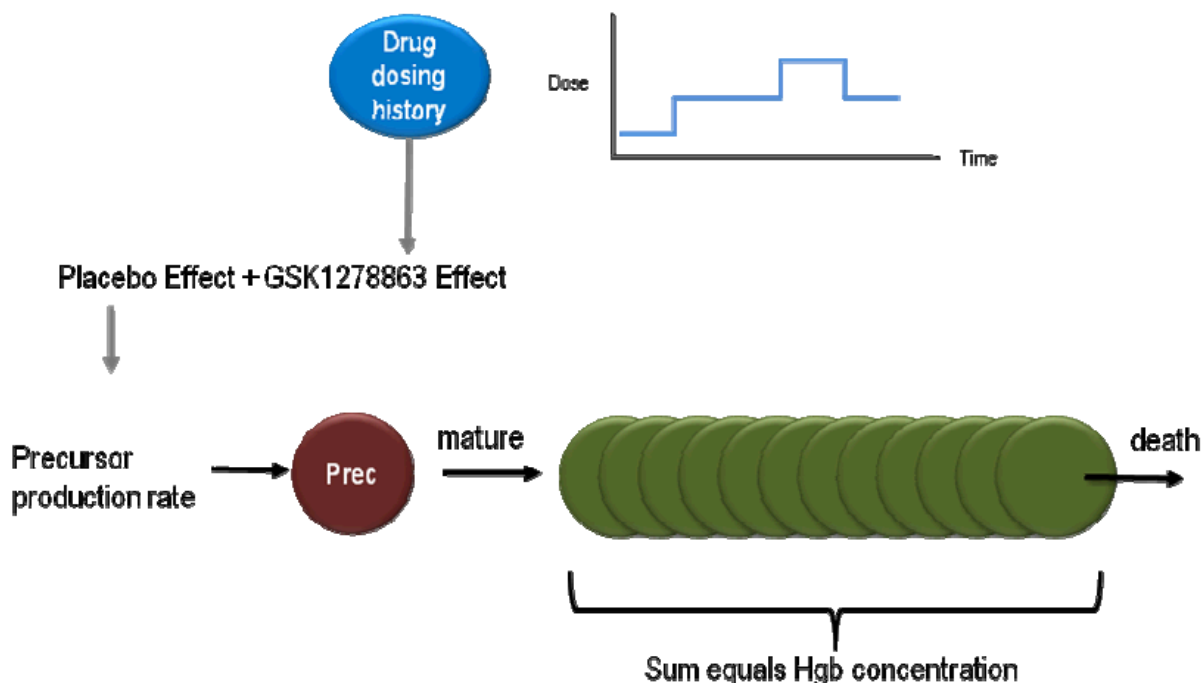
To support TIW dosing for dialysis patients, PopPK simulations were performed. The results showed the weekly AUC is slightly higher with 24 mg QD compared to 48 mg TIW, corresponding to the higher weekly dose in the former (168 mg) compared to the latter (144 mg).

In summary, the PopPK model supports no dose adjustment for the above-mentioned specific populations such as age, gender, body weight, and non-dialysis dependent status but is not sensitive to evaluate drug interactions.

14.5.2. Summary of Applicant's Population Dose response model

The initial daprodustat dose response analysis of Hb in anemic subjects with chronic kidney disease was developed in 2015 using 11,810 Hb observations in a total of 710 subjects. The structural model used to describe the time course of Hb was comprised of a precursor cell compartment, where the baseline production of precursors was a function of baseline Hb, and 12 transit compartments that described the RBC lifespan as shown in [Figure 53](#).

Figure 53. Scheme of Longitudinal Hb Model



Source: Applicant's Population PK/PD report 2015n248947, Figure 3.
Abbreviations: Hb, hemoglobin; Hgb, hemoglobin

Clinical Trial Simulation

To support phase 3 dose selection, the following dosing scheme ([Table 203](#)) was simulated using a clinical trial simulation dose Hb model. The individual dose adjustment scheme used in these simulations is summarized in [Table 204](#). A summary table of key statistics is shown in [Table 205](#). Average Hb and the % of time within the target range were assessed over an evaluation period from Week 28 to 52, as planned in the Phase 3 studies.

Table 203. Starting Doses for Clinical Trial Simulation

ESA status	Prior ESA Dose (Epoetin alfa U/week IV)	HDD GSK1278863 starting dose (mg)	ND GSK1278863 starting dose (mg)
Switch	<2000	4	1
	≥2000 to <10000	6	2
	≥10000 to <20000	8	2
	≥20000	12	4
ESA status	Baseline Hgb (g/dL)	HDD GSK1278863 starting dose (mg)	ND GSK1278863 starting dose (mg)
Naïve	≥8 to <9	4	4
	≥9 to ≤10	2	2
	>10 to ≤11 ^a	1	NA

^aBaseline Hgb of >10 g/dL is only applicable to HDD naïve subjects

Source: Applicant's Population PK/PD report 2015n248947, Table 12.
Abbreviations: ESA, erythropoiesis stimulating agent; HDD, hemodialysis-dependent; Hb, hemoglobin; ND, non-dialysis

Table 204. Dose Adjustment Scheme

HemoCue Hgb at current visit (g/dL)	Hgb change since last visit	GSK1278863 dose adjustment
<9.5	Decreasing or No change	Increase one dose level
<9.5	Increasing	Maintain dose
≥9.5 to <10.0	Any change	Maintain dose
≥9.5 to <10 for two consecutive visits	Decreasing or No change	Increase one dose level
≥10.0 to ≤11.0	Any change	Maintain dose
>11.0 to ≤11.5	Any change	Maintain dose
>11 to ≤11.5 for two consecutive visits	Increasing or No change	Decrease one dose level
>11.5	Decreasing	Maintain dose
>11.5	Increasing or No change	Decrease one dose level
≥12.0	Any change	Temporary hold the dose. Restart at one dose level lower when Hgb < 11.5 g/dL or if Hgb decreases by ≥2 g/dL from previous visit
Any	>2 g/dL increase	Decrease one dose level
Any	>2 g/dL decrease	Increase one dose level

Source: Applicant's Population PK/PD report 2015n248947, Table 13.
Abbreviations: Hgb, hemoglobin

Table 205. Summary Statistics From Simulation Scenarios

Scenario (residual variability proportion)	Population	Mean (SD) Hgb (g/dL) ^a	Mean (SD) %TIR ^b	Mean (SD) %TIR-2 ^c	Rescued (%) ^d	Interrupted (%) ^e	Mean (SD) Number of dose adjustments ^f	Median (min, max) number of dose adjustments ^f
10001 (1.0)	HDD ESA switch	10.32 (0.52)	60.31 (27.19)	71.99 (27.41)	1.2	19.6	2.90(1.90)	3 (0, 9)
10002 (0.25)	HDD ESA switch	10.33 (0.51)	73.74 (31.53)	80.61 (28.52)	1.8	8.5	2.32 (1.83)	2 (0, 7)
10003 (1.0)	ND ESA switch	10.29 (0.45)	63.60 (25.94)	77.42 (24.08)	0	14.5	2.64 (1.79)	2 (0, 9)
10004 (0.25)	ND ESA switch	10.32 (0.41)	76.56 (28.67)	87.05 (23.24)	0	4	2.00 (1.64)	2 (0, 8)
10005 (1.0)	ND ESA naive	10.31 (0.30)	67.27 (23.78)	80.73 (20.53)	0	15.9	2.35 (1.73)	2 (0, 9)
10006 (0.25)	ND ESA naive	10.27 (0.35)	80.59 (27.63)	89.69 (20.85)	0	2.6	1.59 (1.60)	1 (0, 7)
10007 (1.0)	HDD ESA naive	10.36 (0.33)	67.51 (24.31)	80.79 (20.71)	0	16.5	2.13 (1.70)	2 (0, 7)
10008 (0.25)	HDD ESA naive	10.35 (0.34)	81.21 (26.77)	89.41 (20.82)	0	2.7	1.46 (1.53)	1 (0, 8)

^aOver the Week 28 to 52 evaluation period
^bTime In Range (10-11 g/dL) over the Week 28 to 52 evaluation period
^cTime In Range-2 (10-11.5 g/dL) over the Week 28 to 52 evaluation period
^dSubjects meeting rescue criteria in first 52 weeks
^eSubjects that underwent a dose interruption (Hgb >12 g/dL) in first 52 weeks
^fDose adjustments per subjects over first 52 weeks

Source: Applicant's Population PK/PD report 2015n248947, Table 15.
Abbreviations: ESA, erythropoiesis stimulating agent; HDD, hemodialysis-dependent; Hgb, hemoglobin; ND, non-dialysis; SD, standard deviation

Reviewer's Comments

The Applicant's final population dose Hb model developed in 2015 as described above is adequate to describe the Hb concentration time profiles of patients with CKD after treatment with placebo or daprodustat (0.5 to 25 mg QD). Model diagnostics and predictive performance of the model (as assessed by prediction and variability corrected predictive checks) were acceptable. Based on model simulations, the proposed starting doses, dose levels, and dose adjustment scheme appear to be appropriate and were predicted to lead to Hb levels within the target range of 10 to 11 g/dL, which was subsequently confirmed in phase 3 clinical trials.

14.5.3. Summary of Applicant's Exposure-Response Analysis

14.5.3.1. Longitudinal Exposure-Response Analysis to Quantify the Effect of Daprodustat on Hemoglobin Changes in Subjects With Chronic Kidney Disease Undergoing Dialysis From Studies 200807, 204837 and 204836

(b) (4) TIW dosing regimen versus QD in subjects with CKD undergoing dialysis, multivariate longitudinal population exposure-response analysis was conducted using three studies: 200807, 204837 and 204836. The initial daprodustat dose response Hb model in anemic subjects with CKD was developed in 2015 to support phase 3 dose selection (see details in section [14.5.2](#)). The Applicant further updated their 2019 Dose-Hb model with longitudinal Hb data from study 204836 and data from three Japanese Phase 3 studies. Lastly, additional Hb data from 204837 and 200807 studies were used to further update their final 2022 Dose-Hb model. The above dose response models showed consistent parameters estimates and model predictive performance.

For the current longitudinal population concentration-Hb (Conc-Hb) model, the PK data from subgroup populations from the three studies (200807, 204837 and 204836) with corresponding Hb levels were pooled and analyzed using a population approach with evaluation of patient covariates on the model parameters.

Summary statistics of the continuous and categorical covariates that were evaluated in the E-R analysis are shown in [Table 206](#).

Table 206. Selected Baseline and Clinical Patient Characteristics

	Learning Dataset		Validation Dataset
Study	200807	204837	204836
Subjects [n]	203	235	77
Age (y)			
Mean (SD)	54.9 (14.0)	59.7 (14.1)	64.2 (15.1)
[range]	[22-85]	[22-90]	[22-91]
Body Weight (kg)			
Mean (SD)	77.3 (19.7)	76.1 (18.9)	78.4 (22.7)
[range]	[41.2-140.4]	[36.1-138.3]	[50.0-162.4]
Sex [n (%)]			
Male	116 (57.1)	129 (54.9)	49 (63.6)
Female	87 (42.9)	106 (45.1)	28 (36.4)
Race [n (%)]			
African-American	37 (18.2)	39 (16.6)	21 (27.3)
Asian	11 (5.4)	18 (7.7)	1 (1.3)
White-Caucasian	148 (72.9)	170 (72.3)	52 (67.5)
Other	7 (3.4)	5 (2.1)	3 (3.9)
Missing	0 (0)	3 (1.3)	0 (0)
Ethnicity [n (%)]			
Hispanic or Latino	59 (29.1)	62 (26.4)	12 (15.6)
Not Hispanic or Latino	144 (70.9)	170 (72.3)	65 (84.4)
Concomitant medication			
Clopidogrel [n (%)]	30 (14.8)	28 (11.9)	10 (13.0)
Iron [n (%)]	195 (96.1)	205 (87.2)	52 (67.5)
Prior ESA use [n (%)]			
Historic	6 (3.0)	3 (1.3)	0 (0.0)
Recent	197 (97.0)	232 (98.7)	77 (100.0)
Baseline Hemoglobin (g/L)			
Mean (SD)	104 (9.63)	104 (8.22)	106 (7.00)
[range]	[76-131]	[81-134]	[87.5-125.5]
Baseline plasma EPO (mIU/mL)			
Mean (SD)	ND	12.71 (0.95)	11.11 (0.96)
[range]		[2.49-490.95]	[2.49-295.8]
Baseline Hcpidin (ng/mL)			
Mean (SD)	154.5 (0.75)	135.2 (0.79)	385.7 (0.61)
[range]	[8.94-563.9]	[11.2-629.2]	[83.8-1158.1]
Dapro starting dose (mg) [n (%)]			
4	28 (13.8)	0 (0)	NA
6	134 (66)	0 (0)	
8	35 (17.2)	19 (8.1)	
12	6 (3)	161 (68.5)	
16	0 (0)	44 (18.7)	
24	0 (0)	11 (4.7)	

ND: No Data; NA: Not Applicable (no dose titration in study 204836)

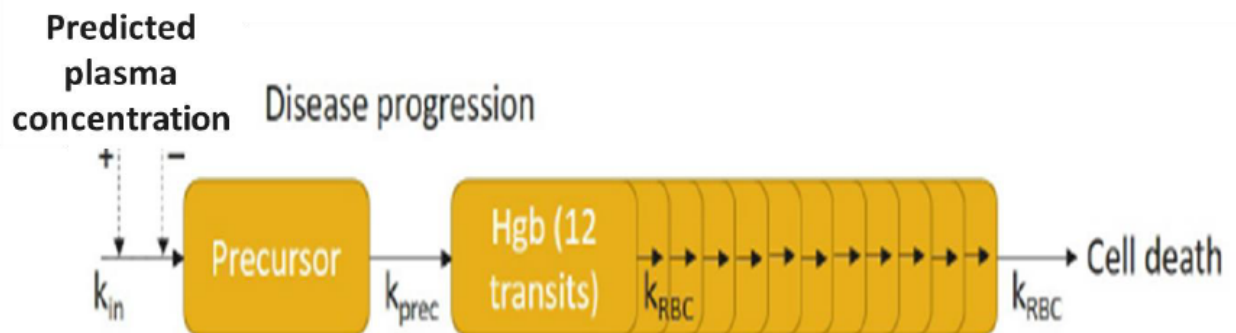
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Source: Applicant's Population PK/PD report TMF-14893649, Table 2.

Abbreviations: EPO, erythropoietin; ESA, erythropoiesis stimulating agent; SD, standard deviation

The final model is illustrated in [Figure 54](#) and includes the estimation of (true) baselines, a linear treatment effect of daprodustat and the full random structure.

Figure 54. Schematic of the Final PK-PD Model



Source: Applicant's Population PK/PD report TMF-14893649, Figure 2.
Abbreviations: Hgb, hemoglobin; PD, pharmacodynamic; PK, pharmacokinetic

The estimated parameter values are listed in [Table 207](#) and are associated with good precision and reasonable shrinkage (i.e., below 30%).

Table 207. Parameter Estimates of the Final PK-PD Model From Learning Dataset

Parameter	Estimate	SE	RSE (%)	95% CI	ρ
<i>Structural, THETAs</i>					
Baseline (BL) HD/PD ESA users (g/L)	106	0.403	0.38	(105, 107)	
True baseline HD/PD ESA users (g/L)	96.6	0.764	0.79	(95.1, 98.1)	
Relative effect of Prior ESA on True baseline (-)	-0.00797 Fixed				
Relative effect of ESA Hyporesponders on apparent baseline for ESA users (-)	-0.0307 Fixed				
Coefficient power model STAGE on (True) BL (-)	-0.107 Fixed				
Coefficient power model Body Weight (BW) on (True) Baseline (-)	0.052 Fixed				
Coefficient power model baseline hepcidin (BHEP) on (True) Baseline (-)	-0.017 Fixed				
Relative effect of Race = Asian on True baseline (-)	-0.0467 Fixed				
Relative effect of Race = Black on True and apparent Baselines (-)	-0.0376 Fixed				
Precursor life-span (Tprc) (days)	3.19 Fixed				
RBC life-span (Trbc) (days)	82.8 Fixed				
Relative effect of Epo use on RBC lifespan (-)	0.1 Fixed				
Coefficient power model baseline BW on RBC lifespan (-)	0.271 Fixed				
Relative effect of HD on RBC lifespan (-)	0.138 Fixed				

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Jesduvroq (daprodustat)

Slope linear concentration effect	0.0198	0.001	5.1	(0.0178, 0.0217)	
Disease progression (/1000 day)	-0.0851 Fixed				
Minimum KIN for disease progression (g/L/day)	-0.678 Fixed				
Relative effect of Epo use of Disease progression (-)	-0.318 Fixed				
Relative effect of HD or PD on Disease progression (-)	-0.573 Fixed				
SD additive residual error study 204837 (g/L)	5.76	0.046	0.80	(5.67, 5.85)	
SD additive residual error study 200807 (g/L)	7.21	0.0386	0.54	(7.14, 7.29)	
<i>Inter-Individual Variability, OMEGA</i>					
ω^2 Add Baseline	47.6	3.93	8.3	(39.9, 55.4)	
ω_{xy} Add Baseline x Add True baseline	28.2	4.84	17	(18.8, 37.7)	0.41
ω^2 Add True baseline	97.2	11.1	11	(75.3, 119)	
ω^2 Exp Slope daprodustat effect	0.619	0.0593	9.6	(0.503, 0.736)	
ω^2 Exp RBC life-span (TRBC)	0.219	0.0255	12	(0.169, 0.269)	
ω^2 Add Disease progression rate first year	0.0425	0.00443	10	(0.0338, 0.0511)	
ω_{xy} Add Disease progression rate first year x Add Disease progression rate second year	0.0176	0.00259	15	(0.0126, 0.0227)	0.71
ω^2 Add Disease progression rate second year	0.0144	0.00168	12	(0.0111, 0.0177)	
ω_{xy} Add Disease progression rate first year x Add Disease progression rate	0.0136	0.00271	20	(0.00825, 0.0189)	0.70
third year and beyond					
ω_{xy} Add Disease progression rate second year x Add Disease progression rate third year and beyond	0.00834	0.00149	18	(0.00542, 0.0112)	0.73
ω^2 Add Disease progression rate third year and beyond	0.00892	0.00173	19	(0.00554, 0.0123)	

The table shows the estimates and standard errors (SE) from NONMEM. Relative standard errors (RSE) was calculated as $(100 \cdot SE / \text{Estimate})$. 95% confidence interval (CI) was calculated as $(\text{Estimate} \pm \Phi(1 - \alpha/2) \cdot SE)$. SE, RSE and 95% CI are displayed only for parameters that were not fixed. Correlation coefficient (ρ) was calculated as $(\omega_{xy} / \sqrt{(\omega_x^2 \cdot \omega_y^2)})$, and displayed only for covariances.

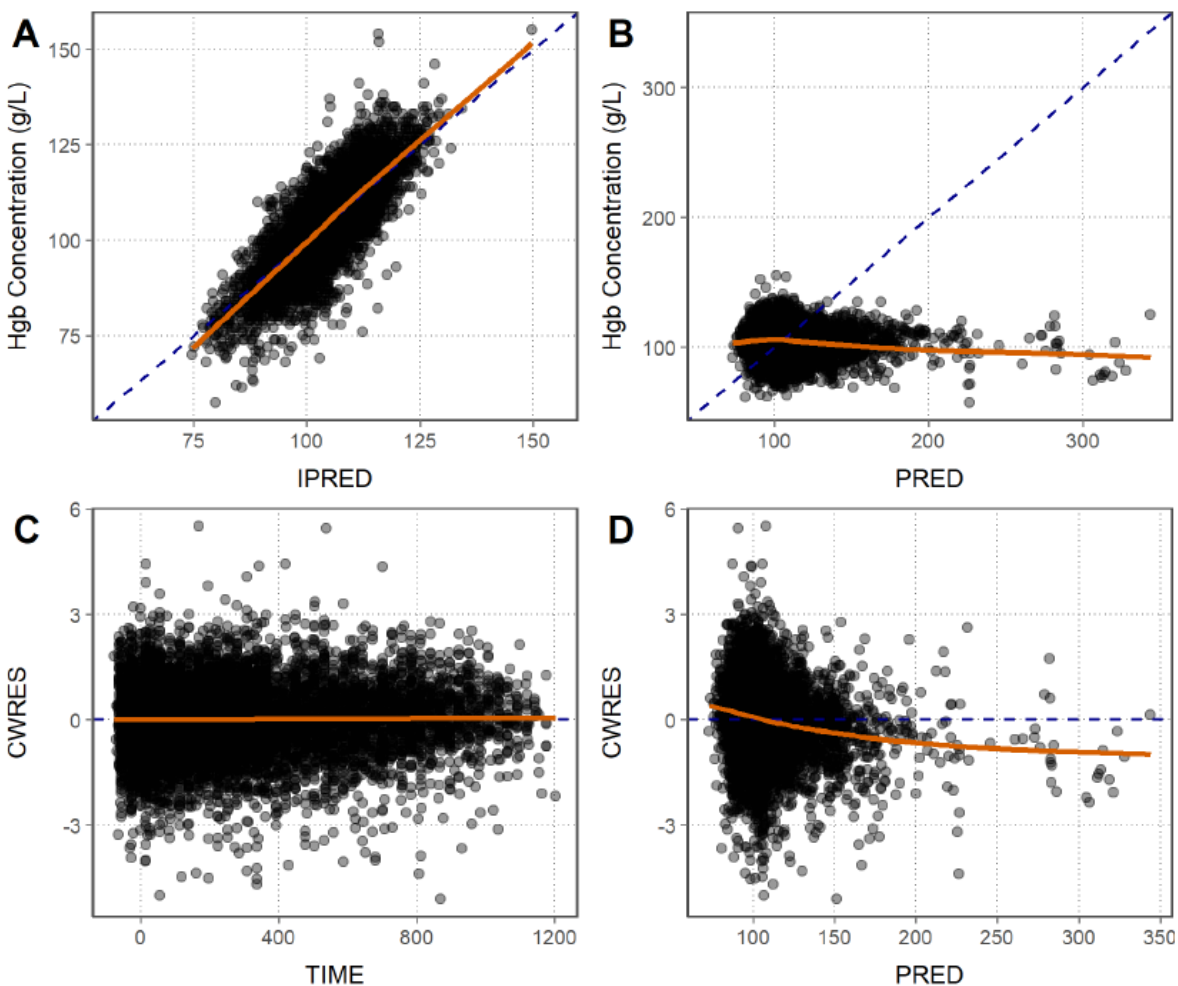
Source: GSK document number [TMF-14904762](#), slide 7; GSK document number [TMF-14904762](#), slide 8

Source: Applicant's Population PK/PD report TMF-14893649, Table 4.

Abbreviations: BL, baseline; BW, body weight; CI, confidence interval; Epo, erythropoietin; ESA, erythropoiesis stimulating agent; HD, hemodialysis; PD, peritoneal dialysis; PK, pharmacokinetic; RBC, red blood cells; RSE, relative standard errors; SE, standard errors

The Goodness-of-Fit plots of the final model showed an adequate fit, as shown in [Figure 55](#) (all data from learning dataset). The VPC based on all data from the learning dataset ([Figure 56](#)) illustrated the prediction percentiles and corresponding 95% CI of simulated concentrations overlaid on the observed daprodustat concentrations and the corresponding 5th and 95th percentiles.

Figure 55. Goodness-of-Fit of the Final PK-PD Model Using All Data From the Learning Dataset (200807 and 204836)



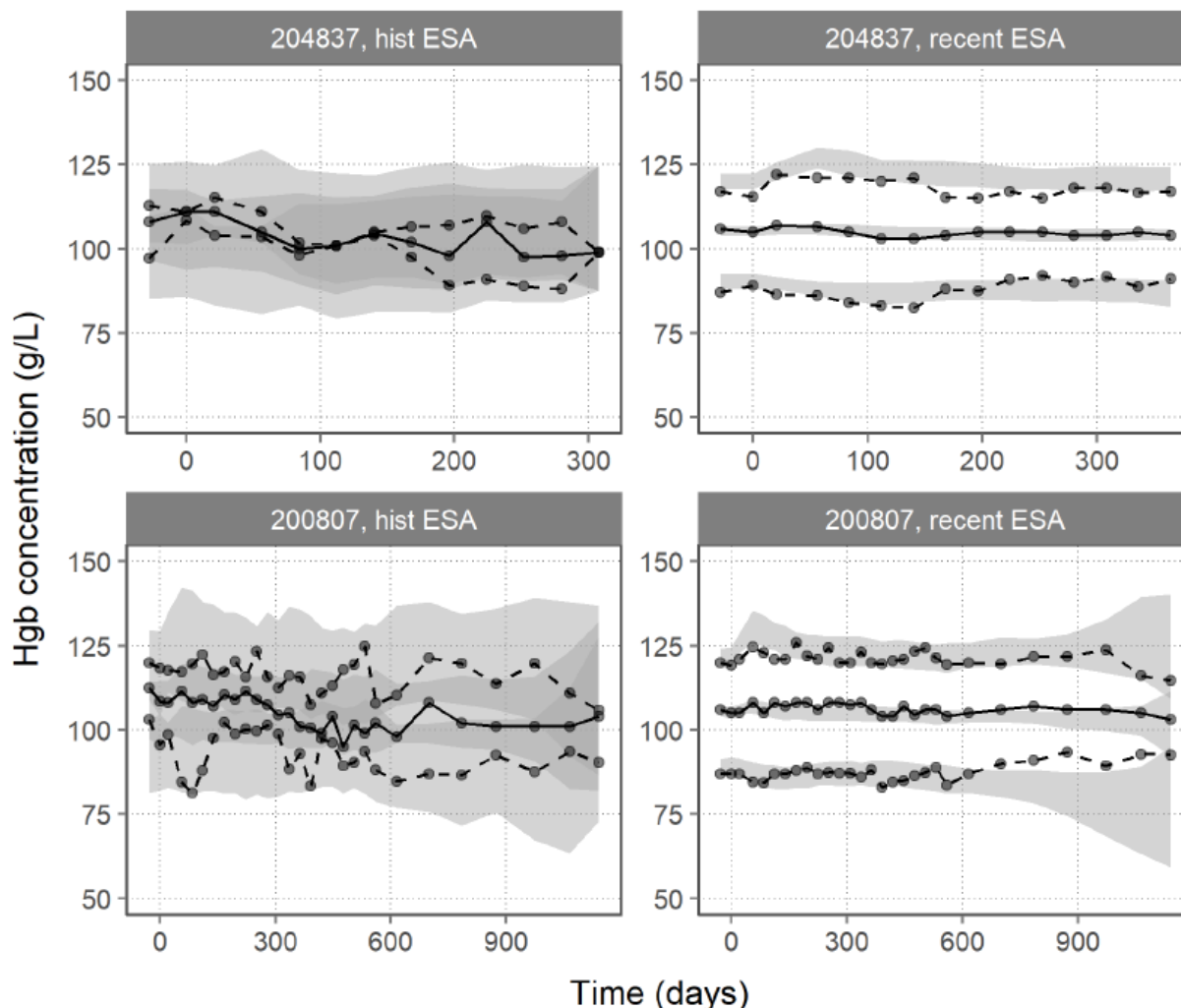
A: Observations versus individual fitted values. B: Observations versus population fitted values. C: Conditional weighted residual versus time (in days). D: Conditional weighted residuals versus population fitted values.

Note that because each subject was dose titrated based on its individual Hgb profile, which may not match the typical profile with the same dosing. For example, based on its individual Hgb baseline a subject would be titrated up or down to move its Hgb level towards the target range. A typical subject with the same dose changes could see its Hgb level moved away from the target range. In other words, these dose changes would not be valid for a typical subject, and hence the population predictions are of limited value here (applies to panels A and B).

Source: GSK document number [TMF-14904762](#), slide 17

Source: Applicant's Population PK/PD report TMF-14893649, Figure 11.
Abbreviations: Hgb, hemoglobin; PD, pharmacodynamic; PK, pharmacokinetic

Figure 56. VPC With Titration of the Final PK-PD Model Applied to the Learning Set, Showing the Mean Dose, Stratified by Study, and ESA Use



Dots and lines: median, 5th and 95th percentile of the observed values. Grey areas: 95% prediction intervals of the same for simulated values, simulating each subject 250 times. Abbreviations: hist ESA = historic ESA user (last use was more than 28 days before study start), recent ESA = recent ESA user (last use was at most 28 days before study start). The historic ESA groups are small, hence the prediction intervals are large and results should be interpreted with caution

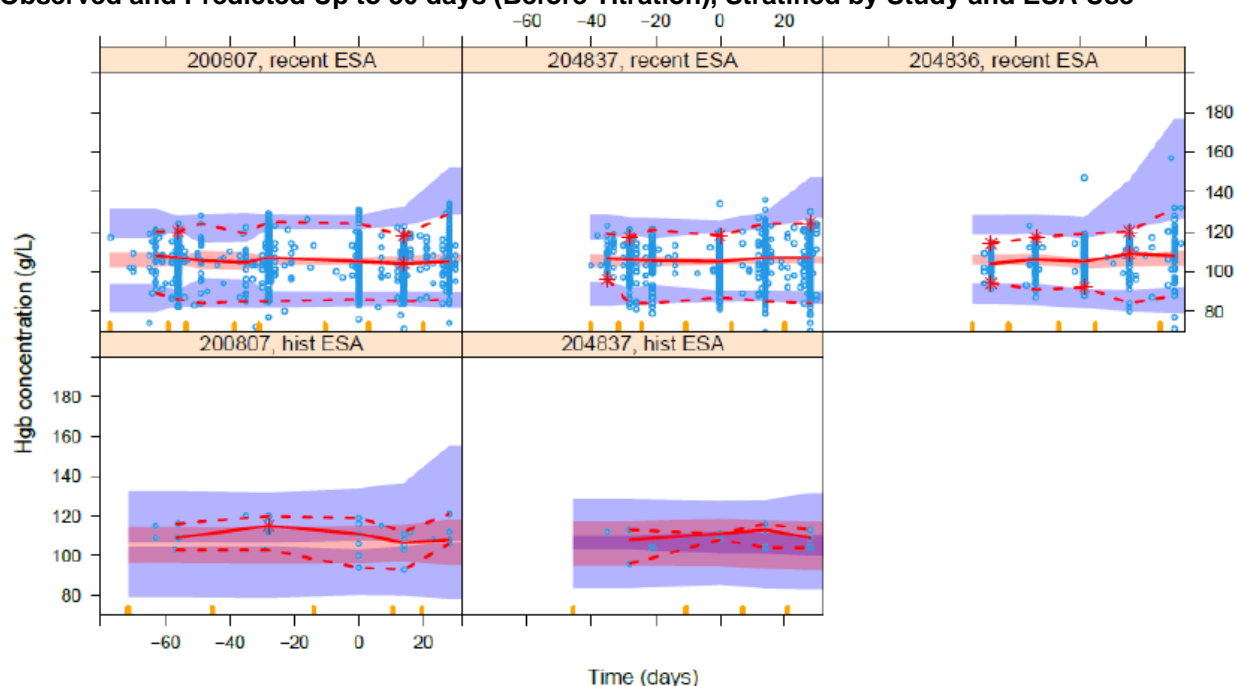
Source: GSK document number [TMF-14904762](#), slide 9

Source: Applicant's Population PK/PD report TMF-14893649, Figure 3.

Abbreviations: ESA, erythropoiesis stimulating agent; Hgb, hemoglobin; PD, pharmacodynamic; PK, pharmacokinetic; VPC, visual predictive check

The VPC obtained after the Bayesian estimation (MAXEVAL=0) of the validation dataset is illustrated in [Figure 57](#) and shows adequacy of the observations with the model predictions over time within the limited study duration of 204836.

Figure 57. VPC (With Titration) After Bayesian Estimation of Study 204836. Hb Time Course Observed and Predicted Up to 30 days (Before Titration), Stratified by Study and ESA Use



Dots and lines: median, 5th and 95th percentile of the observed values. Colored areas: 95% prediction intervals of the same for simulated values, simulating each subject 1000 times. Yellow notches indicate the bins used to summarise the simulated values. Abbreviations: recent ESA = recent ESA user (last use was at most 28 days before study start).

The historic ESA groups are small, hence the prediction intervals are large and results should be interpreted with caution.

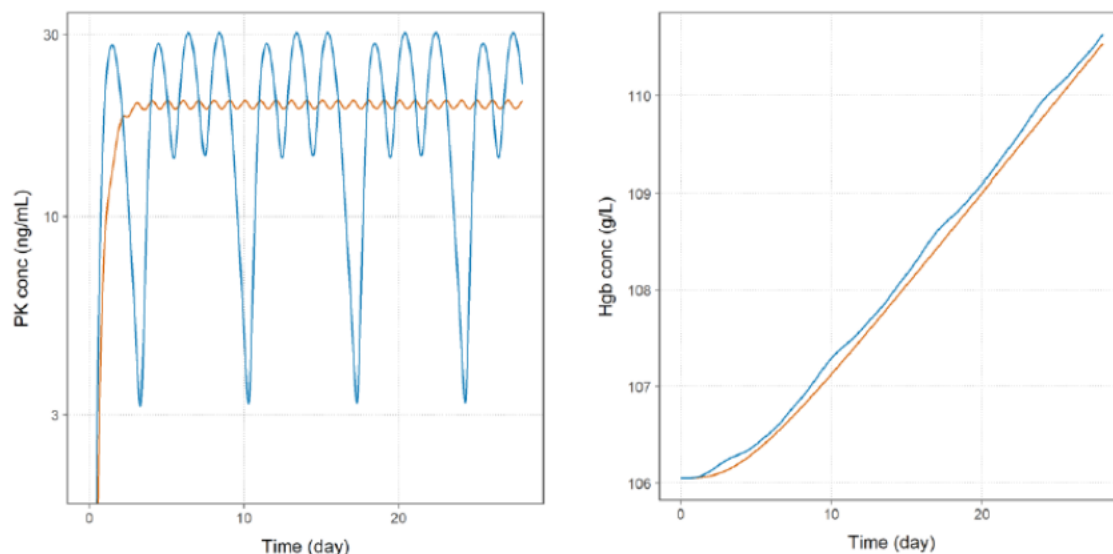
Source: `~/gskevaluation/DAPRODUSTAT_PKPD/SCRIPTS/VPC_script_v1.R`

Source: Applicant's Population PK/PD report TMF-14893649, Figure 6.

Abbreviations: ESA, erythropoiesis stimulating agent; Hb, hemoglobin; Hgb, hemoglobin; PD, pharmacodynamic; PK, pharmacokinetic; VPC, visual predictive check

PK and Hb profiles over time were simulated using the final PK-PD model for a typical subject using 28 mg TIW dosing. The results (Figure 58) show that whilst the peak-trough ratio for the 28 mg TIW dosing is 10-fold (from lowest trough to highest C_{max}) higher than for QD dosing, this translates into minimal, and not clinically relevant fluctuations in Hb levels over time. This indicates that dose (reflecting AUC as D/CL) is the main driver for efficacy.

Figure 58. Simulated PK and Hb Time Profiles After Equivalent QD and TIW Doses for a Typical Subject



The red and blue lines represent 12 mg QD and 28 mg TIW dosing, respectively. Note that the 28 mg TIW dose is not the recommended dose – this dose was selected to be able to compare TIW vs QD dosing at the same total weekly dose (84 mg over 1 week for both 12 mg QD and 28 mg TIW). TIW dosing was on days 1, 4 and 6 of each week.

Source: GSK document number [TMF-14904762](#), slide 22

Source: Applicant's Population PK/PD report TMF-14893649, Figure 8.

Abbreviations: Hb, hemoglobin; PK, pharmacokinetic; QD, once daily, TIW, three times a week

Reviewer's Comments

The Applicant's longitudinal population exposure-response analysis consisting of a linear relationship between daprodustat PK and its effect on Hb appears adequate. Simulations with the final PK-PD model at comparable total weekly doses for QD and TIW indicated that there is no clinically relevant difference in the effect on Hb between the dosing regimens ^{(b) (4)} in subjects with CKD ^{(b) (4)} undergoing dialysis. ^{(b) (4)}

^{(b) (4)} Longitudinal PK/PD analysis and simulation results showed PK fluctuations, as a result of longer inter-dose intervals (TIW vs. QD), do not impact Hb response.

14.5.3.2. Summary of Applicant's Exposure-Response for Safety

To support the proposed TIW dosing regimen with higher maximum concentration (C_{max}) in dialysis-dependent patients, the Applicant conducted multivariate E-R analyses using a logistic regression model and Cox proportional hazards regression model to quantify the relationship between daprodustat PK exposures (e.g., average C_{max} up to an event for patients with an event and average C_{max} during treatment for patients without an event) and safety measures

(thromboembolic event, nonfatal myocardial infarction (MI), and nonfatal hospitalization for HF) by Week 52 based on data from Studies 200807 and 204837.

For each PK Population subject in these two studies, the average cumulative dose (i.e., total dose for each subject divided by duration of dosing up to the time of the safety event or 52 weeks in those without a safety event) was calculated to obtain the average QD or TIW dose and the observed C_{max} from the corresponding PK Day dose was extrapolated to determine the C_{max} at the subject's estimated average QD or TIW dose. The extrapolated average C_{max} up to the time of the event was calculated as the observed C_{max} divided by the dose administered on the PK Day multiplied by average QD or TIW dose. Results are summarized in [Table 208](#). For all safety endpoints, there was a small number of events. For MI and thromboembolic events, the confidence intervals for the hazard ratio (HR) and the odd ratio (OR) included one.

Table 208. Summary of Analysis of the Relationship Between PK Exposure and On-Treatment Safety Measures for Subjects Randomized to Daprodustat From the PK Population in ASCEND-TD and ASCEND-D (First 52 Weeks)

	Dapro (N=441)
Number of subjects with non-missing extrapolated C_{max}	433
Number of Subjects with First Occurrence On-Treatment Thromboembolic Event	39 (9%)
HR (95% CI) [1]	1.000 (0.998, 1.001)
OR (95% CI) [2]	1.000 (0.998, 1.001)
Number of with First Occurrence On-Treatment Myocardial Infarction	15 (3.5%)
HR (95% CI) [1]	0.997 (0.993, 1.001)
OR (95% CI) [2]	0.997 (0.993, 1.001)
Number of with First Occurrence On-Treatment Hospitalization for Heart Failure	10 (2.3%)
HR (95% CI) [1]	0.999 (0.995, 1.003)
OR (95% CI) [2]	0.999 (0.995, 1.003)
Number of with First Occurrence On-Treatment Stroke	4 (0.9%)
HR (95% CI) [1]	0.999 (0.995, 1.004)
OR (95% CI) [2]	0.999 (0.995, 1.004)
Number of with First Occurrence On-Treatment Deep Vein Thrombosis	2 (0.5%)
HR (95% CI) [1]	0.999 (0.992, 1.007)
OR (95% CI) [2]	0.999 (0.992, 1.007)
Number of with First Occurrence On-Treatment Pulmonary Embolism	0

[1] Hazard Ratio is estimated using a Cox proportional hazard regression model with extrapolated average C_{max} up to the time of safety event/week 52 for subjects with no event, baseline weight, and baseline clopidigrol use.

[2] Odds Ratio is estimated using a logistic regression model with extrapolated average C_{max} up to the time of safety event/week 52 for subjects with no event, baseline weight, and baseline clopidigrol use.

Source: Response to Information Request SN#053, Table 1.

Abbreviations: CI, confidence interval; C_{max} , maximum plasma concentration; HR, hazard ratio; OR, odds ratio; PK, pharmacokinetic

For the safety endpoint of pulmonary embolism, no subject had an event in this population, so no model results are available. For the endpoints of hospitalization for heart failure, stroke, and deep vein thrombosis there were too few events in all levels of the covariates. Therefore, results should be interpreted with caution.

Therefore, E-R for safety analysis showed no differences between the average $C_{\max}$ up to the event (in those with the event) vs. the average $C_{\max}$ for 52 weeks (in those without the event); hence daprodustat $C_{\max}$ is not a significant factor in any of these safety measures.

Reviewer's Comments

Applicant's E-R for safety analysis appears reasonable. In summary, there were no statistically significant E-R relationships between daprodustat PK exposures- $C_{\max}$ and safety endpoints, including all stroke (fatal and nonfatal), all deep vein thrombosis (fatal and nonfatal), and all pulmonary embolism (fatal and nonfatal) in study studies 200807/ASCEND-D and 204837/ASCEND-TD.

(b) (4)

14.6. Physiologically Based Pharmacokinetic Modeling Review

Executive Summary

The objective of this review is to evaluate the adequacy of the Applicant's PBPK report regarding evaluation of daprodustat's (GSK1278863) drug-drug interaction with a moderate CYP2C8 inhibitor clopidogrel using physiologically based pharmacokinetic (PBPK) modeling to support the intended uses. Specifically, the Applicant applied the PBPK modeling approach to assess the effects of clopidogrel on the exposure of daprodustat, following multiple dose administration of daprodustat. The Division of Pharmacometrics has reviewed the PBPK report, supporting modeling files, and the Applicant's responses to FDA's information request (IR) submitted on July 15, 2022, and concluded that the effects of clopidogrel on the PK profiles of daprodustat following multiple oral dose administration in healthy subjects were potentially underpredicted because the clopidogrel PBPK model was not reliably validated.

Background

Daprodustat, a HIF PHI, is proposed for the treatment of anemia due to chronic kidney disease in adults on dialysis and not on dialysis. The starting dose of daprodustat is based on the patient's dialysis status and hemoglobin level. For those switching from an erythropoiesis stimulating agent (ESA) to JESDUVROQ, the dose is based on their existing ESA dose. For details of the proposed dose regimen, please refer to the labeling of daprodustat. The dosage form of daprodustat is a tablet with different strengths (1 mg, 2 mg, 4 mg, 6 mg, and 8 mg).

Following oral administration, daprodustat is readily absorbed with median T_{max} in healthy subjects ranging from 1 to 4 hours. Daprodustat exhibited linear PK with generally dose-proportional increases in plasma exposure from 2 mg to 300 mg. The absolute bioavailability of daprodustat is 65%. Daprodustat has an approximately equal distribution between plasma and blood cells. Following intravenous dosing, the volume of distribution at steady state in healthy subjects is 14.3 L (Study 200232). Following oral or intravenous administration of radiolabeled daprodustat to healthy adults, approximately 40% of the total circulating radioactivity in plasma was daprodustat, and the remaining 60% was metabolites. Three metabolites each accounting for more than 10% of circulating drug-related material have been identified (Study 200942). Within seven days of an oral dose of radiolabeled daprodustat, 74% of the radioactivity was recovered in the feces, and 21% in the urine. Approximately 99.5% of the dose was excreted as oxidative metabolites, with the rest accounted for by daprodustat (Study 200232).

Daprodustat clearance was driven by cytochrome P450 (CYP) 2C8 metabolism (in vitro $f_{m_{CYP2C8}}$ of ~96%, CD2010/00300/00) with a minor contribution by CYP3A4 metabolism and urinary excretion (2012N157420_01). Gemfibrozil, a strong CYP2C8 inhibitor, increased daprodustat AUC and C_{max} by 19- and 3.9-fold, respectively (Study PHI113634). Trimethoprim, a weak CYP2C8 inhibitor, increased AUC and C_{max} of daprodustat by 1.5 and 1.3-fold, respectively (Study 200229).

In vitro, daprodustat is not a competitive or time-dependent inhibitor of CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4. Daprodustat inhibited CYP2C8 with a calculated half-maximal inhibitory concentration IC_{50} value of 21 μ M (study UH2008, CD2008). Daprodustat is not an in vitro inducer of CYP1A2, CYP2B6, or CYP3A4 (study CD2008). Daprodustat is a BCRP substrate in vitro but not a substrate of human drug transporters P-gp, OATP1B1, OATP1B3, OATP2B1 OCT1, OAT1, OAT3, OCT2, MATE1, or MATE2-K. Daprodustat is not an inhibitor of transporters P-gp, BCRP, OAT1, OAT3, OCT2, MATE1 and MATE2-K in vitro. Daprodustat is an in vitro inhibitor of human OATP1B1 and OATP1B3 (study UH2008, CD2008).

Methods

PBPK Software

Simcyp v20 (Certara, UK) was used by the Applicant to develop the daprodustat PBPK models and predict the PK of daprodustat with and without CYP2C8 modulators. The reviewer used the same software and version for analyses.

Model Development

During PBPK model development, the ADAM module in Simcyp was used to investigate the mechanistic drug absorption and dissolution profiles. A minimal PBPK model was employed to understand the tissue distribution of daprodustat. The final input parameters for daprodustat are summarized in [Table 209](#).

Table 209. Daprodustat PBPK Model Parameters Used in Simcyp Simulator

Parameter	Value	Source
Phys chem and blood binding		
Molecular Weight (g/mol)	393.4	UD2007/00160/00
Log P	5.28	2018N387402_01
Compound type	Diprotic Acid	
pKa1	3.24, 6.18	2018N387402_01
Blood/Plasma ratio (B/P)	1.23	UH2008/00002/00
Fraction unbound (Fu)	0.004	2012N157420_01
Absorption model	ADAM	
Permeability, Peff = (10 ⁻⁴ cm/s)	2.54	ADMET Predictor v9.0.0.0
Formulation	IR	Capsule or Tablet as appropriate
pH Solubility profile (mg/mL)	See Appendix 9 of PBPK report	N38019-55 and Labware EXP161145
Intrinsic Solubility, S ₀ (mg/mL)	6.015 x 10 ⁻⁶	Optimised in SIVA from pH-solubility profile N38019-55/ Labware EXP161145
Bile Micelle:Buffer partition coefficients (logK _{m:w})	8.274, 4.397	Optimised in SIVA from FaSSiF/FeSSiF solubility
Distribution model	Minimal PBPK	
V _{ss} (L/kg)	0.147	Optimized based on measured V _{dss} value of 0.190 L/kg (2017N352856_01)
V _{sac} (L/kg)	0.036	
SAC, Q (L/h)	0.12	Optimized using sensitivity analysis tool
Elimination model	Enzymatic	
CL _{iv} (L/h)	19.3	2017N352856_01
CL _r (L/h)	0.0119	2012N157420_01
CL _{int} CYP2C8 (μL/min/pmole)	99.97	CL _{iv} from 2017N352856_01 was used to optimize CL _{int} 2C8 and 3A4 parameters with Simcyp Retrograde calculator
CL _{int} CYP3A4 (μL/min/pmole)	0.161	
fmCYP2C8	98.1%	Optimized fm2C8 from CYP phenotyping report (CD2010/00300/00) and gemfibrozil DDI study (2011N115609_00)
fmCYP3A4	1.1%	
Interactions	Included	Enzymes
CYP2C8 K _i (μM)	10.5	(2013N167801_01)

Source: Table 1 of PBPK report 2019N396674.

Abbreviations: BCRP, Breast Cancer Resistance protein (transporter); CL_{po}, oral clearance; CL_r, renal clearance; DLM, Diffusion Layer Model; F_{up}, fraction unbound in plasma; F_{uinc}, fraction unbound in the incubation; GFR, glomerular filtration rate; IR, Immediate release; K_a, AP, acidic phospholipid binding association constant; K_p, tissue partition coefficient; PBPK, physiologically based pharmacokinetic; P-gp, P-glycoprotein (transporter); V_{ss}, volume at steady state

Population, Victim, and Perpetrator Drug Models

The Simcyp software built-in virtual healthy volunteer population and clinically relevant probes substrates, inhibitors or inducers have been utilized. The default PBPK models of gemfibrozil and trimethoprim in Simcyp were used for DDI prediction. The PBPK models of clopidogrel and its glucuronide were developed based on published data (Tornio et al. 2014; Shebley et al. 2017; Varma et al. 2019). Simcyp built-in models of repaglinide and the PBPK models of pioglitazone and montelukast built by the Applicant based on were used to qualify the ability of the clopidogrel PBPK model to simulate DDIs with CYP2C8 substrates as well as a CYP3A4 substrate. No modifications were made to the original models.

PBPK Simulation Trial Design

Simulations for daprodustat PBPK qualification and verification used a fixed study population, where individual subject data were available to match the observed clinical PK. Simulations were designed to replicate the situation in the actual daprodustat clinical studies, with either the exact number of subjects or sufficient subject numbers, sex, proportion of males and females in appropriate virtual populations of healthy volunteer subjects in replicate N trials to have a representative total of at least 100 subjects or more. Where individually matched patient demographic data and PK information were not available (e.g., model application), simulations were conducted using the default ranges within the Simcyp simulator (v20).

Model Refinement and Validation

- The PBPK model was validated against the adult PK studies following a single intravenous dose of 0.05 mg (study 200232), single oral doses of 2 mg, 5 mg, and 15 mg (study PHX111427) and repeated (QD) oral doses of 5 mg, 15 mg, and 100 mg (studies PHI112842, PHI116008 and PHI115573) daprodustat in healthy subjects.
- The PBPK model was optimized and validated with DDI studies in adult healthy subjects treated with gemfibrozil (study PHI113634) and trimethoprim (study 200229) respectively.

Model Application

The daprodustat PBPK model was applied to perform prospective drug-drug risk assessment as a victim after coadministration of clopidogrel

FDA's Assessment

- As stated in the PBPK report by the Applicant that 'the clopidogrel PBPK base model was developed in Simcyp based on published data (Tornio et al. 2014; Shebley et al. 2017; Varma et al. 2019). An information request was issued requesting the Applicant to provide evidence that the cited models of clopidogrel and its glucuronide could simulate the plasma concentration-time profiles and predict the magnitude of interaction observed in drug interaction studies using Simcyp simulator version 20.
- In the PBPK report, PBPK models of pioglitazone and montelukast used to qualify the clopidogrel models are currently not available in Simcyp v20. An information request was issued requesting the Applicant to submit the full documentation of model development and model verification for pioglitazone and montelukast using the Simcyp V20, including comparison of predicted and observed PK profiles, PK parameters and DDIs with CYP inhibitors.

Applicant's Response to FDA's IR and FDA's Assessment

In the response to our IR, the Applicant provided additional details and verification regarding the clopidogrel and clopidogrel-glucuronide Simcyp v20 PBPK models used in PBPK report (2019N396674). Briefly, the Applicant compared the simulated PK parameter values with those observed clinical data extracted from a variety of clinical studies for both clopidogrel and clopidogrel glucuronide. As shown in [Table 210](#), the PK parameters of clopidogrel-glucuronide,

the major driving force for CYP2C8 inhibition, were significantly underpredicted compared with the observed clinical data for several studies. These discrepancies might be potentially explained by between-study variability; as shown in [Figure 59](#), a majority of the observed concentrations fell between the 5th and 95th of the prediction confidence intervals. The ability of the clopidogrel models to predict their interactions with CYP2C8 substrates will be discussed in the Results section.

The Applicant developed PBPK models of pioglitazone and montelukast based on references and verified these models by comparing the simulated PK profiles and parameters with available clinical literature reports after both IV and oral dosing routes. The results demonstrated that the montelukast model could simulate the plasma PK profiles of montelukast reasonably well but the pioglitazone model significantly overpredicted the clearance of pioglitazone following IV administration ([Table 211](#)) and underpredicted pioglitazone AUC following oral administration of multiple doses of 30 mg pioglitazone ([Table 212](#)). However, both montelukast and pioglitazone models could predict the effects of gemfibrozil on their exposure ([Table 213](#)).

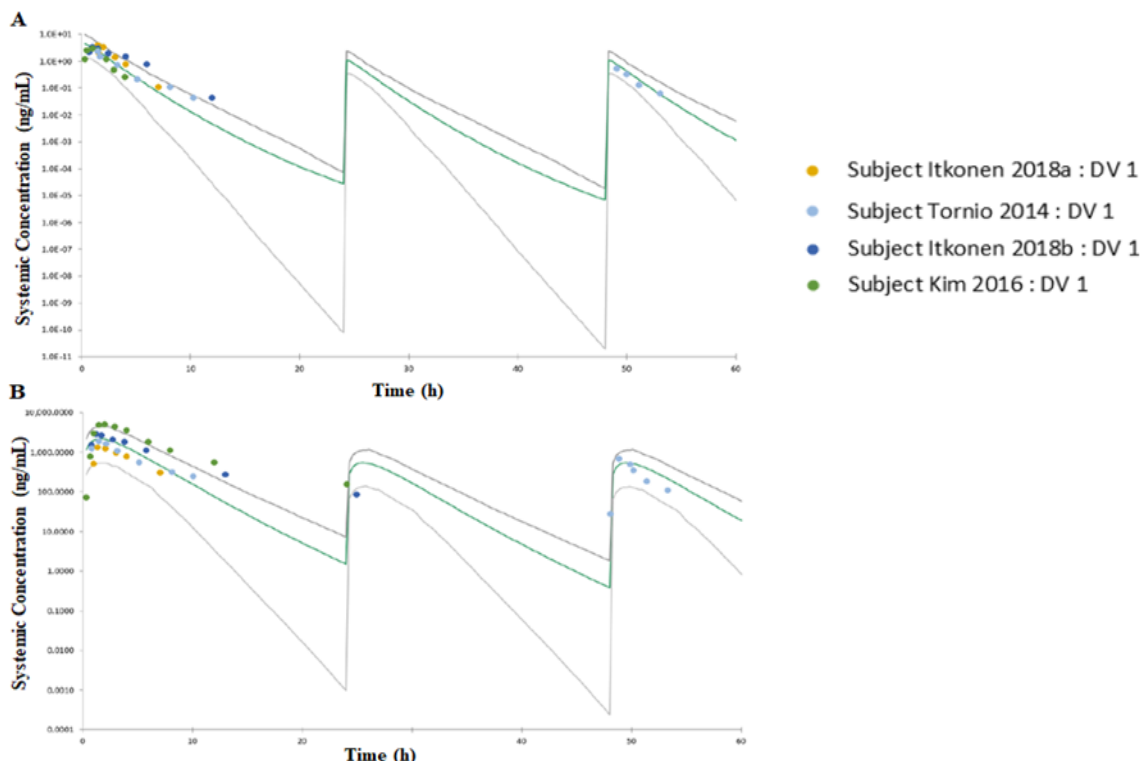
Table 210. Comparison of the Simulated and Observed Clinical Pharmacokinetic Parameters of Clopidogrel and Clopidogrel Glucuronide

Drug	Parameter	Observed Data – Mean - Range		Simulated Data – Mean - Range		Simulated/Observed Ratio	Reference
Clopidogrel	T_{max} (hr) Median (Range)	1.5	0.92 – 2.0	0.35	0.15 – 0.65	0.23	Itkonen, 2018a
	C_{max} (ng/mL) GM (GCV%)	4.49	110	3.90	68	0.87	
	$AUC_{0-∞}$ (ng*h/mL) GM (GCV%)	10.8	94	7.97	63	0.74	
Gluc-Metabolite	T_{max} (hr) Median (Range)	1.75	0.92 – 2.0	1.60	0.75 – 3.05	0.91	
	C_{max} (ng/mL) GM (GCV%)	1420	57	1992	66	1.40	
	$AUC_{0-∞}$ (ng*h/mL) GM (GCV%)	6840	53	9258	69	1.35	
Clopidogrel	T_{max} (hr) Median (Range)	1.5	0.92 – 2.0	0.35	0.15 – 0.65	0.23	Itkonen, 2018b
	C_{max} (ng/mL) Median (Range)	3.53	2.56 – 7.15	3.94	0.82 – 16.02	1.11	
	AUC_{0-T} (ng*h/mL) Median (Range)	13.2	9.25 – 22.9	8.07	2.73 – 41.73	0.61	
Gluc - Metabolite	T_{max} (hr) Median (Range)	1.5	0.92 – 3.0	1.60	0.75 – 3.05	1.07	
	C_{max} (ng/mL) Median (Range)	3290	1470 - 9680	2175.62	415.52 – 9460.20	0.66	
	AUC_{0-T} (ng*h/mL) Median (Range)	16200	7030 - 34200	9980	1577.63 – 34053.39	0.61	
Clopidogrel	T_{max} (hr) Mean ± SD	0.88	± 0.58	0.37	± 0.07	0.42	Kim, 2016
	C_{max} (µg/mL) Mean ± SD	0.0033	± 0.0022	0.0044	± 0.0029	1.33	
	AUC_{0-T} (µg*h/mL) Mean ± SD	0.0061	± 0.0037	0.0086	± 0.0054	1.41	
Glucuronide Metabolite	T_{max} (hr) Mean ± SD	2.06	± 0.62	1.60	± 0.35	0.78	
	C_{max} (µg/mL) Mean ± SD	5.83	± 3.66	2.31	± 1.23	0.40	
	AUC_{0-T} (µg*h/mL) Mean ± SD	29.1	± 14.9	10.95	± 6.11	0.38	
Clopidogrel	T_{max} (hr) Median (Range)	1.5	0.92 – 2.00	0.35	0.20 – 0.65	0.23	Tornio, 2014
	C_{max} (ng/mL) GM (GCV%)	2.9	97	4.02	67	1.39	
	$AUC_{0-∞}$ (ng*h/mL) GM (GCV%)	6.2	87	8.29	61	1.34	
Glucuronide Metabolite	T_{max} (hr) Median (Range)	1.5	0.92 – 2.00	1.65	0.90 – 3.45	1.10	
	C_{max} (ng/mL) GM (GCV%)	1957	35	1870	69	0.95	
	$AUC_{0-∞}$ (ng*h/mL) GM (GCV%)	8722	55	8880	73	1.02	

Source: IR response submitted on July 15, 2022

Abbreviations: AUC, area under the concentration-time curve; C_{max} , maximum plasma concentration; T_{max} , time to maximum concentration

Figure 59. Predicted and Observed Plasma Concentration-Time Profiles of Clopidogrel and its Glucuronide Following a 300 mg Single Oral Dose of Clopidogrel on Day 1 and 75 mg Single Oral Doses of Clopidogrel on Days 2 and 3



Source: IR response submitted on July 15, 2022

^A Overlay of simulated mean plasma PK of clopidogrel (logarithmic plot) with the observed clinical PK.

^B Overlay of simulated mean plasma PK of Clopidogrel-glucuronide (logarithmic plot) with the observed clinical PK. The lines represent the Simcyp v20 simulations and the colored dots are from the reported clinical studies. Green line represents the mean plasma concentrations whereas the grey lines are the 5th-95th percentile.

Table 211. Comparison of the Simulated and Observed Clinical Data After Intravenous Administration of the Drugs

Drug	IV Dose	T _{1/2} h			CL (range) L/h			V _{dss} (range) L/Kg			Reference
		Observed	Simcypv20	Pred/Obs ratio	Observed	Simcypv20	Pred/Obs ratio	Observed	Simcypv20	Pred/Obs ratio	
Pioglitazone	5mg, 2 hour infusion Mean	N/A	3.05 (0.86 -10.8)	N/A	2.47 (1.72 - 4.17)	5.11 (1.10-15.82)	2.07	0.253 (0.199 – 0.299)	0.26	1.03	Eckland, 2000
Montelukast	3 mg, 15 min IV infusion Mean ± SD	4.4 ± 0.9	1.64 ± 0.97	0.37	3.024 ± 0.726	4.69 ± 3.38	1.55	0.1614 ± 0.0128	0.0948 ± 0.0088	0.59	Cheng, 1996

Source: IR response submitted on July 15, 2022

Abbreviations: CL, clearance; IV, intravenous; N/A, not available; SD, standard deviation; T_{1/2}, half-life value; V_{dss}, volume of distribution at steady state

Table 212. Comparison of the Simulated and Observed Clinical Data After Single and Multiple Oral Administration of the Drugs.

Drug	Oral Dose	AUC (range) ng/L.h			C _{max} (range) ng/L			Reference
		Observed	Simcypv20	Pred/Obs ratio	Observed	Simcypv20	Pred/Obs ratio	
Pioglitazone	30mg PO Mean ± SD	8.08 ± 3.41 (µg/mL.h)	7.46 ± 3.51 (µg/mL.h)	0.92	1.12 ± 0.315 (µg/mL)	1.14 ± 0.35 (µg/mL)	1.02	Saha, 2014
Pioglitazone	30 mg QD for 7 days Mean ± SD	13361 ± 4451 (µg/L.h)	8089.2 ± 4391.1 (µg/L.h)	0.61	1174.7 ± 372.8 (µg/L)	1192.7 ± 372.49 (µg/L)	1.02	Hwang, 2020
Montelukast	10 mg PO Single dose Mean ± SD	2916.3 ± 878.9	3111.57 ± 1776.49	1.07	427.5 ± 103.2	462.70 ± 158.76	1.08	Dennison, 2018
Montelukast	10 mg PO Single dose Geometric Mean (Range)	2499.7 (1800.7, 3470.1)	2674.17 (789.16, 6358.30)	1.07	347.5 (233.9, 516.3)	419.27 (187.94, 711.96)	1.21	Bohmer, 2009
Montelukast	10 mg PO Single dose Geometric Mean (Geometric coefficient of variation%)	3020 (28)	2750.16 (76)	0.91	447 (27)	464.22 (45)	1.04	Itkonen, 2018b
Montelukast	10 mg QD for 7 days Geometric Mean ± SD	3504 ± 1198	2814.13 ± 2045.99	0.70	541.5 ± 172.6	469.95 ± 155.55	0.87	Zhao, 1997

Source: IR response submitted on July 15, 2022

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; PO, oral dose; QD, once daily; SD, standard deviation

Table 213. Drug-Drug Interaction Verification for Montelukast and Pioglitazone With Available CYP2C8 Inhibitors and Comparison With Clinical Data

Substrate-Perpetrator	AUC Ratio		C _{max} Ratio		Simulated/Observed Ratio		Reference
	Observed	Simulated	Observed	Simulated	AUC Ratio	C _{max} Ratio	
Montelukast + Gemfibrozil (Geometric Mean)	4.3	3.22	1.5	1.87	0.75	1.25	Karonen, 2010
Pioglitazone + Gemfibrozil (Mean)	3.07	2.63	1.06	1.20	0.86	1.13	*Jaakkola, 2005

Source: IR response submitted on July 15, 2022

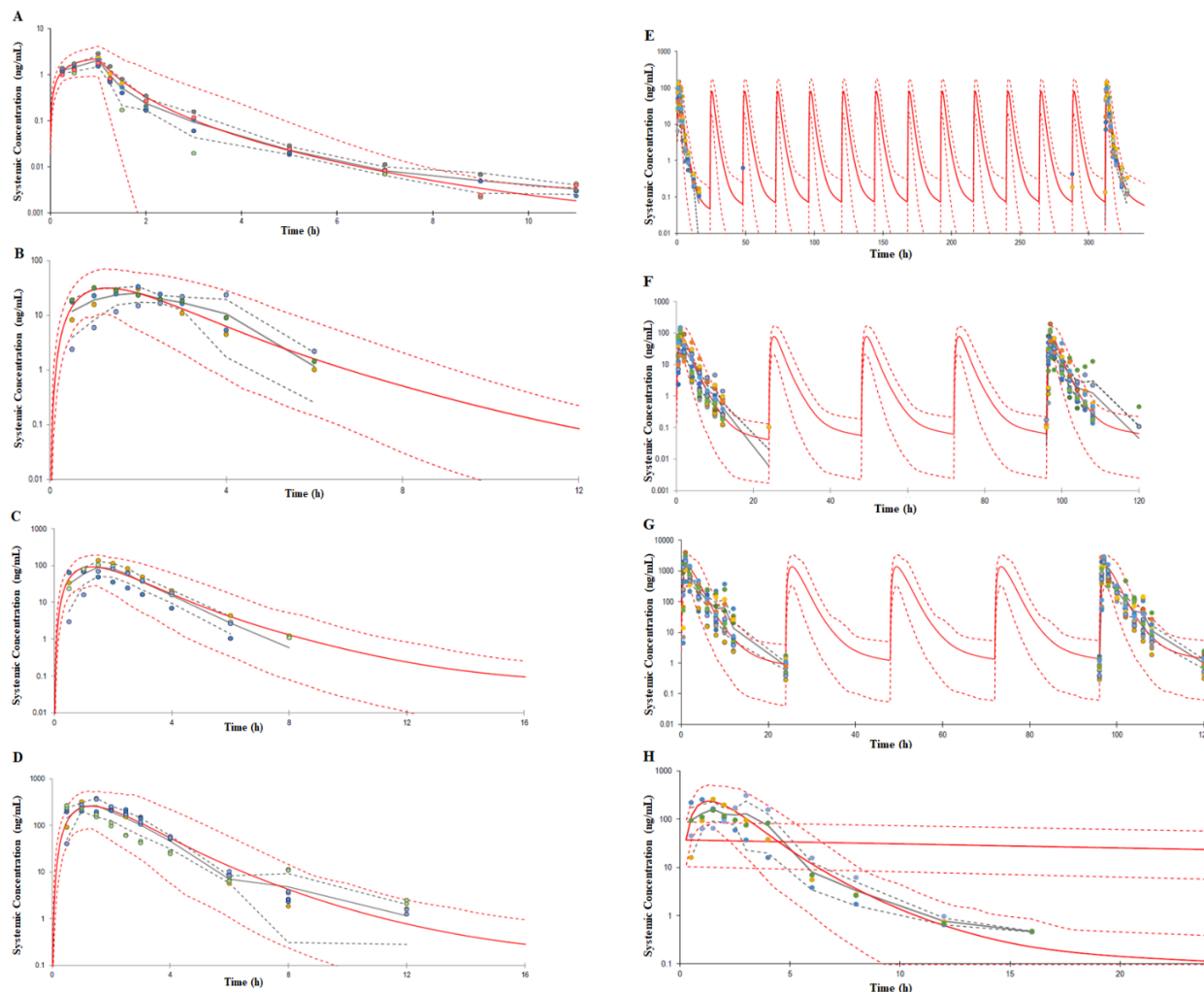
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration

Results

Can Daprodustat PBPK Model Reasonably Describe the PK of Daprodustat?

Yes, PBPK simulations reasonably predicted the PK profiles of daprodustat following a single intravenous dose of 0.05 mg, single oral doses of 2 mg, 5 mg, and 15 mg and multiple (QD) oral doses of 5 mg, 15 mg, and 100 mg of daprodustat in healthy subjects, as demonstrated in [Figure 60](#) and by a reasonable agreement between predicted and observed values for AUC and C_{max} in [Table 214](#).

Figure 60. Predicted and Observed Plasma Concentration-Time Profiles of Daprodustat Following Different Dosing Regimens in Healthy Volunteers



Source: Figures 3-9 from PBPK report 2019N396674. Mean Simcyp simulated (red solid line) $\pm$ 95% confidence interval (red dashed lines) and mean observed (grey solid line) $\pm$ standard deviation (grey dashed lines) and individual (symbols) plasma profiles of daprodustat following 0.05 mg i.v. SD (A), 2 mg oral SD (B), 5 mg oral SD (C), 15 mg oral SD (D), 5 mg oral QD (E), 5 mg oral QD (F), 100 mg oral QD (G) and 15 mg oral SD (H) in healthy volunteers.

Abbreviations: QD, once daily; SD, standard deviation

Table 214. Simulated and Observed Mean PK Parameters and C_{max} and AUC Ratios for Daprodustat

Trial	Dosage	Visit	Observed		Pop	Predicted		Pred/Obs ratio	
			C_{max} (ng/mL)	AUC _(0,t) (ng.h/mL)		C_{max} (ng/mL)	AUC _(0,t) (ng.h/mL)	C_{max}	AUC
200232	0.05 mg SD (i.v.)	Day 1	2.06	2.68	HV	2.26	2.80	1.10	1.04
PHX111427	2 mg SD (oral)	Day 1	29.6	74.7	HV	33.4	78.2	1.13	1.05
	5 mg SD (oral)	Day 1	97.3	205	HV	97.5	228	1.00	1.11

Trial	Dosage	Visit	Observed		Pop	Predicted		Pred/Obs ratio	
			C _{max} (ng/mL)	AUC _(0,t) (ng.h/mL)		C _{max} (ng/mL)	AUC _(0,t) (ng.h/mL)	C _{max}	AUC
PHI112842	15 mg SD (oral)	Day 1	296	654	HV	276	678	0.93	1.04
	15 mg QD (oral)	Day 1	245	522	HV	254	624	1.04	1.20
PHI116008	5 mg QD (oral)	Day 1	80.3	148	HV	83.1	208	1.03	1.41
		Day 5	67.5	138	HV	83.2	209	1.23	1.52
PHI116008	100 mg QD (oral)	Day 1	2122	3690	HV	1520	4267	0.72	1.16
		Day 5	2276	3732	HV	1521	4286	0.68	1.15
PHI115573	5 mg QD (oral)	Day 1	104	203	HV	86.9	216	0.84	1.06
		Day 14	96.3	196	HV	87.0	220	0.90	1.12

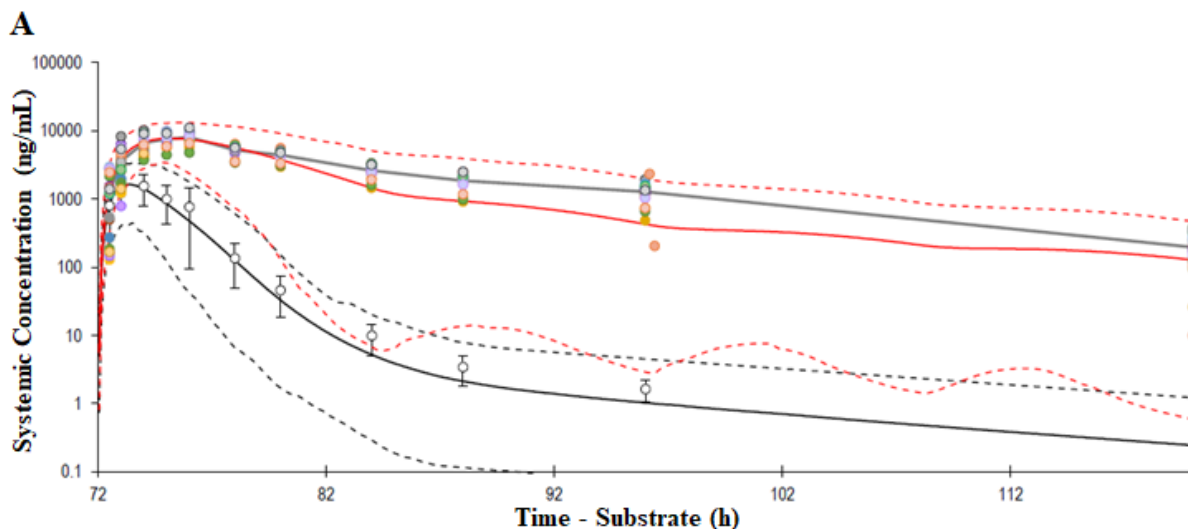
Source: Observed data were from Study 200232, PHX111427, PHI112842, PHI116008 and PHI115573. Simulated data were from PBPK report 2019N396674.

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; HV, healthy volunteers; pop, population; QD, once daily; SD, single dose

Can Daprodustat PBPK Model Reasonably Describe the Effects of Gemfibrozil and Trimethoprim on the PK of Daprodustat in Healthy Subjects?

Yes, the contribution of the CYP2C8 pathway to daprodustat clearance (e.g., assumption of $f_{m,CYP2C8}$) was verified and calibrated using clinical DDI data with gemfibrozil. Briefly, an in vitro study using human liver microsomes attributed approximately 96% metabolism to CYP2C8. Using this value as $f_{m,CYP2C8}$ to determine recombinant CL_{int} values resulted in significant underprediction of the magnitude of DDI with gemfibrozil (predicted AUC ratio of 12-fold versus observed 18.6-fold). The Applicant optimized the $f_{m,CYP2C8}$ value to 98.1% so that the predicted AUC ratio (15.1-fold) was within 20% of the observed AUC ratio ([Table 215](#)). The simulated and observed daprodustat plasma concentration-time profiles in the presence and absence of gemfibrozil are shown in [Figure 61](#). Notably, the increase in contribution via CYP2C8 resulted in overprediction of DDI effect of trimethoprim (200 mg BID) on daprodustat ([Table 215](#)). However, the optimized $f_{m,CYP2C8}$ value is still acceptable, as it may give a conservative estimate of the effect of clopidogrel on daprodustat DDI.

Figure 61. Predicted and Observed Plasma Concentration-Time Profiles of Daprodustat in the Presence and Absence of Gemfibrozil.



Source: (Figures 10 from PBPK report 2019N396674)
Mean Simcyp Simulated (red and black solid lines) $\pm$ 95% confidence interval (red and black dashed lines) and mean observed (symbols and grey lines) plasma profiles of 100 mg daprodustat with and without 600 mg (BID) gemfibrozil in Healthy Volunteers.

Table 215. Simulated vs. Observed DDI of Daprodustat Following Co-Administration of Gemfibrozil, or Trimethoprim From Studies PHI113634 and 200229

Study	Observed		Simulated		Simulated/Observed Ratio	
	AUC _(0-∞)	Ratio C _{max}	AUC _(0-∞)	Ratio C _{max}	AUC _(0-∞)	Ratio C _{max}
Daprodustat + Gemfibrozil	18.6 ^b	3.92 ^b	15.1 ^a	5.14 ^a	0.82	1.31
Daprodustat + Trimethoprim	1.48 ^c	1.28 ^c	2.40 ^a	2.04 ^a	1.62	1.59

Source: Observed data were from Study PHI113634 and 200229. Simulated data were from PBPK report 2019N396674 Geometric Mean Ratio (GMR).

Data is presented as Geometric Least-Square Mean (GLSM) as clinical trial had n = 23 in the daprodustat (100 mg) treatment alone and n = 21 in the daprodustat + gemfibrozil treatment arm (Gemfibrozil 600 mg was dosed twice daily for 5 days and daprodustat 100 mg was administered once on day 4).

Data is presented as Geometric Least-Square Mean (GLSM) as clinical trial had n = 20 in the daprodustat (25 mg) treatment alone and n = 19 in the daprodustat + trimethoprim treatment arm (Trimethoprim 200 mg was dosed twice daily for 5 days and daprodustat 25 mg was administered once on day 4).

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; DDI, drug-drug interaction

Can Daprodustat PBPK Model Predict the Effects of Clopidogrel on the PK of Daprodustat Following Multiple Oral Dose Administration in Healthy Subjects?

The magnitude of interaction between clopidogrel and daprodustat may potentially be underpredicted because the current clopidogrel PBPK model was not reliably validated. As stated in the previous sections, the observed PK profiles of both clopidogrel and clopidogrel-glucuronide could not be consistently reproduced by the PBPK models of clopidogrel and its glucuronide (Table 210). Furthermore, these clopidogrel PBPK models significantly underpredicted repaglinide DDI as indicated in Table 216, even though the predicted clopidogrel and its glucuronide exposure was consistent with that observed in the study (Table 210) and the repaglinide PBPK model was verified (data not shown). These findings suggest that these clopidogrel PBPK models were not well validated and cannot be used confidently to predict the DDI of daprodustat co-administered with clopidogrel.

To further evaluate the potential interaction between clopidogrel and daprodustat, the clinically observed interactions of known CYP2C8 substrates with gemfibrozil or clopidogrel were compared (Table 217). Based on the relative difference in the magnitude of interaction of CYP2C8 substrates with clopidogrel and gemfibrozil, the PBPK model-predicted interaction with clopidogrel following a single 300-mg clopidogrel approximated to the lower bound of the values estimated from the clinical DDI data. Following steady state clopidogrel, there was less data available to evaluate the model prediction. However, based on the results from the interaction between repaglinide and clopidogrel, the interaction was expected to be similar to that observed following a single 300-mg dose of clopidogrel. In addition, because daprodustat is the most sensitive CYP2C8 substrate, increase in its AUC is expected to be greater compared to that observed from repaglinide following the same clopidogrel treatment regimen. Therefore, there is a potential that the interaction between daprodustat and clopidogrel following steady state clopidogrel was underpredicted by the PBPK modeling approach. Taken together, the current clopidogrel PBPK model was not reliably validated and the effects of clopidogrel on the PK of daprodustat following multiple oral dose administration in healthy subjects may be underpredicted.

Table 216. Simulated vs. Observed Pharmacokinetic Parameters for CYP2C8 Substrates After Co-Administration With Clopidogrel

Substrate-Perpetrator	AUC Ratio ¹		C _{max} Ratio ¹		Simulated/Observed Ratio		Reference
	Observed	Simulated	Observed	Simulated	AUC Ratio	C _{max} Ratio	
Pioglitazone + Clopidogrel	2.14 ^{2,4} (1.76, 2.61)	2.29 ^{2,4} (2.17, 2.42)	1.02 ² (0.77, 1.35)	1.19 ² (1.17, 1.21)	1.07	1.17	(Itkonen et al. 2016)
Repaglinide + Clopidogrel	4.8 ^{2,5} (3.7, 6.2)	1.94 ^{2,5} (1.86, 2.02)	3.8 ² (2.8, 5.1)	1.48 ² (1.45, 1.52)	0.40	0.39	(Tornio et al. 2014)
	3.8 ^{3,5} (2.8, 5.1)	1.53 ^{3,5} (1.49, 1.57)	2.0 ³ (1.3, 3.1)	1.30 ³ (1.28, 1.32)	0.40	0.65	
Montelukast + Clopidogrel	1.98 ^{2,4} (1.72, 2.28)	1.58 ^{2,4} (1.52, 1.64)	0.98 ² (0.82, 1.18)	1.21 ² (1.19, 1.23)	0.80	1.23	(Itkonen et al. 2018)

Source: Appendix 14 from PBPK report 2019N396674.

¹ Geometric Mean (90% CI)

² PK parameters taken following 300 mg treatment of clopidogrel.

³ PK parameters taken following 75 mg treatment of clopidogrel.

⁴ AUC_{0-∞}

⁵ AUC₀₋₉ hours

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration

Table 217. Drug Interactions Between CYP2C8 Substrates and Gemfibrozil and Clopidogrel

CYP2C8 Substrates	GEM (500 mg BID 5 days)		Pubmed Reference	CLP (300 mg Day 1)		Pubmed Reference	CLP (300 mg Day 1, 75 mg QD 2 days)		Pubmed Reference
	Substrate dosing date	Substrate on Day 3		Substrate on Day 1	Substrate on Day 3				
Drug	AUCR	C _{max} R		AUCR	C _{max} R		AUCR	C _{max} R	
Daprodustat (MD)	18.5	3.9	Study PHI113634	[7.61]*	[3.34]*	PBPK report	[4.28] ^{b/} [3.87] [†]	[2.69] ^{b/} [2.56] [†]	PBPK report
Dasabuvirn (SD)	11.3	2.02	(Menon et al. 2015)	4.66	1.63	(Itkonen et al. 2019a)			

CYP2C8 Substrates Substrate dosing date	GEM (500 mg BID 5 days)		Pubmed Reference	CLP (300 mg Day 1)		Pubmed Reference	CLP (300 mg Day 1, 75 mg QD 2 days)		Pubmed Reference
	Substrate on Day 3	AUCR		C _{max} R	Substrate on Day 1		AUCR	C _{max} R	
Repaglinide (SD)	8.09	2.39	(Niemi et al. 2003)	5.1	2.53	(Tornio et al. 2014)	3.95	2.02	(Tornio et al. 2014)
Desloratadine (SD)	4.61	1.74	(Itkonen et al. 2019b)	2.8	1.64	(Itkonen et al. 2019b)			
Montelukast (SD)	4.54	1.53	(Karonen et al. 2010)	1.98	0.982	(Itkonen et al. 2018)			
Pioglitazone (SD)	3.37	1.1		2.15		(Itkonen et al. 2016)			

Source: Simulated data were from PBPK report 2019N396674, and observed data were queried from the University of Washington Numbers in [] were simulated values. Drug Interaction Database (DIDB) by FDA reviewers.

* Daprodustat was given 4 mg QD for 14 days and daprodustat PK was assessed on day 8 following a 300 mg loading dose of clopidogrel

^b Daprodustat was given 4 mg QD for 14 days. On day 8, a 300 mg loading dose of clopidogrel was administered, followed by 75 mg QD clopidogrel from day 9 to day 14. Daprodustat PK was assessed on day 9.

[†] A 300 mg loading dose of clopidogrel was administered on day 1 followed by 75 mg QD clopidogrel from day 2 to day 9. 4 mg QD of daprodustat was administered on days 8 and 9. Daprodustat PK was assessed on day 8.

Abbreviations: AUC, area under the concentration-time curve; AUCR, AUC ratio; BID, twice daily; CLP, clopidogrel; C_{max}, maximum plasma concentration; C_{max}R, C_{max} ratio; GEM, gemfibrozil; MD, multiple dose; PBPK, physiologically based pharmacokinetic; QD, once daily; SD, single dose

14.7. Pharmacogenetics

Not applicable.

15. Study/Trial Design

15.1. Study 200808/ASCEND-ND

Protocol Amendments

There were 4 global amendments and 5 “country specific” local amendments to the original protocol (dated June 2016). Protocol Amendment 05 (dated July 20, 2020) was used to prepare the clinical study report (CSR) submitted with the application.

Amendment 01

Amendment 01 was implemented September 20, 2016 and applied to: Austria, Belgium, Czech Republic, Denmark, Estonia, Germany, Hungary, Italy, Norway, Poland, Portugal, Romania, Spain, Sweden and the United Kingdom. The main changes to the protocol included clarification of the end of the study definition/criteria. The study rationale and inclusion criteria were revised. Additional guidance was added to the iron management criteria, and a new exploratory objective to compare the effect of daprodustat to rhEPO on delayed graft function (DGF) after deceased donor kidney transplantation was added.

Amendment 02

Amendment 02 was implemented October 12, 2016 and applied to all countries and sites. It also included country-specific requirements for France and Czech Republic. The main changes to the protocol included the changes from country-specific Amendment 1. The section relating to darbepoetin alfa pre-filled syringe incidents, malfunctions and user errors was deleted. The Schedules of Assessments were modified to include new timepoints and prompts. The negatively adjudicated events reporting process was updated. Historical kidney ultrasound and a provision for a more sensitive imaging study (e.g., MRI, CT) to assess entry criteria were added.

Amendment 03

Amendment 03 was implemented October 5, 2017 and applied to all countries and sites. The main changes to the protocol included a provision for possible adjustment to the Dose Adjustment Algorithm triggers for Hb values 7.5 g/dL to <9.5 g/dL based on the review of blinded instream aggregate Hb data. Retest values for Hb and transferrin saturation (TSAT) to determine eligibility at Week -8 were added. Exclusion criteria were broadened. A provision was added for unexpected circumstances where the supply to the site was interrupted. New darbepoetin alfa dose strengths (not available in all countries) were added. Directions regarding randomized treatment and study continuation for participants away from the research site for an extended period of time were added.

Clarifications were made to the timeframe for iron management criteria. Visit windows were revised and clarified. The FSH level to confirm menopause was updated.

The Schedules of Assessments were modified for clarification and to include new activities and prompts. The additional inclusion and eligible visits to collect PK samples in PK sub-studies were clarified. The patient reported outcome (PRO) section was updated to add healthcare resource utilization data.

The statistical section was revised to change from two-sided testing at the 5% level to one-sided testing at the 2.5% level; to clarify progression of CKD endpoint population and analysis model and for secondary endpoints; to change significance levels to p-values; to correct the timepoint for various PROs; to add a more complete description of the adjustments to statistical model; and to add additional text regarding the interim analysis process. Exploratory endpoints around Hb variability, iron parameters, transfusions, and dose adjustment scheme were added.

Adjustments to the objectives, endpoints and analysis of the ABPM sub-study were made.

Amendment 04

Amendment 04 was implemented August 16, 2019 and applied to all countries and sites. The protocol was modified to add retest values for Hb at Day 1, an additional opportunity for TSAT to determine eligibility at Week -8, and revised the definition of current uncontrolled hypertension in order to determine eligibility. Hypothetical risk information, new required assessments, and randomized treatment stopping criteria for participants with ADPKD were added.

Worsening of hypertension was added as an adverse event of special interest.

Renal progression via change in eGFR was added as a secondary objective/endpoint.

For participants transitioning to dialysis, clarified the last historical clearance (Kt/V_{urea}) measurement obtained per standard of care can be used. Stated recruitment in the ABPM sub-study was closed in Amendment 4. Entry criteria to PK sub-study was revised; if the sub-study recruitment did not make its pre-specified target, depending on the number of participants with data, a limited number of analyses and data summaries may be produced.

Amendment 05

Amendment 05 was implemented July 30, 2020 and applied to all countries and sites. The protocol was modified to revise the major adverse cardiovascular events (MACE) non-inferiority margin; as a result, the target MACE was also revised (to 664 events). The primary rationale for these changes was to accelerate the closeout of the study in consideration of the COVID-19 pandemic and its impact on study integrity and data quality. Additionally, this change was aligned with similar studies that had already compared the effects of HIF-PHIs on MACE versus rhEPO, based on publicly available information. Analysis of the Hb co-primary endpoint and the multiplicity adjustment strategy were updated. Pregnancy reporting timelines were updated to align with revised Sponsor timings.

It should be noted that because the adjudication process for MACE may have taken time, some MACE related analyses contain more than 664 events.

Protocol Deviations

Significant protocol deviations were reported for 66% of subjects in the daprodustat arm and 68% of subjects in the darbepoetin alfa arm. The majority of protocol deviations were due to study procedures/assessments (44%) of total population (N=3872) and were balanced between arms.

In Study 200808 (ASCEND-ND), data on 17 study participants were excluded from all analyses as a valid informed consent was never obtained. During the conduct of the study, three sites (223710, 228130, and 239601) were closed early due to good clinical practice (GCP) non-compliance issues including suspected fraud at one site. The site (23601) with suspected fraud randomized one subject and the data from this site were included in the study displays. No additional analyses were performed to exclude the participant's data from this site. Data from site 228130 were included in the study displays except for one screen-failed participant who did not have a valid informed consent form. Data from site 223710 were not included in the data displays due to invalid consent obtained from 14 participants at this site.

Additional quality issues relating to GCP noncompliance (e.g., informed consent, dosing errors, late reporting of serious adverse events (SAEs), unreported temperature excursions, central lab issues, late reporting of individual case safety reports, missing research records) were identified at other sites and corrective actions taken, and issues were reviewed for root causes and addressed by Applicant.

15.2. Study 200807/ASCEND-D

Protocol Amendments

There were 3 global amendments and 4 “country-specific” local amendments to the original protocol (dated June 9, 2016). Protocol Amendment 04 (dated July 30, 2020) was used to prepare the CSR. A copy of Protocol Amendment 04 and each of the protocol amendments is found in the modular appendix. A brief summary of each protocol amendment is provided below.

Amendment 01

Amendment 01 was implemented September 20, 2016 and applied to: Austria, Belgium, Czech Republic, Denmark, Estonia, Germany, Hungary, Italy, Norway, Poland, Portugal, Romania, Spain, Sweden and the United Kingdom. The main changes to the protocol included clarification of the end of the study definition/criteria. The study rationale and inclusion criteria were revised. Additional guidance was added to the iron management criteria, and a new exploratory objective to compare the effect of daprodustat to rhEPO on delayed graft function (DGF) after deceased donor kidney transplantation was added.

Amendment 02

Amendment 02 was implemented October 12, 2016 and applied to all countries and sites. It also included country-specific requirements for France and Czech Republic. The main changes to the protocol included the changes from country-specific Amendment 1. The section relating to darbepoetin alfa pre-filled syringe incidents, malfunctions and user errors was deleted. The Schedules of Assessments were modified to include new timepoints and prompts. Text was added to clarify participants randomized to rhEPO who transition from hemodialysis to peritoneal dialysis change from epoetin alfa to darbepoetin alfa. Text relating to the timing of dialysis in relation to the study visit was amended. The negatively adjudicated events reporting process was updated. Historical kidney ultrasound and a provision for a more sensitive imaging study (e.g., MRI, CT) to assess entry criteria were added.

Amendment 03

Amendment 03 was implemented October 5, 2017 and applied to all countries and sites. The main changes to the protocol included a provision for possible adjustment to the Dose Adjustment Algorithm triggers for Hb values 7.5 g/dL to <9.5 g/dL based on the review of blinded instream aggregate Hb data. Retest values for Hb and TSAT to determine eligibility at Week -8 were added. Exclusion criteria were broadened. Guidance for those receiving hemodialysis two times a week randomized to the epoetin alfa arm and to require three times a week epoetin alfa dosing was added. A provision was added for unexpected circumstances where the supply to the site was interrupted. New darbepoetin alfa dose strengths (not available in all countries) were added. Directions regarding randomized treatment and study continuation for participants away from the research site for an extended period of time were added.

Clarifications were made to the timeframe for iron management criteria, the “frequency of dialysis” inclusion criterion, the valid study visit days by dialysis type, and the Baseline dose for the purposes of the rescue algorithm for participants switching from hemodialysis to peritoneal

dialysis who are randomized to rhEPO. Visit windows were revised and clarified. The FSH level to confirm menopause was updated.

The Schedules of Assessments were modified for clarification and to include new activities and prompts. The additional inclusion and eligible visits to collect PK samples in PK sub-studies were clarified. The PRO section was updated to add healthcare resource utilization data.

The statistical section was revised to change from two-sided testing at the 5% level to one-sided testing at the 2.5% level; to correct the comparator for the Null and Alternative hypotheses; to change significance levels to p-values; to add a more complete description of the adjustments to statistical model; to update hyporesponder analyses; and to add additional text regarding the interim analysis process. Exploratory endpoints around Hb variability, iron parameters, transfusions, and dose adjustment scheme were added. Adjustments to the objectives, endpoints and analysis of the ABPM sub-study were made.

Amendment 04

Amendment 04 was implemented July 30, 2020 and applied to all countries and sites.

The protocol was modified to add retest values for Hb and TSAT in order to determine eligibility. The MACE non-inferiority margin was revised; as a result, the target MACE was also revised (to 664 events). The primary rationale for these changes was to accelerate the closeout of the study in consideration of the COVID-19 pandemic and its impact on study integrity and data quality. Additionally, this change was aligned with similar studies that had already compared the effects of HIF-PHIs on MACE versus rhEPO, based on publicly available information. Analysis of the Hb co-primary endpoint and the multiplicity adjustment strategy were updated.

Please note that the protocol amendment was approved after 664 events had already occurred during the study. This means that some MACE related analyses contain more than 664 events.

Reviewer's Comments

The amendments did not negatively impact the study design, conduct or results of the study.

Protocol Deviations

Significant protocol deviations were reported for 7% of the participants in the daprodustat arm and 81% of participants in the rhEPO arm. The majority of protocol deviations were due to study procedures/assessments (44%) of the total population (N=2964) and were balanced between arms. The protocol deviations recorded likely did not have an impact on the interpretation of the results.

Eight sites were closed due to ongoing GCP non-compliance and three of the 8 closed sites were closed due to research conduct, which included suspected fraud. The data from these 3 sites were included in the primary study displays and to assess the impact of the sites on the overall study conclusion, a supportive analysis was conducted, excluding the data from these 3 sites. The results from supportive analyses, conducted by the Applicant, did not show a significant impact on the primary endpoints when all randomized participants were excluded from these sites.

16. Efficacy

16.1. Additional Efficacy Analyses

16.1.1. ASCEND-D

A supportive analysis was performed for the primary intent-to-treat analysis of post-randomization mean change in Hb between Baseline and over the Evaluation Period (EP), repeating the analysis using a shorter EP (Week 28 to 36). For each study the lower boundary of the 95% CI for the treatment difference was greater than the prospectively defined non-inferiority margin of -0.75 g/dL, thus demonstrating non-inferiority of daprodustat (see [Table 218](#)). Results were consistent with the conclusion from the primary analysis.

Table 218. Analysis of Post-Randomization Hb Change From Baseline to the Alternative EP (Weeks 28 to 36) for Study 200807/ASCEND-D

Parameter	Daprodustat (N=1487)	rhEPO ^c (N=1477)
Number of participants with baseline and EP Hb, n (%) ^a	1487 (100)	1477 (100)
Baseline Hb (g/dL) mean (SD)	10.35 (0.970)	10.39 (0.981)
EP (Weeks 28-36) Hb (g/dL) mean (SD) ^a	10.65 (0.934)	10.44 (0.932)
Adjusted mean change from baseline (SE) ^b	0.29 (0.026)	0.07 (0.026)
Adjusted mean treatment difference (dapro - rhEPO) ^b		0.22
Two-sided 95% CI for adjusted mean difference ^b	(0.15, 0.29)	

Source: Sponsor CSR

^a Hb values during the EP include both observed and imputed values.

^b Based on an ANCOVA model with terms for treatment, Baseline Hb, dialysis type and region.

^c Epoetin alfa in HD participants or darbepoetin alfa in PD participants

Abbreviations: CI, confidence interval; EP, evaluation period; Hb, hemoglobin; rhEPO, recombinant human erythropoietin; SD, standard deviation; SE, standard error

Additional primary endpoint supportive analyses included an on-treatment analysis using only evaluable Hb values, a per protocol Population analysis using only evaluable Hb values, a shorter EP (Weeks 28 to 36) analysis using only evaluable Hb values, and a post-randomization (on- and off-treatment) observed case analysis. Results were consistent with the non-inferiority conclusion from the primary analysis (see [Table 219](#)).

Table 219. Primary Endpoint Hb Supportive Analyses for Study 200807/ASCEND-D

Primary Endpoint	Daprodustat (N=1487)	rhEPO ^c (N=1477)
Evaluable Hb (Weeks 28 to 52), ITT Population		
Number of participants with Baseline and EP Hb, n (%) ^a	1238 (83)	1247 (84)
Adjusted mean change from Baseline (SE) ^b	0.27 (0.022)	0.06 (0.022)
Adjusted mean treatment difference (dapro - darbe) ^b		0.21
Two-sided 95% CI for adjusted mean difference	(0.15, 0.27)	
Evaluable Hb (Weeks 28 to 52), PP Population		
Number of participants with Baseline and EP Hb, n (%) ^a	934 (100)	949 (100)
Adjusted mean change from Baseline (SE) ^b	0.30 (0.022)	0.13 (0.022)
Adjusted mean treatment difference (dapro - darbe) ^b		0.17
Two-sided 95% CI for adjusted mean difference ^b	(0.11, 0.23)	

Primary Endpoint	Daprodustat (N=1487)	rhEPO^c (N=1477)
Evaluable Hb Alternative EP (Weeks 28 to 36), ITT Population		
Number of participants with Baseline and EP Hb, n (%) ^a	1231 (83)	1236 (84)
Adjusted mean change from Baseline (SE) ^b	0.30 (0.026)	0.05 (0.026)
Adjusted mean treatment difference (dapro - darbe) ^b		0.25
Two-sided 95% CI for adjusted mean difference ^b	(0.18,0.32)	
Post-randomization Hb (Observed Cases only), ITT Population		
Number of participants with Baseline and EP Hb, n (%)	1364 (92)	1352 (92)
Adjusted mean change from Baseline (SE) ^b	0.28 (0.023)	0.09 (0.023)
Adjusted mean treatment difference (dapro - darbe) ^b		0.19
Two-sided 95% CI for adjusted mean difference ^b	(0.13,0.25)	

Source: Sponsor CSR

^a Hb values during the EP include observed values only.

^b Based on an ANCOVA model with terms for treatment, Baseline Hb, dialysis type and region.

^c Epoetin alfa in HD participants or darbepoetin alfa in PD participants

Abbreviations: CI, confidence interval; EP, evaluation period; Hb, hemoglobin; ITT, intent-to-treat; rhEPO, recombinant human erythropoietin; SE, standard errors

16.1.2. ASCEND-ND

Efficacy Results – Secondary Endpoints

A supportive secondary analysis was performed for the primary intent-to-treat analysis of post-randomization mean change in Hb between Baseline and over the EP, repeating the analysis using a shorter EP (Week 28 to 36). The lower boundary of the 95% CI for the treatment difference was greater than the prospectively defined non-inferiority margin of -0.75 g/dL, thus demonstrating non-inferiority of daprodustat to darbepoetin alfa in mean change in Hb between Baseline and over the EP ([Table 220](#)). Results were consistent with the conclusion from the primary analysis.

Table 220. Analysis of Post-Randomization Hb Change From Baseline to the Alternative EP (Weeks 28 to 36) for Study 200808/ASCEND-ND

Parameter	Daprodustat (N=1937)	Darbepoetin (N=1935)
Number of participants with baseline and EP Hb, n (%) ^a	1937 (100)	1935 (100)
Baseline Hb (g/dL) mean (SD)	9.87 (0.940)	9.85 (0.948)
EP (Weeks 28-36) Hb (g/dL) mean (SD) ^a	10.56 (0.783)	10.46 (0.864)
Adjusted mean change from baseline (SE) ^b	0.70 (0.022)	0.60 (0.022)
Adjusted mean treatment difference (dapro-darbe) ^b		0.10
Two-sided 95% CI for adjusted mean difference ^b	(0.03, 0.16)	

Source: Sponsor CSR

^a Post-randomization values include on and off treatment values. Observed and imputed Hb values are used in this analysis. There were no missing Baseline values in the analysis.

^b Based on an ANCOVA model with terms for treatment, baseline Hb, current ESA use and region.

Abbreviations: CI, confidence interval; EP, evaluation period; Hb, hemoglobin; SD, standard deviation; SE, standard errors

Additional supportive secondary analyses included an on-treatment analysis using only evaluable Hb values, a per protocol Population analysis using only evaluable Hb values, a shorter EP (Weeks 28 to 36) analysis using only evaluable Hb values, and a post-randomization (on- and off-treatment) observed case analysis ([Table 221](#)). Results were consistent with the non-inferiority conclusion from the primary analysis

Table 221. Primary Endpoint Hb Supportive Analyses for Study 200808/ASCEND-ND

Primary Endpoint	Daprodustat (N=1937)	Darbepoetin (N=1935)
Evaluable Hb (Weeks 28 to 52), ITT population		
Number of participants with baseline and EP Hb, n (%) ^a	1491 (77)	1520 (79)
Adjusted mean change from baseline (SE) ^b	0.70 (0.019)	0.64 (0.019)
Adjusted mean treatment difference (dapro - darbe) ^b		0.07
Two-sided 95% CI for adjusted mean difference	(0.01, 0.12)	
Evaluable Hb (Weeks 28 to 52), PP population		
Number of participants with baseline and EP Hb, n (%) ^a	982 (100)	1083 (100)
Adjusted mean change from baseline (SE) ^b	0.76 (0.020)	0.67 (0.019)
Adjusted mean treatment difference (dapro - darbe) ^b		0.09
Two-sided 95% CI for adjusted mean difference ^b	(0.04, 0.14)	
Evaluable Hb Alternative EP (Weeks 28 to 36), ITT population		
Number of participants with baseline and EP Hb, n (%) ^a	1483 (77)	1503 (78)
Adjusted mean change from baseline (SE) ^b	0.69 (0.022)	0.62 (0.022)
Adjusted mean treatment difference (dapro - darbe) ^b		0.08
Two-sided 95% CI for adjusted mean difference ^b	(0.01, 0.014)	
Post-randomization Hb (Observed Cases only), ITT population		
Number of participants with baseline and EP Hb, n (%)	1576 (81)	1578 (82)
Adjusted mean change from baseline (SE) ^b	0.71 (0.020)	0.63 (0.020)
Adjusted mean treatment difference (dapro - darbe) ^b		0.08
Two-sided 95% CI for adjusted mean difference ^b	(0.03, 0.13)	

Source: Sponsor CSR

Note: Evaluable values are on-treatment values that are not taken within the 8 weeks following a red blood cell or whole blood transfusion or a post-randomization non-randomized ESA treatment.

Note: Post-randomization values include on and off treatment values.

^a Hb values during the EP include observed values only.

^b Based on an ANCOVA model with terms for treatment, baseline Hb, current ESA use and region.

Abbreviations: CI, confidence interval; EP, evaluation period; Hb, hemoglobin; ITT, intent-to-treat; SD, standard deviation; SE, standard errors

16.1.3. Secondary Endpoints ASCEND-NHQ

Efficacy Results – Secondary Endpoints

For the analysis of post-randomization Hb change from Baseline to Week 28 daprodustat was nominally superior to placebo. The adjusted mean treatment difference (daprodustat - placebo) was 1.36 (95% CI: 1.16, 1.55; $p < 0.0001$) ([Table 222](#)).

Table 222. Analysis of Post-Randomization Hb Change From Baseline at Week 28 for Study 205270/ASCEND-NHQ

	Placebo N=307	Daprodustat N=307
Number of participants in the analysis ^a , n (%) ^a	301 (98)	299 (97)
Baseline Hb (g/dL) mean (SD)	9.71 (0.73)	9.74 (0.64)
Week 28 Hb (g/dL) mean (SD) ^b	9.96 (1.19)	11.32 (1.09)
Adjusted mean change from baseline (SE) ^c	0.20 (0.07)	1.56 (0.07)
Adjusted mean treatment difference (dapro-placebo) ^c		1.36
Two-sided 95% CI for adjusted mean difference ^c		1.16, 1.55
One-sided superiority p-value ^{c,d}		<0.0001

Source: Sponsor CSR

^a To be included in the analysis, participants must have a non-missing baseline Hb value, at least one scheduled non-missing post-baseline Hb value, and non-missing values for model covariates.

^b Week 28 Hb mean and SD from participants with baseline and Week 28 Hb values.

^c Week 28 values based on a MMRM model fitted from baseline up to Week 28, with factors for treatment, time, region, baseline Hb and baseline Hb by time and treatment by time interactions. One-sided p-value based on test of null hypothesis: (daprodustat-placebo) ≤ 0 vs alternative: difference > 0.

^d Superiority was achieved if the one-sided p-value was < 0.025.

Abbreviations: CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; Hb, hemoglobin; MMRM, mixed model repeated measures; SD, standard deviation; SE, standard error

The analysis of Hb responders during the EP showed that the difference in response rate (daprodustat - placebo) was 0.45 (95% CI: 0.37, 0.52) ([Table 223](#)). The difference was nominally statistically significant (one-sided p<0.0001).

Table 223. Analysis of Hb Responders During the EP Using Evaluable Hb for Study 205270/ASCEND-NHQ

	Placebo N=307	Daprodustat N=307
Participants with evaluable Hb during the EP, n (%)	217 (71)	252 (82)
Hb responder: participants with mean EP Hb 11-12 g/dL, n (%)	17 (8)	132 (52)
Difference in response rate (dapro-placebo)		0.45
Two-sided 95% CI for difference in response rate		0.37, 0.52
One-sided p-value ^{a,b}		<0.0001

Source: Sponsor CSR

Note: Evaluable values are on-treatment values that were not taken within the 8 weeks following a red blood cell or whole blood transfusion or a post-randomization non-randomized ESA treatment.

^a Treatment group comparisons are based on a CMH test adjusted for treatment group, and region. One-sided p-value based on test of null hypothesis: (daprodustat-placebo) ≤ 0 vs alternative: difference > 0.

^b The one-sided p-value was compared to 0.025 to assess nominal significance.

Abbreviations: CI, confidence interval; CMH, Cochran-Mantel-Haenszel; CSR, clinical study report; Dapro, daprodustat; EP, evaluation period; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin

Daprodustat was nominally superior to placebo for the secondary endpoint of percentage of time Hb within the analysis range during the EP according to the Hodges-Lehmann estimate of the median treatment difference (daprodustat-placebo). Daprodustat was also nominally superior to placebo for percentage of time Hb within the analysis range according to the van Elteren's test (one-sided p<0.0001) ([Table 224](#)).

Table 224. Analysis of % Time Hb Within the Analysis Range During the EP Using Evaluable Hb Values for Study 205270/ASCEND-NHQ

	Placebo N=307	Daprodustat N=307
n (%) ^a	216 (70)	252 (82)
Median (%) time in range (11-12 g/dL)	0.0	53.6
Hodges-Lehmann estimate of treatment difference (dapro-placebo) (%)		38.8
Hodges-Lehmann two-sided asymptotic 95% CI (%) ^b	25.0, 54.6	
One-sided p-value ^b	<0.0001	

Source: Sponsor CSR

Note: Evaluable values are on-treatment values that are not taken within the 8 weeks following a red blood cell or whole blood transfusion or a post-randomization non-randomized ESA treatment.

^a Number of participants with a treatment stop date that was on or after their Week 24 visit date, and had at least two evaluable Hb values on different days, where at least one evaluable Hb value is contained within the EP and another evaluable Hb value occurs within the range of the Week 12 visit through Week 28 visit, inclusive.

^b A nominal superiority was achieved if the lower limit of the Hodges-Lehmann two-sided 95% CI is above 0 and the one-sided p-value from the van Elteren test is < 0.025.

Abbreviations: CI, confidence interval; CSR, clinical study report; Dapro, daprodustat; EP, evaluation period; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin

Permanently stopping randomized treatment due to meeting rescue criteria was reported in 2 participants (<1%) in the daprodustat group and in 26 participants (8%) in the placebo group. There was a lower risk of stopping randomized treatment due to meeting rescue criteria with daprodustat compared with placebo and this lower risk achieved nominal significance with a hazard ratio of 0.07 (95% CI: 0.02, 0.30; one-sided p=0.0002) ([Table 225](#)).

Table 225. Analysis of Time to Permanently Stopping Randomized Treatment Due to Meeting Rescue Criteria for Study 205270/ASCEND-NHQ

	Placebo N=307	Daprodustat N=307
Number of participants, n (%)	307	307
Permanently stopping randomized treatment due to meeting rescue criteria, n (%)	26 (8)	2 (<1)
Censored, n (%)	281 (92)	305 (>99)
Incidence rate per 100 PY (two-sided 95% CI)	18.88 (12.33, 27.66)	1.33 (0.16, 4.82)
Hazard ratio		
Estimate ^a	0.07	
Two-sided 95% CI for hazard ratio	0.02, 0.30	
One-sided p-value ^b	0.0002	
Absolute rate difference per 100 PY (two-sided 95% CI) ^c	-17.5 (-25.0, -10.1)	

Source: Sponsor CSR

^a Hazard ratio is estimated using a Cox proportional hazard regression model adjusted for treatment group, and region. A hazard ratio <1 indicates a lower risk with daprodustat compared to placebo.

^b One-sided p-value based on Wald test of null hypothesis: (daprodustat/placebo) ≥1 vs alternative: ratio<1.

^c A rate difference <0 indicates a lower risk with daprodustat compared to placebo.

Abbreviations: CI, confidence interval; CSR, clinical study report; PY, person-year

More participants in the daprodustat group had an improvement in SF-36 v2.0 Vitality domain score ≥6 points from Baseline at Week 28 than those in the placebo group, and the treatment difference between the groups favored daprodustat (see [Table 60](#)). However, the review team has concern about the Applicant's proposed improvement threshold (refer to Section [6.3.2](#) for more details).

Table 226. Analysis of Participants Having an Improvement in On-Treatment Observed and Imputed SF-36 v2.0 Vitality Domain ≥ 6 From Baseline at Week 28 for Study 205270/ASCEND-NHQ

0-100 Scoring	Placebo N=307	Daprodustat N=307
Number of participants with baseline and Week 28 SF-36 v2.0 Vitality domain score ^a , n (%)	307 (100)	307 (100)
Number of participants with an improvement in SF-36 v2.0 Vitality domain ≥ 6 from baseline at Week 28, n (%)	122 (40)	179 (58)
Difference in response rate (%) (dapro-placebo) ^b		13
Two-sided 95% CI for difference in response rate (%)		4, 22
One-sided p-value ^{b,c}		0.0049

Source: Sponsor CSR

^a SF-36 v2.0 Vitality domain data includes imputed values.

^b Treatment group comparisons are based on a Cochran-Mantel-Haenszel test adjusted for treatment group, and region. One-sided p-value based on test of null hypothesis: (daprodustat-placebo) ≤ 0 vs alternative : difference > 0 .

^c Superiority for the difference between the daprodustat and placebo treatment groups was established if the one-sided p-value is less than 0.025.

Abbreviations: CI, confidence interval; CSR, clinical study report; Dapro, daprodustat

16.2. ASCEND-NHQ Statistical Analysis Plan

Sample Size Determination

Approximately 600 participants were planned to be randomized (300 per arm) to receive daprodustat or placebo, who would be treated to achieve and maintain Hb between 11 and 12 g/dL.

Under the assumption that up to 10% of participants would withdraw from the study before Week 24 (start of EP), 600 randomized participants would result in at least 540 evaluable participants.

For the primary endpoint, the expected difference in mean Hb change from baseline and the EP, between arms, was 1.0 g/dL and the anticipated between participant standard deviation was 1.5 g/dL, based on historical rhEPO trials and daprodustat clinical trial experience to date. The planned study size would provide more than 99% power for superiority on Hb. In addition, it was anticipated that the smallest difference in mean Hb change between arms that would meet the statistical criterion for superiority would be 0.25 g/dL.

For the first key secondary endpoint of % of participants having a Hb increase of ≥ 1.0 g/dL from baseline to EP, 540 evaluable participants would provide $> 90\%$ power to detect a 20% difference between daprodustat and placebo, assuming 30% of placebo arm participants would have a Hb increase of ≥ 1.0 g/dL. In that case, the smallest difference between daprodustat and placebo that would meet the statistical criterion for superiority would be 8%.

Missing Data Handling for Primary Efficacy Endpoint

Multiple imputation analysis was performed using all available Hb values (on and off-treatment) and conducted under a set of assumptions about missing Hb values.

- For each of the missing dataset (out of the 200 imputed as indicated above), the missing scheduled Hb values through Week 28 were imputed based on different scenarios. The ANCOVA regression had region, treatment, baseline Hb, off treatment status, and prior scheduled (possibly imputed) Hb values as covariates. Missing data were handled as either being on-treatment or off treatment, with the former being imputed with only on-treatment data and the latter being imputed with only off-treatment data
 - Missing data were classified as being on-treatment, if
 - Patients died, used non-randomized ESA Medication, received blood transfusions while on treatment
 - Study Drug Interruption (e.g., temporarily stopped treatment due to COVID, then restarted)
 - Missing data were classified as being off-treatment, if
 - Treatment discontinuation
 - Patients died, used non-randomized ESA Medication, received blood transfusions after treatment discontinuation
- The low and high cutoff Hb values of 6 g/dL and 15 g/dL were applied to all imputed Hb values.
- EP Hb values were computed and compared across treatment groups using the primary ANCOVA model described above. Rubin’s rules were used to combine results of the imputed datasets using SAS PROC MIANALYZE procedure. As a result, a single estimated treatment difference and its standard error was produced, with which a 95% CI was calculated.

Analysis for Primary Efficacy Endpoint

The primary efficacy estimand was the effect of daprodustat treatment relative to placebo regardless of adherence to treatment, the use of non-randomized ESA medication for any reason including rescue therapy, or the use of blood transfusions, in participants defined by the inclusion/exclusion criteria and assuming participants did not die before the end of the EP.

The primary efficacy endpoint was the mean change in Hb between baseline and over the EP (mean of all available [on and off treatment] Hb values for Week 24 through Week 28, inclusive, including any unscheduled Hb values that were taken during this time period). To quantify the difference in mean Hb change, an ANCOVA model adjusted for treatment, baseline Hb, and region was used. The primary model provided a two-sided 95% CI and one-sided superiority p-value for the difference in the primary Hb endpoint between the daprodustat and placebo arm. Superiority would be established if the one-sided p-value was less than 0.025.

Sensitivity Analysis

A sensitivity analysis that considers an “on-drug” efficacy estimand was conducted. In this analysis, only Hb values measured while the participant was taking randomized study medication was included. Missing Hb values were imputed based on the reasons for the missing Hb data.

Analyses for Secondary Efficacy Endpoints

For the endpoint of % of participants having a Hb increase of ≥ 1.0 g/dL from baseline to EP, the EP Hb value was defined as the mean of all available Hb values (on- and off-treatment) during

the EP (Week 24 to Week 28, inclusive). A CMH chi-squared test that adjusted for treatment and region was used to make comparisons between the treatment groups. A treatment policy strategy was used as in the primary analysis to include all on- and off-treatment Hb values of the ITT populations, including values taken after discontinuation of study treatment/rescue therapy. Missing data were imputed as described for the primary efficacy analysis.

For the key secondary endpoint of mean change in SF-36 v2.0 Vitality domain score between baseline and Week 28, a hypothetical strategy, including only on-treatment values, was used assuming participants had not had intercurrent events of death prior to the end of the EP and randomized treatment discontinuation prior to the end of the EP. Data collected after discontinuation of study treatment/rescue medication were not included in the analysis and were treated as missing. The SF-36 v2.0 Vitality domain scores after discontinuation of study treatment were imputed based on the conservative assumption that there was no change in the domain scores after the intercurrent event, so the last available on-treatment values (or baseline values if there was no on-treatment value) were used in the imputation. Missing SF-36 v2.0 Vitality domain scores were imputed based on a missing at random assumption. An ANCOVA model was used including factors for baseline score, treatment, and region. The analysis was performed for both 0-100 scoring and norm-based scoring. The 0-100 scoring was considered as the primary results and norm-based as supportive results.

Additional Secondary Endpoints

For the Hb responder analysis, mean Hb during the EP was defined as the on-drug supportive analysis. Responders were participants with a mean Hb during the EP that fell within the Hb analysis range of 11 to 12 g/dL. A CMH chi-squared test that adjusted for treatment and region was used to compare the number and % of responders between the treatment groups. The one-sided CMH p-value was compared to 0.025 to assess nominal significance.

For the time to stopping study treatment due to meeting rescue criteria, a Cox proportional hazards model adjusted for the baseline categorical values of treatment and region was used. Confidence intervals (CIs) for the rate per 100 person-years was also reported. For within-group rates, the two-sided 95% CI was obtained using an exact Poisson method. For difference in rates between treatments, the two-sided 95% CI was constructed with a normal approximation using Wald's method. One-sided p-values were compared to 0.025 to assess nominal significance.

Secondary endpoints included the mean change from baseline in Patient Global Impression of Severity (PGI-S) score and each Chronic Kidney Disease Anemia Questionnaire (CKD-AQ) domain score and single items were analyzed using a mixed model repeated measures approach with an unstructured covariance matrix to compare the difference in means between arms. The models were fitted to data collected after Baseline up to Week 28, using on-treatment values and excluded the measures taken after discontinuation of study treatment. The models included factors for treatment, time, region, treatment by time interaction, corresponding baseline score values (i.e., PGI-S score, CKD-AQ domain, and CKD-AQ single items score in the corresponding model), and the baseline score by time interaction. Analyses were done with the MIXED procedure in SAS, using the Kenward-Roger option to estimate denominator degrees of freedom and standard errors. A point estimate and two-sided 95% CI for the adjusted mean treatment difference (daprodustat - placebo) and a one-sided superiority p-value for this test at

Week 28 were provided. One-sided p-values were compared to 0.025 to assess nominal significance.

For the % of participants having an improvement in SF-36 v2.0 Vitality domain score ≥ 6 from baseline at Week 28, a CMH chi-squared test that adjusted for treatment and region was used to make comparisons between the treatment groups. This was based on the 0-100 scoring. As with the second key secondary endpoint, on-treatment SF-36 v2.0 Vitality domain scores were used and excluded the measures taken after discontinuation of study treatment.

The mean change from baseline in SF-36 v2.0 Vitality domain scores (individual items of the Vitality domain, overall score of the Physical Function domain), EuroQol 5 Dimension 5 Level (EQ-5D-5L) score, and EuroQol Visual Analogue Scale (EQ-VAS) score were analyzed using a mixed model repeated measures approach with an unstructured covariance matrix to compare the difference in means between arms. The models were fitted to HRQoL data collected after Baseline up to Week 28, using on treatment values and excluded the measures taken after discontinuation of study treatment. The model included factors for treatment, time, region, treatment by time interaction, corresponding baseline value (HRQoL parameter or utility scores), and the baseline value by time interaction term.

Multiple Comparison

The primary endpoint based on mean change from baseline on Hb value was tested first for superiority using a one-sided 2.5% significance level. Conditional on achieving statistical significance, the two key secondary endpoints would be tested for superiority using a one-sided 2.5% significance level sequentially. The additional secondary/exploratory endpoints were not adjusted for multiplicity. Summary statistics and nominal one-sided 2.5% significance levels were used to describe the results of these treatment comparisons.

Subgroup Analysis

Subgroup analysis including age, sex, and race was performed using all available Hb values (on- and off- treatment, including imputed values) and separately using evaluable Hb values only. The ANCOVA models used in the primary analysis was used and adjusted for treatment, baseline Hb, region, subgroup, and subgroup treatment interaction term.

For the first key secondary endpoint (% of participants having a Hb increase of ≥ 1.0 g/dL from baseline to EP), subgroup analysis was performed using all available Hb values (on- and off-treatment). A CMH chi-squared test that adjusted for treatment and subgroup was used to make comparisons between the treatment groups.

For the second key secondary endpoint, subgroup analyses were performed using an ANCOVA model that included factors for baseline score, treatment, region, subgroup, and subgroup treatment interaction.

16.2.1. Review of the 36-item Short Form Health Survey version 2.0 (SF-36 v2.0) Vitality Domain for Evaluation of Clinical Benefit

The Applicant used the SF-36 v2.0 Vitality domain to assess the secondary efficacy PRO endpoint in ASCEND-NHQ. This instrument was reviewed for content validity, other measurement properties, and meaningful within-patient change.

16.2.1.1. Instrument Description

The SF-36 v2.0 Vitality domain ([Figure 64](#) in Section [16.3.1](#)) is a 4-item domain within the SF-36 v2.0, which is designed to assess some aspects of fatigue related to fullness of life, energy, feeling tired, and feeling worn out. Each item is rated on a 5-point verbal rating scale ranging from “None of the time” to “All of the time.” The recall period is the previous 7 days (“past week”).

Each item score of the SF-36 v2.0 Vitality domain ranges between 1 to 5. The four items in the SF-36 v2.0 Vitality domain are first summed to calculate a raw domain score that ranges from 4 to 20. Of note, the positively worded items (feeling “full of life” and having energy) are reverse scored (i.e., a response value of 1 corresponds to a raw score value of 5). The raw domain score is then converted to a 0-100 scaled score by the formula shown below, where higher scores indicate better health, and lower scores indicate more disability (or worse health state):

0-100 scaled SF-36 Vitality score = ((SF-36 Vitality domain raw sum score) – 4)/16*100)

To limit missing data at the item-level, the SF-36 v2.0 was administered in an electronic format and respondents were not permitted to skip items.

16.2.1.2. Content Validity

The Applicant completed the following instrument development activities to evaluate the content validity of the SF-36 Vitality domain in patients with anemia associated with CKD:

- A literature review
- Patient input (published qualitative study, reanalysis of existing patient interview transcripts)

The review team evaluated the data generated from these instrument development activities. Both literature and patient input (from published qualitative study and reanalysis of patient interview transcripts) confirmed that the content of the SF-36 v2.0 Vitality domain was relevant to the patient experience with anemia due to CKD. While the SF-36 v2.0 has been widely used across different diseases, the Applicant did not cognitively test the SF-36 v2.0 with patients with anemia due to CKD to evaluate patient comprehension. However, the Applicant cited a published qualitative study that conducted cognitive interviews in patients with type 2 diabetes and anemia due to CKD; this literature provides limited details regarding patient comprehension of the SF-36 v2.0 Vitality domain.

Refer to the full COA review by the DCOA for detailed results of the content validity of the SF-36 v2.0 Vitality domain.

16.2.1.3. Other Measurement Properties (Reliability, Construct Validity, Responsiveness)

The Applicant evaluated the other measurement properties of the SF-36 v2.0 Vitality domain using combined data from two phase 3 trials in adult patients with anemia associated with CKD:

- ASCEND-ND (n=350): A randomized, open-label, active controlled, parallel-group, multi-center, event-driven study in non-dialysis subjects with anemia associated with CKD.
- ASCEND-ID (n=100): A randomized, open-label, active-controlled, parallel-group, multi-center study in subjects with anemia associated with CKD who were not routine ESA users and were initiating dialysis.

The Applicant's rationale for combining the data from the two trials was to ensure a full representation in the sample among a broad range of patients with CKD across the continuum of the disease—from non-dialysis to those who were previously not on dialysis, but subsequently required the initiation of dialysis. It's unclear why data from ASCEND-D were not included as well.

Two 'refresh samples²¹' were used to evaluate the measurement properties of the SF-36 v2.0 Vitality domain. Both refresh samples 1 and 2 comprised 350 ASCEND-ND participants. A further 100 ASCEND-ID participants were also included in refresh sample 1. Of the 100 ASCEND-ID participants included in refresh sample 1, 50 were randomly selected to also be included in refresh sample 2. For more details regarding the refresh samples, refer to Section 2.2 in Appendix 20 of the Applicant's SF-36 PRO Dossier ("EVA-28889-01: Confirmation of the Psychometric Properties of the SF-36 Vitality Scale Among Patients with Chronic Kidney Disease" report Version 1.0 dated July 26, 2021).

Based on FDA's assessment, the quantitative sample used for psychometric analyses is not fully comparable to the study population of ASCEND-NHQ. Despite this, the reliability (internal consistency and test-retest reliability) and convergent validity of the SF-36 v2.0 Vitality domain appear to be within acceptable and reasonable ranges. The results for assessment of known groups validity (a form of construct validity) were difficult to interpret as the Applicant's selected known groups (i.e., Hb, CKD stage, dialysis status, PGI-S, CKD-AQ Items 1 and 2) did not adequately categorize patients by the severity of fatigue-related symptoms. The Applicant did not evaluate the responsiveness of the SF-36 v2.0 Vitality domain using the refresh samples. Instead, the Applicant cited publications to support the responsiveness of this instrument. The review team acknowledges that the responsiveness of this instrument has been widely published, however, it remains difficult to fully evaluate the responsiveness of this instrument solely on literature given the considerable variability observed in the context of use (e.g., design of studies).

²¹ The samples are termed 'refresh' as they were also used to confirm psychometric properties of the CKD-AQ.

Refer to the full COA review by the DCOA for detailed discussion of the other measurement properties of the SF-36 v2.0 Vitality domain.

16.2.1.4. Interpretation of Meaningful Within-Patient Score Changes

The Applicant proposed that a 6.0-point reduction (improvement) in the SF-36 v2.0 Vitality domain is a meaningful within-patient score change. Accordingly, the Applicant performed the following analyses to support the proposed threshold range for meaningful within-patient change in the SF-36 Vitality domain score using combined data from ASCEND-ND and ASCEND-ID:

Anchor-based analyses
Distribution-based analyses

This section focuses on the anchor-based analyses as these are the primary methods used by FDA to interpret meaningful within-patient score changes in PRO endpoints.

[Table 227](#) summarizes the anchors utilized by the Applicant and their corresponding target COA.

Copies of the anchor scales are provided in Section [16.3.2](#) of this review.

Table 227. Summary of Anchor Scales

Endpoint Concept/Attribute (COA Type/Name if Any)	Anchor (Concept)	Anchor Response Scale	Recall Period (Target/Anchor)	Timing (Target/Anchor)
SF-36 v2.0 Vitality domain (frequency of fatigue-related symptoms)	PGI-S (overall CKD symptom severity)	5-point VRS: Absent, Mild, Moderate, Severe, Very severe	Previous week/ Previous 24 hours	Week 28/ Week 28
	PGI-C (change in severity of CKD)	7-point VRS: Very much improved, Moderately improved, Minimally improved, No change, Minimally worse, Moderately worse, Very much worse	Previous week/ Since start of study	Week 28/ Week 28
	CKD-AQ Item 1 (frequency of tiredness or exhaustion)	5-point VRS: None of the time, A little of the time, Some of the time, Most of the time, All of the time	Previous week/ Previous 7 days	Week 28/ Week 28
	CKD-AQ Item 2 (frequency of low or no energy)	5-point VRS: None of the time, A little of the time, Some of the time, Most of the time, All of the time	Previous week/ Previous 7 days	Week 28/ Week 28

Source: FDA reviewer table

Abbreviations: CKD, Chronic Kidney Disease; CKD-AQ, Chronic Kidney Disease Anemia Questionnaire; PGI-C, Patient Global Impression of Change; PGI-S, Patient Global Impression of Severity; VRS, Verbal Rating Scale

The Applicant conducted anchor-based analyses using combined data from Studies ASCEND-ND and ASCEND-ID (refer to Section [16.2.1.3](#) above regarding discussion on “refresh samples 1 and 2”). The SF-36 PRO Dossier states that “*In determining these thresholds of meaningful change, data from refresh sample 1 was prioritized as this sample had the broadest range of CKD severity (including more patients with severe CKD).*” [Table 228](#) shows the mean change in

the SF-36 Vitality Domain scores corresponding to a 1-category and 2-category improvement in the anchor scales by refresh samples 1 and 2.

Table 228. Anchor-Based Meaningful-Change Thresholds for the Change From Baseline at Week 28 in the SF-36 Vitality Domain Score: Refresh Samples 1 and 2

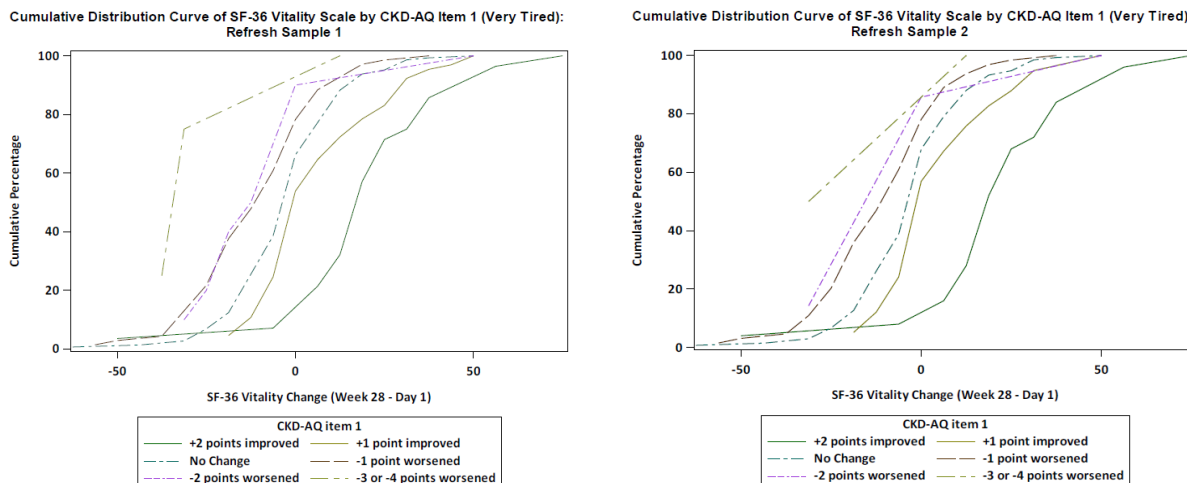
Anchor	Sf-36 Vitality Δ (Week 28 – Day 1)			
	Refresh Sample 1		Refresh Sample 2	
	N	Mean (SD)	N	Mean (SD)
+2 PGI-S Δ Week 28	10	11.90 (28.18)	9	17.40 (23.55)
+1 PGI-S Δ Week 28	49	6.10 (20.98)	42	3.70 (19.18)
Very much improved PGI-C at Week 28	37	6.80 (20.75)	30	8.20 (18.39)
Moderately improved PGI-C at Week 28	90	4.50 (17.60)	83	3.60 (16.59)
+2 CKD-AQ Item 1 (very tired) Δ Week 28	28	21.20 (22.65)	25	23.30 (20.60)
+1 CKD-AQ Item 1 (very tired) Δ Week 28	65	7.7 (16.82)	58	5.4 (13.76)
+2 CKD-AQ Item 2 (low or no energy) Δ Week 28	28	18.10 (17.46)	25	17.80 (17.29)
+1 CKD-AQ Item 2 (low or no energy) Δ Week 28	77	4.70 (18.23)	67	3.0 (15.96)

Source: SF-36 PRO dossier.

Abbreviations: CKD-AQ, Chronic Kidney Disease Anemia Questionnaire; PGI-C, Patient Global Impression of Change; PGI-S, Patient Global Impression of Severity; SD, standard deviation

Given that no qualitative evidence was provided by the Applicant for what constitutes a meaningful change on the anchors from the patients’ perspective, FDA took a conservative approach by considering a 2-category (rather than a 1-category) improvement on the anchor scales to support the anchor-based meaningful change analyses. As shown in the eCDF plots ([Figure 62](#) and [Figure 63](#)) of the cumulative distribution curves of SF-36 Vitality Domain scores by anchor change categories, there is a large separation between the “2-category improvement” curve and the “no change” curve, while the separation between the “1-category improvement” curve and the “no change” curve is minimal.

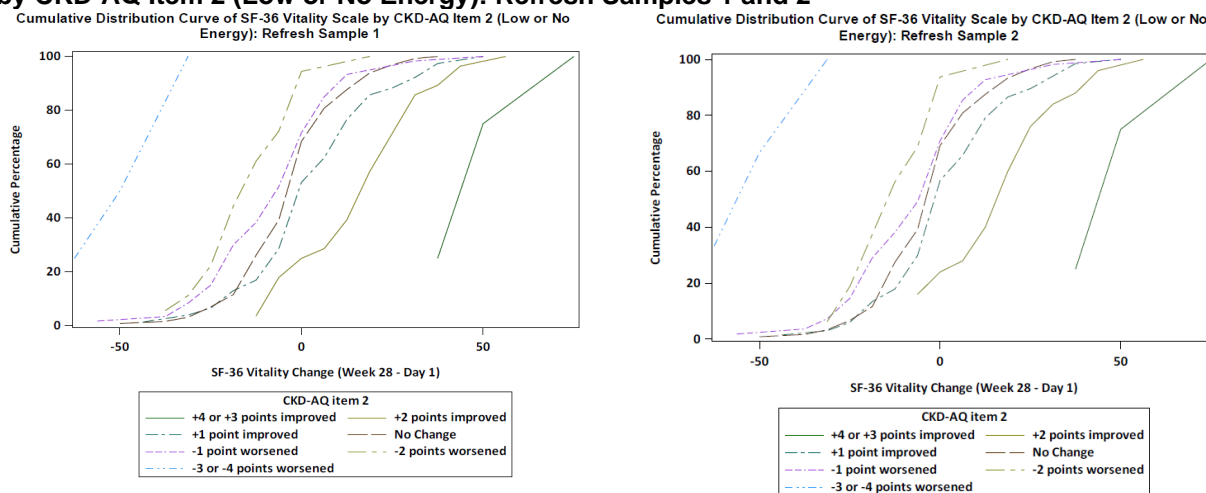
Figure 62. eCDF Plot of the Change From Baseline at Week 28 in the SF-36 Vitality Domain Score by CKD-AQ Item 1 (Very Tired): Refresh Samples 1 and 2



Source: Figure 1 (Page 38) and Figure 2 (Page 39) of the EVA-28889-01 Confirmation of Psychometric Properties of the SF-36 VT in CKD Patients.

Abbreviations: CKD-AQ, Chronic Kidney Disease Anemia Questionnaire; eCDF, empirical Cumulative Distribution Function

Figure 63. eCDF Plot of the Change From Baseline at Week 28 in the SF-36 Vitality Domain Score by CKD-AQ Item 2 (Low or No Energy): Refresh Samples 1 and 2



Source: Figure 3 (Page 40) and Figure 4 (Page 41) of the EVA-28889-01 Confirmation of Psychometric Properties of the SF-36 VT in CKD Patients.

Abbreviations: CKD-AQ, Chronic Kidney Disease Anemia Questionnaire; eCDF, empirical Cumulative Distribution Function

As mentioned in Section 6.3.2, FDA requested additional anchor-based analyses using data from Study ASCEND-NHQ. Based on the Applicant’s results (Table 229, Table 230), with baseline symptom severity taken into account (Table 231, Table 232), FDA derived a threshold range of 12.5- to 18.75-point improvement by considering the mean and median values of change from baseline in the SF-36 Vitality Domain scores that correspond to a 2-category improvement in the anchors.

Table 229. Distribution of Change From Baseline at Week 28 for On-Treatment SF-36 Vitality Domain Score by CKD-AQ Item 1 (Very Tired): Study ASCEND-NHQ

SF-36 Vitality CFB at Week 28

CKD-AQ Item 1 CFB at Week 28	N	Mean	SD	Min.	P10	P25	Median	P75	P90	Max.
4 points improved	1	12.500		12.50	12.500	12.500	12.500	12.500	12.500	12.50
3 points improved	10	30.000	30.3052	-18.75	-9.375	18.750	25.000	43.750	75.000	87.50
2 points improved	38	16.447	20.0517	-37.50	-6.250	6.250	12.500	31.250	37.500	81.25
1 point improved	118	8.316	16.1799	-25.00	-12.500	-6.250	6.250	18.750	31.250	50.00
No Change	154	3.612	16.9519	-31.25	-12.500	-6.250	0.000	12.500	25.000	62.50
1 point worsened	64	-3.125	20.3211	-62.50	-31.250	-12.500	-3.125	6.250	18.750	43.75
2 points worsened	13	-9.615	22.3338	-43.75	-37.500	-31.250	-6.250	0.000	18.750	31.25
3 points worsened	2	-65.625	13.2583	-75.00	-75.000	-75.000	-65.625	-56.250	-56.250	-56.25
4 points worsened	0									

Source: Table 69.309007 of the Applicant's response to information request dated August 5, 2022.
Abbreviations: CFB, change from baseline; CKD-AQ, Chronic Kidney Disease Anemia Questionnaire; SD, standard deviation

Table 230. Distribution of Change From Baseline at Week 28 for On-Treatment SF-36 Vitality Domain Score by CKD-AQ Item 2 (Low or No Energy): Study ASCEND-NHQ

SF-36 Vitality CFB at Week 28

CKD-AQ Item 2 CFB at Week 28	N	Mean	SD	Min.	P10	P25	Median	P75	P90	Max.
4 points improved	1	12.500		12.50	12.500	12.500	12.500	12.500	12.500	12.50
3 points improved	7	35.714	29.0282	-6.25	-6.250	12.500	37.500	43.750	87.500	87.50
2 points improved	41	17.530	21.5702	-25.00	-12.500	0.000	18.750	31.250	43.750	81.25
1 point improved	109	9.518	19.3686	-37.50	-12.500	-6.250	6.250	18.750	37.500	62.50
No Change	157	2.986	14.9046	-43.75	-12.500	-6.250	0.000	12.500	25.000	56.25
1 point worsened	60	-1.042	14.3340	-31.25	-18.750	-6.250	0.000	6.250	15.625	31.25
2 points worsened	20	-6.875	22.2962	-43.75	-40.625	-28.125	-6.250	12.500	18.750	25.00
3 points worsened	5	-53.750	16.8866	-75.00	-75.000	-62.500	-56.250	-43.750	-31.250	-31.25
4 points worsened	0									

Source: Table 69.309008 of the Applicant's response to information request dated August 5, 2022.
Abbreviations: CFB, change from baseline; CKD-AQ, Chronic Kidney Disease Anemia Questionnaire; SD, standard deviation

Table 231. Distribution of Change From Baseline at Week 28 for On-Treatment SF-36 Vitality Domain Scores by Baseline CKD-AQ Item 1 (Very Tired) for Patients Who Had a 2-Category Improvement From Baseline in CKD-AQ Item 1: Study ASCEND-NHQ

SF-36 Vitality CFB at Week 28

CKD-AQ Item 1 at Baseline	N	Mean	SD	Min	P10	P25	Median	P75	P90	Max
Overall	38	16.447	20.0517	-37.50	-6.250	6.250	12.500	31.250	37.500	81.25
Some of the time	11	15.341	14.0767	-6.25	-6.250	6.250	12.500	25.000	31.250	37.50
Most of the time	25	14.500	18.8987	-37.50	-6.250	6.250	12.500	31.250	37.500	43.75
All of the time	2	46.875	48.6136	12.50	12.500	12.500	46.875	81.250	81.250	81.25

Source: Table 69.309023 of the Applicant's response to information request dated August 12, 2022.
Abbreviations: CFB, change from baseline; CKD-AQ, Chronic Kidney Disease Anemia Questionnaire; SD, standard deviation

Table 232. Distribution of Change From Baseline at Week 28 for On-Treatment SF-36 Vitality Domain Scores by Baseline CKD-AQ Item 2 (Low or No Energy) for Patients Who had a 2-Category Improvement From Baseline in CKD-AQ Item 2: Study ASCEND-NHQ

SF-36 Vitality CFB at Week 28

CKD-AQ Item 2 at Baseline	N	Mean	SD	Min	P10	P25	Median	P75	P90	Max
Overall	41	17.530	21.5702	-25.00	-12.50	0.000	18.750	31.250	43.750	81.25
Some of the time	8	11.719	16.1734	-25.00	-25.00	9.375	15.625	21.875	25.000	25.00
Most of the time	31	17.137	20.3465	-18.75	-12.50	0.000	18.750	37.500	43.750	56.25
All of the time	2	46.875	48.6136	12.50	12.50	12.500	46.875	81.250	81.250	81.25

Source: Table 69.309024 of the Applicant's response to information request dated August 12, 2022.
Abbreviations: CFB, change from baseline; CKD-AQ, Chronic Kidney Disease Anemia Questionnaire; SD, standard deviation

16.2.1.5. Conclusion

(b) (4)

FDA determined that the results of ASCEND-NHQ did not substantiate a clinically meaningful improvement of daprodustat against placebo due to the minimal magnitude of changes observed at the item- and domain-level (using raw and transformed score scales), modest to small between arm differences in response rates.

16.3. Patient-Reported Outcome Instruments

16.3.1. Patient-Reported Outcome Instruments Used for Secondary Endpoints in ASCEND-NHQ

Figure 64. SF-36 v2.0 Vitality Domain

Item wording	Response options
9) These questions are about how you feel and how things have been with you <u>during the past week</u> . For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the <u>past week</u> ...	
a) Did you feel full of life?	• All of the time • Most of the time • Some of the time • A little of the time • None of the time
e) Did you have a lot of energy?	• All of the time • Most of the time • Some of the time • A little of the time • None of the time
g) Did you feel worn out?	• All of the time • Most of the time • Some of the time • A little of the time • None of the time
i) Did you feel tired?	• All of the time • Most of the time • Some of the time • A little of the time • None of the time

Source: Section 3.1.2 of the Applicant's SF-36 PRO Dossier.

16.3.2. Anchor Scales Used in ASCEND-NHQ for Evaluation of Meaningful Within-Patient Score Change

Figure 65. PGI-S

PATIENT GLOBAL IMPRESSION OF SEVERITY VERSION 1
CHRONIC KIDNEY DISEASE

Overall, how would you rate the severity of your chronic kidney disease symptoms during the past 24 hours?

- Absent
- Mild
- Moderate
- Severe
- Very Severe

Source: Appendix B of Appendix 25 of the Applicant's SF-36 PRO Dossier.
Abbreviations: PGI-S, Patient Global Impression of Severity

Figure 66. PGI-C

PATIENT GLOBAL IMPRESSION OF CHANGE VERSION 1
CHRONIC KIDNEY DISEASE

Overall, how would you rate the change in severity of your chronic kidney disease symptoms since you first started this study?

- Very much improved
- Moderately improved
- Minimally improved
- No change
- Minimally worse
- Moderately worse
- Very much worse

Source: Applicant's response to FDA Information Request dated June 16, 2020 in IND 101291 [DARRTS Reference ID: 4625669; SDN 338, eCTD 0333, June 19, 2020].
Abbreviations: PGI-C, Patient Global Impression of Change

Figure 67. Chronic Kidney Disease and Anemia Questionnaire Items 1 and 2

Item wording	Response options
1. In the <u>past 7 days</u> , how much of the time were you <u>very tired</u> ?	a) None of the time b) A little bit of the time c) Some of the time d) Most of the time e) All of the time
2. In the <u>past 7 days</u> , how much of the time were you <u>low or no energy</u> ?	a) None of the time b) A little bit of the time c) Some of the time d) Most of the time e) All of the time

Source: CKD-AQ Items 1 and 2 (taken from the full CKD-AQ instrument), Appendix A of Appendix 25 of the Applicant's SF-36 PRO Dossier.

16.4. Anemia Rescue Algorithm

Table 233. Rescue Algorithm for Anemia Management

Evaluate participant for rescue if:
HemoCue Hb remains <9 g/dL (at a scheduled study visit, Week 4 onwards) despite three^a consecutive dose increases above the starting^b or post-rescue^c dose (where HemoCue Hb is <9 g/dL prior to each dose increase) OR HemoCue Hb is <7.5 g/dL despite a dose increase at the prior study visit.

Step 1: While continuing randomized treatment (increase dose if HemoCue Hb <7.5 g/dL;
Initial otherwise maintain current dose), intervene with one or more of the following as dictated by
Intervention clinical comorbidities

- Single course of IV iron up to 1000 mg (in addition to the iron management criteria)
- Transfusion of up to two units of PRBC if clinically indicated
- Allow additional 4 weeks on randomized treatment (NOTE: this is a required choice; can be combined with either or both of the above).

Step 2: Check HemoCue Hb 4 weeks $\pm$ 1 week from last study visit; earlier checks of Hb may be
Rescue obtained to advise further intervention as clinically indicated.

Randomized treatment should be permanently discontinued, and the participant should be rescued according to local clinical practice if either,

- If HemoCue Hb remains <9 g/dL despite initial intervention based on the average of two HemoCue Hb values^d

OR

- More than two units of PRBC were needed for transfusion (and was not related to acute bleeding).

Source: Adapted from Table 10 of the ASCEND-ND CSR and Table 10 of the ASCEND-D CSR.

^a Two consecutive dose increases if starting/post-rescue dose is daprodustat 12 mg or darbepoetin alfa 200 μ g over 4 weeks or epoetin alfa 42,000 U per week; one dose increase if starting/post-rescue dose is daprodustat 16 mg or darbepoetin alfa 300 μ g over 4 weeks or epoetin alfa 48,000 U per week; and no prior dose increase if starting/post-rescue dose is daprodustat 24 mg or darbepoetin alfa 400 μ g over 4 weeks or epoetin alfa 60,000 U per week (top dose)

^b For participants who switched from HD to peritoneal dialysis who were randomized to rhEPO, the baseline dose for the purposes of the rescue algorithm was the new darbepoetin alfa dose.

^c For participants who previously were evaluated for rescue and who are able to continue in the trial, "post-rescue" dose is the dose of randomized treatment that a participant is receiving at the study visit after initial intervention.

^d Repeat HemoCue Hb at the same study visit to confirm Hb (using the same sample); take the average of two values.

Abbreviations: CSR, clinical study report; Hb, hemoglobin; PRBC, packed red blood cells

17. Clinical Safety

17.1. Missing Data Sensitivity Analyses

The Applicant conducted pre-specified tipping point sensitivity analyses under a range of missing data assumptions, in addition to non-informative censoring, to determine the assumptions that would change the non-inferiority conclusions for MACE. Assumptions about missing outcomes in the daprodustat and control arms varied independently and included scenarios where subjects with missing data on daprodustat had worse outcomes than subjects with missing data on control. Patients who prematurely withdrew²² from the study without a known first MACE prior to study withdrawal were multiply imputed in bootstrap samples under

²² Withdrawal includes withdrawal of consent, lost to follow-up, and study site closure

a range of event rate scenarios. As requested by the FDA during the NDA review, the follow-up period for all subjects was from randomization to the date of study completion/withdrawal, excluding deaths after this time.

For each treatment arm, the assumed event rates ranged from all prematurely censored subjects had a MACE at the time of censoring to all prematurely censored subjects completed the study without experiencing the event. These event rates, including a censored-at-random scenario, were quantified using the log-hazard rate ratio comparing prematurely censored subjects to observed subjects (Jackson et al. 2014). For each pair (daprodustat, control) of log-hazard rate ratio, the imputed time to first occurrence of MACE in a bootstrap sample was then compared between treatment groups using the primary analysis Cox proportional hazards model.²³ Rubin's rules combined the result (hazard ratio) of the Cox proportional hazards model for 200 imputed datasets. HR estimates were reported with the assumed hazard rate ratio and number of events for the missing subjects in each treatment arm. Interpretation of sensitivity analysis results required an assessment of the plausibility of the assumptions regarding: (1) missing subjects' event rate relative to observed subjects' event rate in each treatment arm; and (2) missing subjects' event rate in the daprodustat arm relative to the control arm.

Using the same imputation method, the Applicant also explored the impact of missing secondary safety endpoint information for the adjudicated endpoints: all-cause mortality, CV mortality, MI, stroke, hospitalization for heart failure, and thromboembolic event. Unlike MACE, for which missing data assumptions that changed (tipped) the non-inferiority conclusion could be identified, secondary safety endpoints were tested but not controlled for multiple comparisons; therefore, the determination of which missing data assumptions were plausible and would result in a concerning hazard ratio required an abundance of judgement, because there was not a multiplicity controlled test as a point of reference. In that regard, the interpretation of sensitivity analyses for secondary safety endpoints were limited and not presented. Only MACE tipping point analyses results are shown.

ASCEND-ND

In the primary analysis of MACE, 61/1937 (3%) daprodustat and 61/1935 (3%) darbepoetin alfa subjects were censored. The time to event analysis results of MACE, with deaths excluded after study completion/withdrawal, was HR 1.05 (CI 0.93, 1.21)²⁴ with incidence rates of: daprodustat 367/1937 (19%); darbepoetin alfa 356/1935 (18%). Note that the HR estimate is slightly different from the pre-specified primary analysis result of MACE that included deaths after study completion/withdrawal (see discussion in Section 7.4). Sensitivity analyses (Figure 68) to the non-informative censoring assumption in the MACE primary analysis showed that the results (HR 1.09; 95% CI 0.4, 1.26) tipped, i.e., did not rule out the 1.25 risk margin, when daprodustat-treated, censored subjects were assumed to have 19/61 (31%) MACE and a (worse) hazard rate of 1.7 times the daprodustat-treated, observed subjects, and darbepoetin alfa-treated, censored

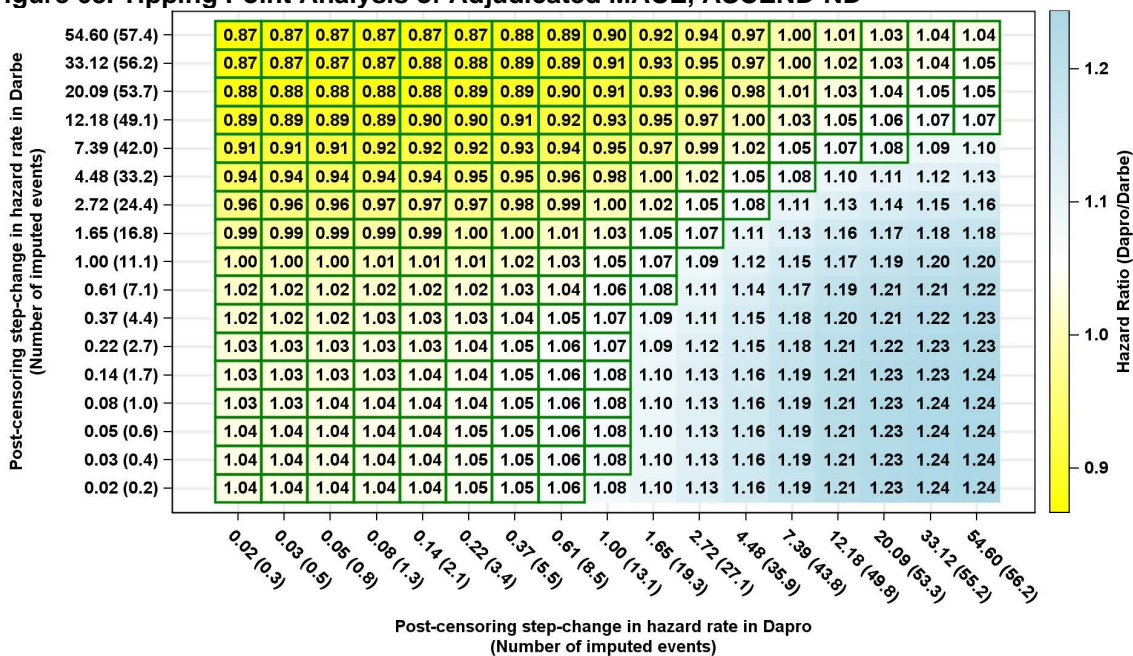
²³ Primary analysis of time to first MACE was a Cox proportional hazards model controlling for treatment and adjusting for the following baseline variables used in stratified randomization: region (Asia Pacific, Eastern Europe/South Africa, Western Europe/Canada/ANZ, Latin America, USA), current ESA use (ASCEND-ND only: User, Non-user), and dialysis type (ASCEND-D only: HD, peritoneal dialysis).

²⁴ Sponsor response (dated June 10, 2022) to information request

subjects were assumed to have 4/61 (7%) MACE and a (better) hazard rate of 0.4 times the darbepoetin alfa-treated, observed subjects. A less extreme assumption (daprodustat 1.7, darbepoetin alfa 0.6) resulted in HR 1.08 (95% CI 0.93, 1.25), which did not tip the MACE results away from non-inferiority (Figure 69, estimate 1.08 boxed in green). Note that assuming a hazard rate ratio of 1.0 in each arm is equivalent to the non-informative censoring assumption.

Comment: The MACE rate assumptions in the daprodustat and darbepoetin alfa arms (1.7 vs 0.4 times the event rate of observed subjects) that censored darbepoetin alfa-treated subjects have a lower MACE rate than the observed darbepoetin alfa-treated subjects could be considered implausible for a tipping point in an active-controlled study. A limitation of these analyses was that the Applicant did not consider patients that failed to have a CV endpoint assessment during or after the EOS window to be missing outcome data. Although including these subjects could increase the number of subjects missing to be approximately 5%²⁵ in each treatment arm, missing data were low and similar across arms. The primary analysis result for MACE, assuming non-informative censoring, was tipped only under fairly extreme assumptions of daprodustat having more MACE while the active control having less MACE than the observed subjects. Therefore, the primary analysis assuming non-informative censoring could be relied upon for interpretation.

Figure 68. Tipping Point Analysis of Adjudicated MACE, ASCEND-ND



Note: Non-inferiority concluded at the area with the green borders.
Source: Sponsor Response (dated June 24, 2022) to Information Request, Figure 68.222024
Abbreviations: Dapro, daprodustat; Darbe, darbepoetin alfa; MACE, major adverse cardiovascular event

ASCEND-D

In the primary analysis of MACE, 107/1487 (7%) daprodustat and 105/1477 (7%) ESA subjects were censored. The time to event analysis results of MACE with deaths excluded after study

²⁵ Sponsor response (dated June 20, 2022) to information request

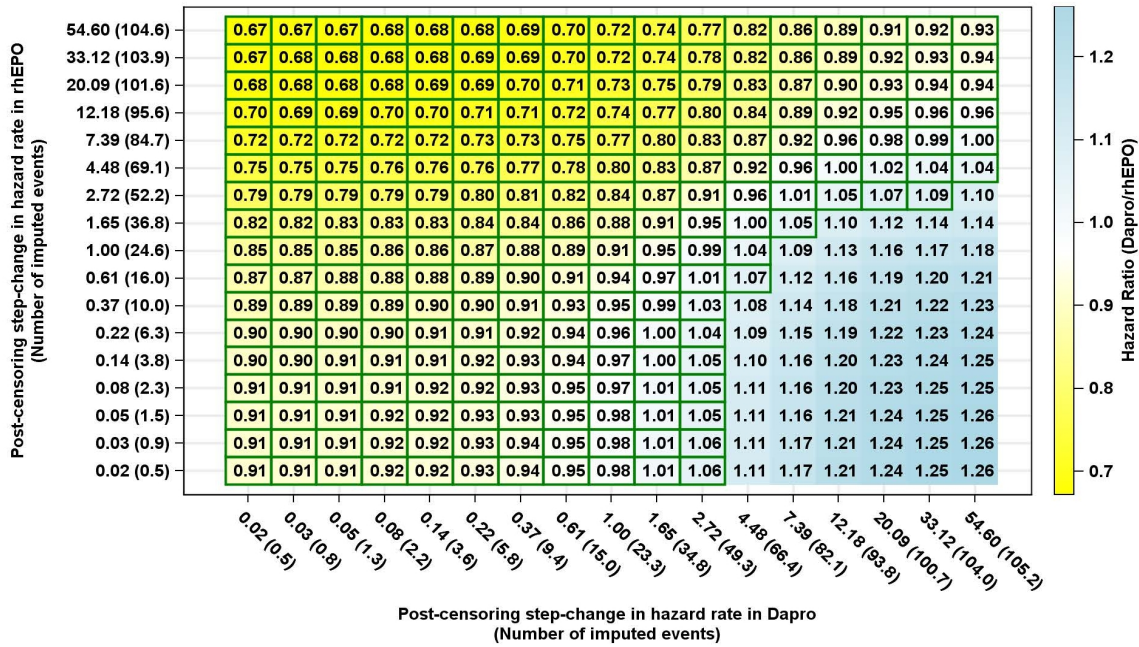
completion/withdrawal was: HR 0.92 (CI 0.79, 1.06)²⁶ with incidence rates of: daprodustat 360/1487 (24%); ESA 387/1477 (26%). Note that the HR estimate is slightly different from the pre-specified primary analysis result of MACE including deaths after study completion/withdrawal (see discussion in Section [7.6.1.1](#)). Sensitivity analyses to the non-informative censoring assumption in the MACE primary analysis showed that the results (HR 1.08; 95% CI 0.94, 1.25) tipped, i.e., did not rule out the 1.25 risk margin, when censored subjects in the daprodustat arm were assumed to have 66/107 (62%) MACE and a (worse) hazard rate of 4.5 times the observed subjects in the daprodustat arm and censored subjects in the ESA arm were assumed to have 10/105 (10%) and a (better) hazard rate of 0.4 times the observed subjects in the ESA arm.

Comment: The MACE rate assumptions in the daprodustat and ESA arm (4.5 versus 0.4 times the event rate of observed subjects) could be considered implausible for a tipping point, because censored daprodustat-treated subjects would need to have a high event rate of 4.5 times higher than the observed daprodustat-treated subjects. A limitation of these analyses was that the Applicant did not consider patients who failed to have a CV endpoint assessment during or after the EOS window to be missing outcome data. Including these subjects could increase the number of subjects missing to be approximately 11%²⁷ in each treatment arm, however, the rates of missing subjects were similar across the arms. In summary, the primary analysis result for MACE, assuming non-informative censoring, was tipped only under extreme assumptions of daprodustat having more MACE while the active control having less MACE than the observed subjects. Therefore, the primary analysis assuming non-informative censoring could be relied upon for interpretation.

²⁶ Sponsor response (dated June 10, 2022) to information request

²⁷ Sponsor response (dated June 20, 2022) to information request

Figure 69. Tipping Point Analysis of Adjudicated MACE, ASCEND-D



Note: Non-inferiority concluded at the area with the green borders.

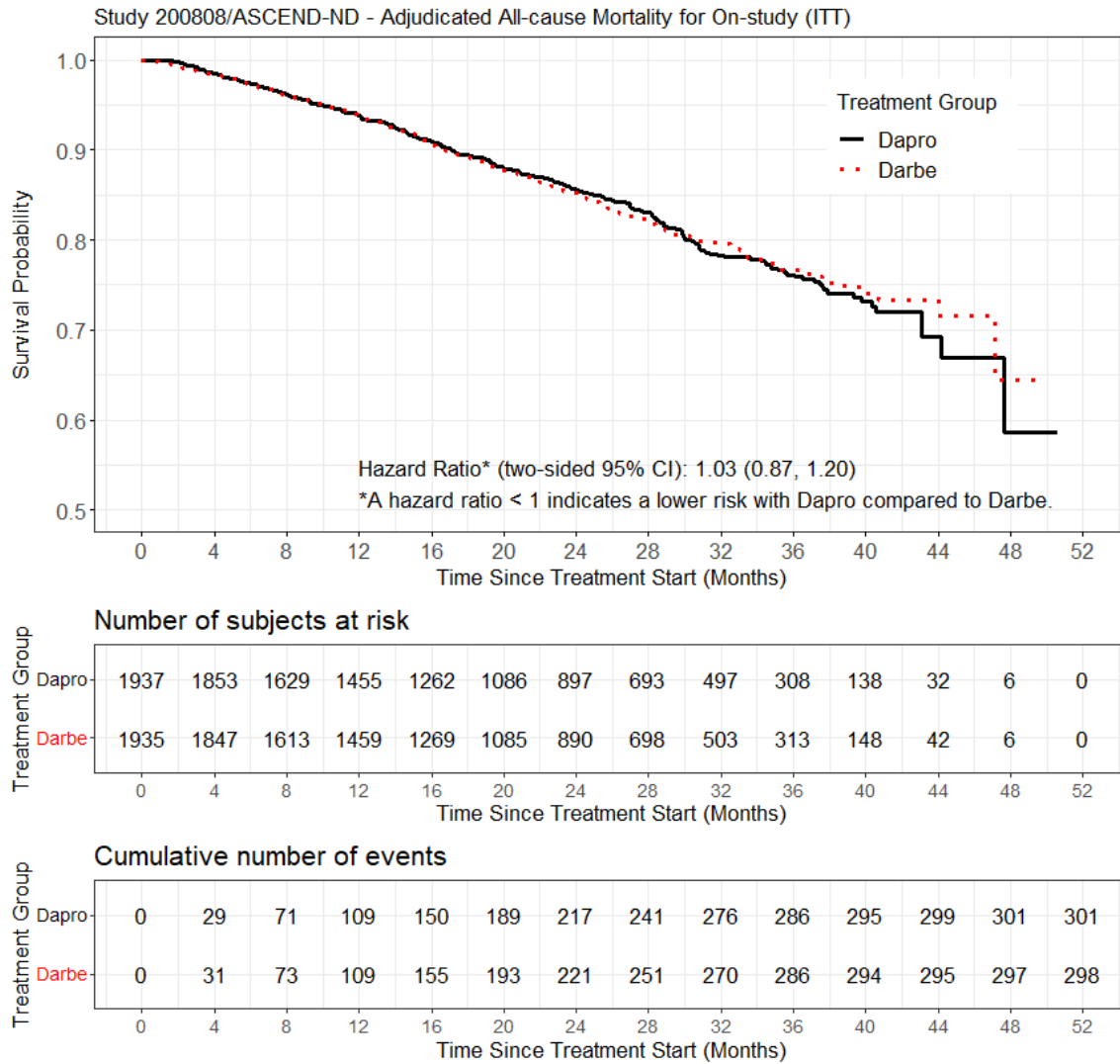
Source: Applicant Response (dated June 30, 2022) to Information Request, Figure 68.222024

Abbreviations: Dapro, daprodustat; MACE, major adverse cardiovascular event; rhEPO, recombinant human erythropoietin

17.2. Time to All-Cause Mortality K-M Figures

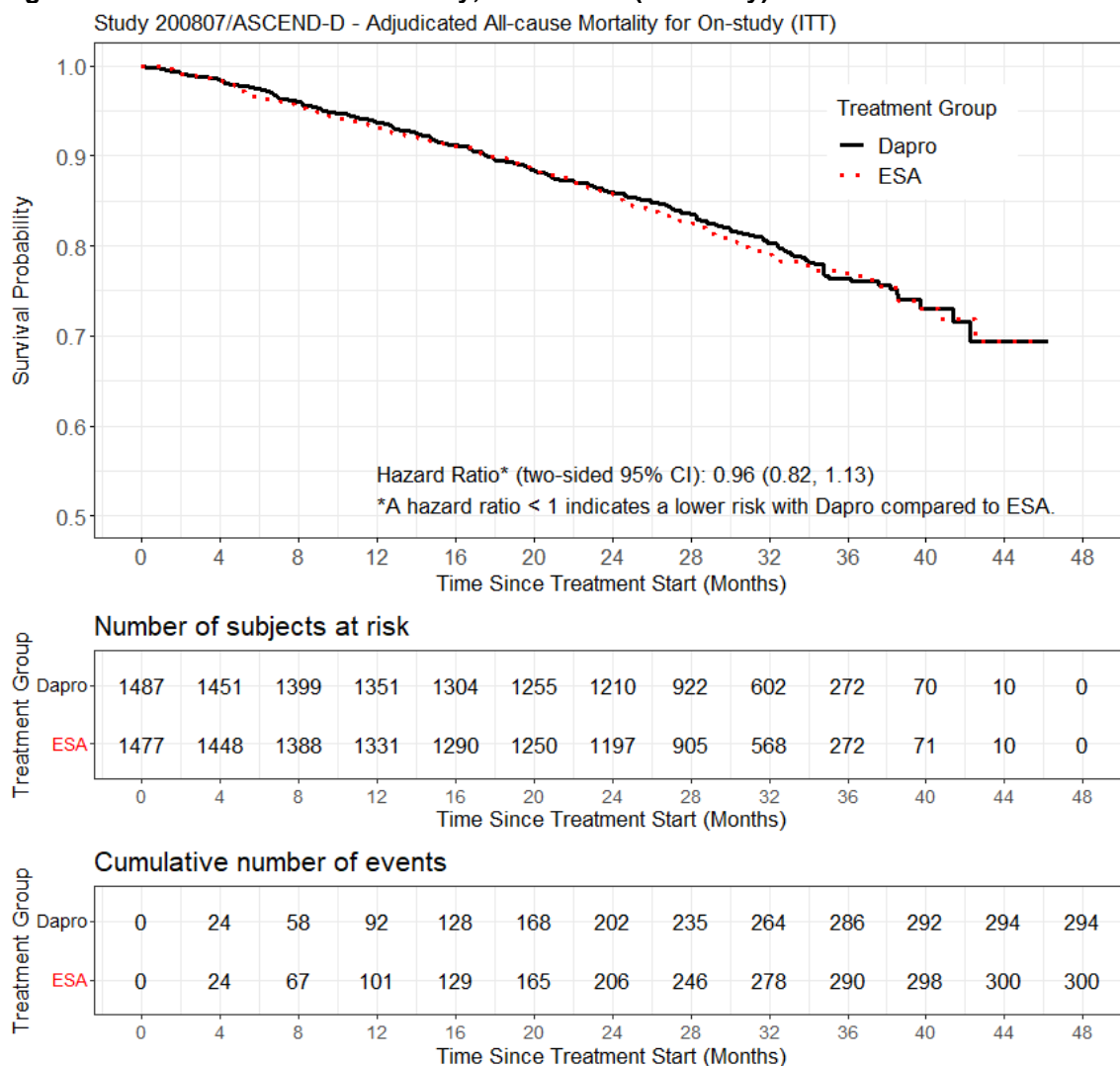
Kaplan-Meier figures for time to all-cause mortality (on-study analysis) are presented in [Figure 70](#) and [Figure 71](#) for the ASCEND-ND and ASCEND-D trials.

Figure 70. Time to All-Cause Mortality, ASCEND-ND (On-Study)



Source: FDA analysis.
 Abbreviations: CI, confidence interval; Dapro, Daprodustat; Darbe, darbepoetin alfa; ITT, intention-to-treat

Figure 71. Time to All-Cause Mortality, ASCEND-D (On-Study)



Source: FDA analysis.
Abbreviations: CI, confidence interval; Dapro, Daprodustat; ESA, erythropoiesis-stimulating agent; ITT, intention-to-treat

17.3. CV Events in Relation to Average Dose Summaries Exploratory Analyses

We explored the relationship between dose level and each adjudicated CV endpoint in studies ASCEND-D and ASCEND-ND. The endpoints included MACE, all-cause mortality, CV mortality, myocardial infarction, stroke, hospitalization for heart failure, and thromboembolic events. For subjects who experience more than one adverse event with the same preferred term or more than one adverse event within a grouping of preferred terms, the first adverse event was used for analysis, and exposure time used in the calculation of total patient-years was censored at the date of the first adverse event. We focused on the dosing frequency adjusted on-treatment CV period, which is the endpoint ascertainment window defined as the period starting from the

treatment start date to the earlier of the date of study completion/withdrawal or last non-zero dose date + dosing frequency²⁸ + 28 days (OT + Dosing Frequency + 28).

Dose was analyzed by:

- Average dose while receiving treatment
- Average dose received during the 4 weeks preceding the event
- Average dose received during the 2 weeks preceding the event
- Average dose at the time of the event

In “average dose while receiving treatment” analysis, overall average weekly study drug dose was calculated for each subject until the first occurrence of the event for subjects with the event or until the end of the ascertainment window for subjects without the event. Each of these overall averages were assigned to one of five fixed dose categories.²⁹ The number of events were tabulated by for each dose category in each treatment group and expressed as a percent of subjects with events.

Because these drugs were titrated to effect throughout the treatment period, alternative analyses were conducted using a moving average of the dose. In “average dose received during the 4 weeks preceding the event” analysis, average weekly dose during the 4 weeks prior to the event of interest was calculated for every subject until the first occurrence of the event of interest for subjects with the event or until the end of the ascertainment window for subjects without the event. For example, the dose at Week 16 was the average dose during Weeks 13 through 16. The average weekly dose was assigned to one of five fixed dose categories. A similar analysis was conducted using two-week intervals preceding the event, which was referred to as “average dose received during the 2 weeks preceding the event.” In “average dose at the time of the event” analysis, the weekly dose at the time of the event was defined as the dose on the day the event occurred, converted to a weekly dose, and assigned to a dose category. In these three analyses, the count of subjects with an event, total person-years (PY), rate per PY and rate per 100 PY were calculated for each dose category and each treatment group.

Summary tables and figures were created for each endpoint by treatment arm (daprodustat, control) for each study (ASCEND-ND, ASCEND-D). Results are not shown.

Summary: Higher percentages or incidence rates observed at higher dose categories for some CV endpoints including MACE in both treatment arms. Limitations with the interpretability of these analyses included: (1) potential bias in treatment (daprodustat vs. control) effect comparison using an on-treatment analysis that has the potential to break the integrity of randomization and introduce bias as there may differences between treatment arms in the types of subjects that adhere to treatment; (2) potential confounding by disease severity in the relationship between CV

²⁸ 1 day (daprodustat); 7, 14, or 28 days (darbepoetin alfa); 2 or 7 days (epoetin alfa).

²⁹ Sponsor determined the dose categories based on the possible doses in each treatment arm of each study. Dose categories differed by arm and can differ by the dose categorization (while receiving treatment, 4 weeks preceding the event, 2 weeks preceding the event, at the time of event). Categories were chosen to “balance the representation of a range of doses across the categories, while having sufficient amount of observation time in each dose category to permit meaningful examination of the data.”

event by dose level; and (3) data sparsity for most events except for MACE and all-cause mortality.

17.4. CV Events in Relation to Average Hemoglobin Summaries

We explored the relationship between hemoglobin (Hb) level and each adjudicated CV endpoint in studies ASCEND-D and ASCEND-ND. The endpoints included MACE, all-cause mortality, CV mortality, myocardial infarction, stroke, hospitalization for heart failure, and thromboembolic events. For subjects who experience more than one adverse event with the same preferred term or more than one adverse event within a grouping of preferred terms, the first adverse event was used for analysis, and exposure time used in the calculation of total patient-years was censored at the date of the first adverse event. We focused on the dosing frequency adjusted on-treatment CV period, which is the endpoint ascertainment window defined as the period starting from the treatment start date to the earlier of the date of study completion/withdrawal or last non-zero dose date + dosing frequency³⁰ + 28 days (OT + Dosing Frequency + 28).

Hb was analyzed by:

- Hb just prior to the event
- Hb rate of change over the 4 weeks preceding the event
 - Separate analysis for rates of increases and decreases
- Hb rate of change over the 12 weeks preceding the event
 - Separate analysis for rates of increases and decreases

For each treatment arm, Hb just prior to the event of interest was defined as the last on-treatment Hb just prior to the event. This was calculated for every subject until the first occurrence of the event for subjects with the event or until end of treatment of subjects without the event. The number of events were tabulated for each Hb quintile, determined from all Hb values, indicating how many subjects had an event in that quintile for their given Hb associated with the event. Total PY, rate per PY, and rate per 100 PY were calculated for each quintile.

Another Hb analysis explored the Hb rate of change (g/dL/week) over the 4 weeks preceding the event. Using the subject's treatment start date as the anchor date, a 4-week interval was created up to either the event of interest or the end of the ascertainment window if they did not have an event. Then a Hb slope was calculated by fitting a linear regression line through the Hb values obtained during the 4 weeks. For example, the slope at Week 8 was calculated from Hb values obtained on Weeks 5, 6, 7, 8. The slopes were categorized as either positive (including zero) or negative slopes. Quintiles were created based on the positive slopes and separately, for the negative slopes. The number of events, total PY, rate per PY, and rate per 100 PY were calculated for each Hb quintile. The analyses were repeated for rate of change over the 12 weeks

³⁰ 1 day (daprodustat); 7, 14, or 28 days (darbepoetin alfa); 2 or 7 days (epoetin alfa).

preceding the event. Of note, Hb slopes could be only estimated for a limited number of events, because Hb assessments were scheduled less frequently later in the trials (at least every 4 weeks during the first year of the trial and at least every 12 weeks thereafter), and two values were necessary to calculate a slope.

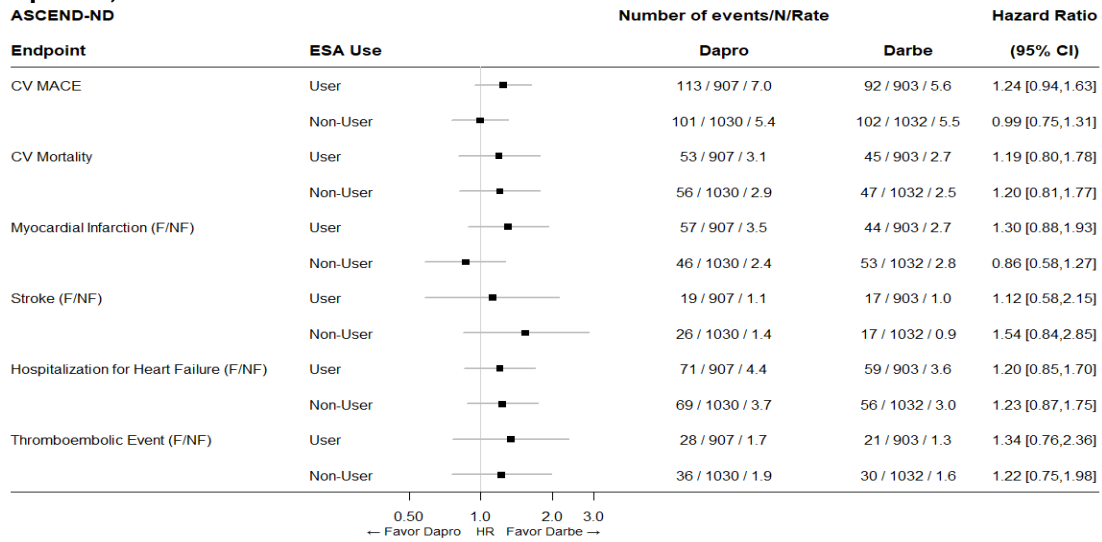
Summary tables and figures were created for each endpoint by treatment arm (daprodustat, control) for each study (ASCEND-ND, ASCEND-D). Results are not shown.

Summary: Higher incidence rates observed at lower Hb quintiles for some CV endpoints including MACE in both treatment arms. Limitations with the interpretability of these analyses included: (1) potential bias in treatment (daprodustat vs. control) effect comparison using an on-treatment analysis that has the potential to break the integrity of randomization and introduce bias as there may be differences between treatment arms in the types of subjects that adhere to treatment; (2) potential confounding by disease severity in the relationship between CV event by Hb level; and (3) data sparsity for most events except for MACE and all-cause mortality.

17.5. Subgroup Analyses by ESA Use – ASCEND-ND

To further explore CV endpoints among baseline ESA users versus non-users in the NDD population, exploratory subgroup analyses were conducted for each adjudicated CV endpoint. For all these CV endpoints, a higher incidence was observed with daprodustat compared to darbepoetin alfa among ESA users and non-users at randomization, except CV MACE and MI among ESA non-users ([Figure 72](#)). The variability (CI widths) of HR estimates depended on the number of events and sample sizes, with lower precision for endpoints like stroke and thromboembolic events. In summary, analyses of ESA use subgroups did not indicate a consistently higher risk among ESA users compared to non-users, but the elevated HR estimates in both subgroups across multiple CV endpoints corroborated the unfavorable results in the overall study population.

Figure 72. ESA Use at Randomization (User, Non-User) Subgroup Analyses for All Adjudicated CV Endpoints, ASCEND-ND



Source: FDA analysis.

CV MACE: composite of CV mortality, nonfatal MI, and nonfatal stroke.

Rate: Incidence rate per 100 person-years.

Abbreviations: CI, confidence interval; CV, cardiovascular; Dapro, daprodustat; Darbe, darbepoetin alfa; ESA, erythropoietin-stimulating agent; F/NF, fatal or nonfatal; MACE, major cardiovascular adverse event

17.6. Cardiovascular Endpoint Analyses

Table 234. Summary of Results for Adjudicated Cardiovascular Safety Endpoints, ASCEND-ND and ASCEND-D

	200807/ASCEND-D				200808/ASCEND-ND			
	Daprodustat N=1487	rhEPO N=1477	Daprodustat vs rhEPO		Daprodustat N=1937	Darbepoetin alfa N=1935	Daprodustat vs. Darbepoetin alfa	
	n (%) n/100 PY	n (%) n/100 PY	Rate Diff Per 100 PY (95% CI)	HR (95% CI)	n (%) n/100 PY	n (%) n/100 PY	Rate Diff Per 100 PY (95% CI)	HR (95% CI)
MACE	374 (25.2) 11.07	394 (26.7) 11.86	-0.78 (-2.41, 0.84)	0.93 (0.81, 1.07)	378 (19.5) 10.86	371 (19.2) 10.63	0.23 (-1.31, 1.77)	1.03 (0.89, 1.19)
MACE or thromboembolic events	497 (33.4) 15.84	543 (36.8) 17.85	-2.01 (-4.06, 0.04)	0.88 (0.78, 1.00)	422 (21.8) 12.34	405 (20.9) 11.77	0.57 (-1.08, 2.21)	1.06 (0.93, 1.22)
MACE or hospitalization for heart failure (HF)	425 (28.6) 12.98	433 (29.3) 13.38	-0.40 (-2.16, 1.36)	0.97 (0.85, 1.11)	444 (22.9) 13.16	417 (21.6) 12.22	0.94 (-0.76, 2.63)	1.09 (0.95, 1.24)
All-cause mortality	294 (19.8) 8.32	300 (20.3) 8.59	-0.28 (-1.63, 1.08)	0.96 (0.82, 1.13)	301 (15.5) 8.35	298 (15.4) 8.27	0.08 (-1.25, 1.41)	1.03 (0.87, 1.20)

Source: FDA analysis

CV MACE: Composite of CV mortality, nonfatal MI, and nonfatal stroke.

Abbreviations: CI, confidence interval; CV, cardiovascular; Diff, difference; HR, hazard ratio; MACE, major adverse cardiovascular event; MI, myocardial infarction; N, number of subjects; n, number of subjects with event; PY, person-year; rhEPO, recombinant human erythropoietin

Table 235. Summary of Results for Adjudicated Cardiovascular Safety Endpoints, ASCEND-D

CV Safety Endpoints	Daprodustat	ESA	Rate Diff	Hazard Ratio
	N=1487 n [IR]	N=1477 n [IR]	Per 100 PY (95% CI)	(95% CI)
CV MACE	226 [6.7]	257 [7.7]	-1.0 (-2.3, 0.2)	0.86 (0.72, 1.03)
CV mortality	117 [3.3]	121 [3.5]	-0.2 (-1.0, 0.7)	0.95 (0.74, 1.23)
Fatal or nonfatal myocardial infarction	114 [3.3]	137 [4.1]	-0.7 (-1.7, 0.2)	0.81 (0.63, 1.04)
Fatal or nonfatal stroke	43 [1.2]	51 [1.5]	-0.2 (-0.8, 0.3)	0.84 (0.56, 1.25)
Hospitalization for heart failure	112 [3.3]	101 [3.0]	0.3 (-0.6, 1.1)	1.10 (0.84, 1.45)
Thromboembolic event	185 [5.7]	215 [6.75]	-1.1 (-2.3, 0.1)	0.84 (0.69, 1.02)
Vascular access thrombosis	164 [5.0]	201 [6.3]	-1.3 (-2.4, -0.1)	0.80 (0.65, 0.98)

Source: FDA analysis.

CV MACE: Composite of CV mortality, nonfatal MI, and nonfatal stroke.

Abbreviations: CV, cardiovascular; CI, confidence interval; ESA, erythropoietin-stimulating agents; IR, incidence rate per 100 PY; MACE, major adverse cardiovascular event; N, number of subjects; n, number of subjects with event; PY, person-year

17.7. On-Study and On-Treatment Analyses of CV Endpoints

Table 236. On-Study and On-Treatment Analyses of CV Endpoints, ASCEND-ND, ASCEND-D

CV Endpoints	200807/ASCEND-D			200808/ASCEND-ND		
	Daprodustat N=1487 n [IR]	ESA N=1477 n [IR]	Daprodustat vs ESA HR (95% CI)	Daprodustat N=1937 n [IR]	Darbedustat N=1935 n [IR]	Daprodustat vs Darbepoetin alfa HR (95% CI)
CV MACE						
On study	226 [6.7]	257 [7.7]	0.86 (0.72, 1.03)	214 [6.1]	194 [5.6]	1.11 (0.91, 1.34)
OT+28	175 [6.8]	207 [7.9]	0.86 (0.70, 1.05)	170 [6.2]	134 [4.8]	1.30 (1.04, 1.63)
CV mortality						
On study	117 [3.3]	121 [3.5]	0.95 (0.74, 1.23)	109 [3.0]	92 [2.6]	1.20 (0.91, 1.58)
OT+28	80 [3.0]	86 [3.2]	0.95 (0.70, 1.29)	66 [2.3]	51 [1.8]	1.34 (0.93, 1.93)
Fatal or non-fatal MI						
On study	114 [3.3]	137 [4.1]	0.81 (0.63, 1.04)	103 [2.9]	97 [2.8]	1.06 (0.80, 1.40)
OT+28	90 [3.5]	112 [4.3]	0.83 (0.63, 1.09)	96 [3.5]	75 [2.7]	1.30 (0.96, 1.76)
Fatal or non-fatal stroke						
On study	43 [1.2]	51 [1.5]	0.84 (0.56, 1.25)	45 [1.3]	34 [1.0]	1.33 (0.85, 2.07)
OT+28	33 [1.2]	42 [1.6]	0.80 (0.51, 1.26)	35 [1.2]	22 [0.8]	1.64 (0.96, 2.80)
Hospitalization for heart failure						
On study	112 [3.3]	101 [3.0]	1.10 (0.84, 1.45)	140 [4.0]	115 [3.3]	1.22 (0.95, 1.56)
OT+28	89 [3.4]	90 [3.4]	1.03 (0.77, 1.38)	118 [4.3]	99 [3.5]	1.20 (0.92, 1.57)

CV Endpoints	200807/ASCEND-D			200808/ASCEND-ND		
	Daprodustat N=1487 n [IR]	ESA N=1477 n [IR]	Daprodustat vs ESA HR (95% CI)	Daprodustat N=1937 n [IR]	Darbedustat N=1935 n [IR]	Daprodustat vs Darbepoetin alfa HR (95% CI)
Thromboembolic event						
On study	185 [5.7]	215 [6.8]	0.84 (0.69, 1.02)	64 [1.8]	51 [1.4]	1.27 (0.88, 1.84)
OT+28	158 [6.3]	185 [7.4]	0.86 (0.70, 1.07)	54 [1.9]	38 [1.3]	1.50 (0.99, 2.27)
Vascular access thrombosis						
On study	164 [5.0]	201 [6.3]	0.80 (0.65, 0.98)	46 [1.3]	31 [0.9]	1.49 (0.94, 2.35)
OT+28	145 [5.8]	176 [7.0]	0.84 (0.67, 1.04)	39 [1.4]	25 [0.9]	1.61 (0.97, 2.66)

Source: FDA analysis

CV MACE: composite of CV mortality, nonfatal MI, and nonfatal stroke

Abbreviations: CI, confidence interval; CV, cardiovascular; ESA erythropoietin-stimulating agent; IR, incidence rate per 100 PY; MI, myocardial infarction; N, number of subjects; n, number of subjects with event; OT, on-treatment; PY, person-year

18. Clinical Virology

Not applicable.

19. Clinical Microbiology

Not applicable.

20. Mechanism of Action/Drug Resistance

Not applicable.

21. Other Drug Development Considerations

Not applicable.

22. Data Integrity–Related Consults (Office of Scientific Investigations, Other Inspections)

Data quality was ensured by the Applicant through periodic monitoring with primary source verification of trial data at all trial sites. Throughout the application review period, data quality was evaluated by the clinical review team, who independently reviewed the provided datasets and noted no anomalies in enrollment characteristics, patterns of protocol violations reported, patterns of efficacy reporting or patterns of SAE reporting.

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Consults were obtained from the Office of Scientific Investigations and Office of Study Integrity and Surveillance and described below.

Office of Clinical Investigations

Four clinical investigator sites (Drs. Barton Cox Brezina, Anjay Rastogi, Chao Sun and Kendra Hendon) were inspected for Studies 200807/ASCEND-D and 200808/ASCEND-ND.

From the review by Anthony Orenca M.D., Ph.D., F.A.C.P., Min Lu, M.D., M.P.H. and Jenn Sellers, M.D., Ph.D. “Based on clinical inspections, the study data derived from the above clinical investigator sites appear reliable. The study data submitted to the Agency for assessment are acceptable in support of the proposed indication.”

Office of Study Integrity and Surveillance (OSIS)

From the review “The Office of Study Integrity and Surveillance (OSIS) arranged the inspection of study 213022 (NDA 216951) conducted at Altasciences Clinical Kansas, Inc. and PPD Development, L.P.

At Altasciences Clinical Kansas, Inc, a Form FDA 483 was not issued at the close-out of the inspection. However, there was verbal discussion with site’s management regarding the maintenance of blinding codes onsite. The discussion item did not impact the reliability of the data from the audited study. At PPD Development, L.P., no objectionable conditions were observed, and a Form FDA 483 was not issued at its inspection close-out.”

The review concluded that the data for study 213022, “ A Two-Part, Randomized, Double-Blind, Single-Dose, Crossover Study to Compare Daprodustat Formulations Produced by Two Methods of Manufacture for Bioequivalence and Dissolution in Healthy Adult Volunteers” from both clinical sites are reliable.

23. Labeling: Key Changes and Considerations

This Prescribing Information (PI) review includes a high-level summary of the rationale for major changes to the finalized PI as compared to the Applicant’s draft PI. The PI was reviewed to ensure that PI meets regulatory/statutory requirements, is consistent (if appropriate) with labeling guidance, conveys clinically meaningful and scientifically accurate information needed for the safe and effective use of the drug, and provides clear and concise information for the healthcare practitioner.

2 Pages of Draft Labeling have been Withheld in Full as B4 (CCI/TS) immediately following this page

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Integrated Review Template, version 3.0 (05/25/2022)

[To complete this review, detailed instructions are available in the [Integrated Review Template How-to Guide.](#)]

23.1. Approved Labeling Types

Upon approval of this application, the following labeling documents will be FDA-approved:

United States Prescribing Information
Medication Guide

24. Postmarketing Requirements and Commitments

Draft Daprodustat PMRs for Dialysis Dependent Population

Final language will be in the action letter.

PMR 1: Conduct a prospective observational study to characterize the long-term safety (up to 5 years follow up) of JESDUVROQ in adults with dialysis dependent chronic kidney disease treated with the approved dosing regimen of JESDUVROQ (b) (4). Specific safety outcomes of interest include: the risk of major adverse cardiovascular events (MACE) defined as all-cause mortality, nonfatal myocardial infarction and nonfatal stroke; thrombotic vascular events to include vascular access thrombosis; hospitalization for heart failure; serious gastrointestinal bleeds and eye disorders (proliferative retinopathy). The study population should include adults previously treated with erythropoiesis-stimulating agents (ESAs) and adults naïve to ESAs. The effect of baseline and maximum achieved hemoglobin on the specified safety outcomes should be evaluated. Submit an integrated safety dataset and patient level data including JESDUVROQ dosing, treatment-emergent serious adverse events (SAEs), adverse events leading to treatment discontinuation, and outcomes of SAEs with your final report.

Draft Protocol Submission:	08/2023
Final Protocol Submission:	02/2024
Interim Report Submission:	07/2028
Study Completion:	07/2031
Final Report Submission:	07/2032

PMR 2: Conduct an observational study (up to 5 years follow up) to assess the risk for malignancy (hematological and non-hematological) in dialysis dependent chronic kidney disease adults with anemia treated with JESDUVROQ versus an erythropoiesis-stimulating agent (ESA) comparator arm. The study should include an assessment of primary malignancies among adults with no cancer history (including assessment by type and location), and the impact of JESDUVROQ on progression-free survival, and overall survival in adults with prior cancers.

Draft Protocol Submission:	12/2024
Final Protocol Submission:	06/2025
Study Completion:	10/2031
Final Report Submission:	10/2032

PMR 3: Conduct a worldwide descriptive study to collect prospective and retrospective data on women exposed to JESDUVROQ during pregnancy to assess the risk of pregnancy and maternal complications, adverse effects on the developing fetus and neonate, and adverse effects on the infant. Assess infant outcomes through at least the first year of life. The minimum number of patients will be specified in the protocol.

Draft Protocol Submission:	08/2023
Final Protocol Submission:	02/2024
Interim Report Submission:	09/2027
Study Completion:	08/2029
Final Report Submission:	09/2030

PREA PMR: Conduct a trial to evaluate the pharmacokinetics and safety of daprodustat for the treatment of anemia associated with chronic kidney disease in children and adolescents aged 3 months to under 18 years requiring dialysis. (b) (4)

Study Completion:	08/2029
Final Report Submission:	02/2030

(b) (4)

We request that you submit all adverse events received or otherwise obtained from foreign or domestic sources of possible thrombotic vascular events (TVE), heart failure (HF), gastrointestinal erosion (GE), and hypertensive crisis as 15-day “Alert reports” (described under 21 CFR 314.80(c)(1)). Include a summary and analysis of these adverse events (i.e., TVE, HHF, GE and hypertensive crisis) that are serious in the narrative portion of your periodic adverse drug experience report (PADER) required under 21 CFR 314.80(c)(2), quarterly during the first 3 years post-approval and annually thereafter, through the 5 year following the initial U.S. approval date.

The summary analyses should include an assessment of all new information obtained during the reporting interval and cumulatively since initial U.S. approval related to these adverse events (i.e., TVE, HHF, GE and hypertensive crisis) with the aim of further characterizing these risks (e.g., indication, temporal association, action taken, outcome, de-challenge/re-challenge, confounders, underlying risk factors, use in unapproved populations such as non-dialysis-dependent patients, and assessment of causality).

We also request that, in the narrative portion of your annual PADERS, you submit annual reports of daprodustat utilization rates, including off-label use, amongst females of reproductive potential (i.e., females aged 15 to 50 years) calculated cumulatively from the initial U.S. approval date annually through the 5th year following the initial U.S. approval date

25. Financial Disclosure

Table 238. Covered Clinical Studies: [ASCEND-D (200807); ASCEND-ND (200808)]

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: (includes sub-I) ASCEND-D: 1904; ASCEND-ND: 2144		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 1		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c), and (f)):		
Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0		
Significant payments of other sorts: 1		
Proprietary interest in the product tested held by investigator: 0		
Significant equity interest held by investigator: 0		
Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): ASCEND-D: 28; ASCEND-ND: 13		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

Abbreviation: FDA, Food and Drug Administration

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Guidance for Industry

Guidance for Industry *Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims* (December 2009)

Guidance for Industry *E9(R1) Statistical Principles for Clinical Trials: Addendum: Estimands and Sensitivity Analysis in Clinical Trials* (May 2021)

27. Review Team

Table 239. Reviewers of Integrated Assessment

Role	Name(s)
Regulatory project manager	Caden Brennen
Nonclinical reviewer	Bo Lee
Nonclinical team leader	Natalie Simpson, Pedro DelValle
OCP reviewer(s)	Guansheng Liu, Snehal Samant, Yuzhuo Pan
OCP team leader(s)	Sudharshan Hariharan, Jiang Liu, Ying-Hong Wang
Clinical reviewer	Justin Penzenstadler, Patricia Oneal
Clinical team leader	Tanya Wroblewski
Biometrics reviewer	Sarabdeep Singh, Thanh Tran
Biometrics team leader	Yeh Fong Chen, Clara Kim
Cross-disciplinary team leader	Tanya Wroblewski
Division director (pharm/tox)	Calvin Elmore
Associate Division director (OCP)	Jayabharathi Vaidyanathan
Division director (OB)	Mark Levenson, Mat Soukup
Division director (clinical)	Ann Farrell
Office director (or designated signatory authority)	Hylton Joffe

Abbreviations: OCP, Office of Clinical Pharmacology; OB, Office of Biostatistics

Table 240. Additional Reviewers of Application

Office or Discipline	Name(s)
OPQ	Sharmista Chatterjee, Theodore Carver
OPMA	Manufacturing Process and Facilities: Liya Tang, Feiyan Jin, Haitao Li Biopharmaceutics: Debasis Ghosh, Haritha Mandula Drug Substance: Ben Zhang, Zhengfu Wang Drug Product: Akm Khairuzzaman, Mohan Sapru
OPDP	Melissa Khashei
OSI	Anthony Orenca, Min Lu, Jenn Sellers
OSE	DEPI: Kate Gelperin, Orestis Panagiotou, Fang Tian DPV: Danielle Molnar, Jamie Klucken DRM: Carla Darling, Jacqueline Sheppard DMEPA: Devin Kane
Other	ADL: Virginia Kwitkowski DPMH: Jane Liedtka, Denise Pica-Branco CDS: Megan Peach, Qunshu (Alena) Zhang DCOA: Yasmin Choudhry, Selena Daniels DMPP: Jessica Chung, Barbara Fuller OB DBIII PFSS: Xin Yuan, Lili Garrard OB AIS: Liping Sun, Hye Soo Cho

Abbreviations: OPQ, Office of Pharmaceutical Quality; OPDP, Office of Prescription Drug Promotion; OSI, Office of Scientific Investigations; OSE, Office of Surveillance and Epidemiology; DEPI, Division of Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DRISK, Division of Risk Management

27.1. Reviewer Signatures

Table 27-241 Signatures of Reviewers

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Regulatory Project Manager Discipline Primary Reviewer	Caden Brennen OCHEN DNH	Sections: 12, 27	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Caden Brennen		Digitally signed by Caden Brennen Date: 2/1/2023 8:11 AM EST GUID: 202321131133		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Discipline Tertiary Reviewer	Ann Farrell OCHEN DNH	Sections: Clinical	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Ann Farrell		Digitally signed by Ann Farrell Date: 2/1/2023 8:12 AM EST GUID: 202321131211		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Regulatory Project Manager Discipline DPMS	Julie Van Der Waag ORO DROCHEN	Sections: 12, 27	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.	
Signature: Julie Van Der Waag		Digitally signed by Julie Van Der Waag		
		Date: 2/1/2023 8:12 AM EST GUID: 202321131221		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Tertiary Reviewer	Mark Levenson OB DBIX	Sections: 7.4, 7.6, 7.7, 17, 6	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Mark Levenson		Digitally signed by Mark Levenson		
		Date: 2/1/2023 8:14 AM EST GUID: 202321131419		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharm-tox/Non-clinical Discipline Secondary Reviewer	Natalie Simpson OCHEN DPTCHEN	Sections: 7.1, 13.1, 13.2, 5.1, 8.4	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Natalie Simpson			Digitally signed by Natalie Simpson	
			Date: 2/1/2023 8:15 AM EST GUID: 20232113150	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Discipline Primary Reviewer	Justin Penzenstadler OCHEN DDLO	Sections: 2, 3, 6, 7, 15,16,17,22,24,25	Based on my assessment of the application: ☐ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.	
Signature: Justin Penzenstadler			Digitally signed by Justin Penzenstadler	
			Date: 2/1/2023 8:20 AM EST GUID: 202321132028	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Primary Reviewer	Snehal Samant OCP DCEP	Sections: 5.2, 6.1, 8.1,8.2, 14.1 - 14.7, 20, 23	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Snehal Samant			Digitally signed by Snehal Samant	
			Date: 2/1/2023 8:21 AM EST GUID: 202321132131	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharm-tox/Non-clinical Discipline Tertiary Reviewer	Calvin Elmore OCHEN DPTCHEN	Sections: 7.1, 13.1, 13.2, 5.1, 8.4	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Calvin Elmore			Digitally signed by Calvin Elmore	
			Date: 2/1/2023 8:27 AM EST GUID: 202321132725	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Secondary Reviewer	Yeh Fong Chen OB DBIX	Sections: 7.4, 7.6, 7.7, 17, 6	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Yeh Fong Chen			Digitally signed by Yeh Fong Chen	
			Date: 2/1/2023 8:29 AM EST GUID: 202321132958	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharm-tox/Non-clinical Discipline Primary Reviewer	Bo Yeon Lee OCHEN DPTCHEN	Sections: 7.1, 13.1, 13.2, 5.1, 8.4	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Bo Yeon Lee			Digitally signed by Bo Yeon Lee	
			Date: 2/1/2023 8:31 AM EST GUID: 202321133127	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Outcomes Assessment Reviewer Discipline Secondary Reviewer	Selena Daniels ODES DCOA	Sections: Review Sections 3.1, 4, Review Sections 6.2.2, 6.4, Review Section 16.1	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.	
Signature: Selena Daniels			Digitally signed by Selena Daniels	
			Date: 2/1/2023 8:33 AM EST GUID: 202321133329	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Primary Reviewer	Guansheng Liu OCP DPM	Sections: 5.2, 6.1, 8.1,8.2, 14.1 - 14.7, 20, 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Guansheng Liu			Digitally signed by Guansheng Liu	
			Date: 2/1/2023 8:36 AM EST GUID: 202321133642	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Deputy Director	Matthew Soukup OB DBVII	Sections: 7.4, 7.6, 7.7, 17, 6	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☒ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Matthew Soukup			Digitally signed by Matthew Soukup	
			Date: 2/1/2023 8:43 AM EST GUID: 202321134359	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Secondary Reviewer	Sudharshan Hariharan OCP DCEP	Sections: 5.2, 6.1, 8.1,8.2, 14.1 - 14.7, 20, 23	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Sudharshan Hariharan			Digitally signed by Sudharshan Hariharan	
			Date: 2/1/2023 8:56 AM EST GUID: 202321135632	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Tertiary Reviewer	Jayabharathi Vaidyanathan OCP DCEP	Sections: 5.2, 6.1, 8.1,8.2, 14.1 - 14.7, 20, 23	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Jayabharathi Vaidyanathan Digitally signed by Jayabharathi Vaidyanathan				
Date: 2/1/2023 9:02 AM EST GUID: 20232114236				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Associate Director for Labeling Discipline Primary Reviewer	Virginia Kwitkowski OCHEN DNH	Sections: 23	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Virginia Kwitkowski Digitally signed by Virginia Kwitkowski				
Date: 2/1/2023 9:46 AM EST GUID: 202321144646				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Associate Director for Labeling Discipline Primary Reviewer	Virginia Kwitkowski OCHEN DNH	Sections: 23	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Virginia Kwitkowski		Digitally signed by Virginia Kwitkowski Date: 2/1/2023 9:47 AM EST GUID: 202321144721		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Secondary Reviewer	Ying-Hong Wang OCP DPM	Sections: 5.2, 6.1, 8.1,8.2, 14.1 - 14.7, 20, 23	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Ying-Hong Wang		Digitally signed by Ying-Hong Wang Date: 2/1/2023 10:19 AM EST GUID: 202321151938		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Associate Director	Jiang Liu OCP DPM	Sections: 5.2, 6.1, 8.1,8.2, 14.1 - 14.7, 20, 23	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Jiang Liu			Digitally signed by Jiang Liu Date: 2/1/2023 10:25 AM EST GUID: 202321152556	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Consultant Discipline Primary Reviewer	Xin Yuan OB DBIII	Sections: 6.3.2, 16.2.1	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Xin Yuan			Digitally signed by Xin Yuan Date: 2/1/2023 10:31 AM EST GUID: 20232115316	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Consultant Discipline Secondary Reviewer	Lili Garrard OB DBIII	Sections: 6.3.2, 16.2.1	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Lili Garrard			Digitally signed by Lili Garrard Date: 2/1/2023 10:33 AM EST GUID: 202321153344	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Secondary Reviewer	Yuzhuo Pan OCP DPM	Sections: 5.2, 6.1, 8.1,8.2, 14.1 - 14.7, 20, 23	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Yuzhuo Pan			Digitally signed by Yuzhuo Pan Date: 2/1/2023 10:39 AM EST GUID: 202321153931	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Discipline Secondary Reviewer	Tanya Wroblewski OCHEN DNH	Sections: Tanya Wroblewski	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Tanya Wroblewski		Digitally signed by Tanya Wroblewski Date: 2/1/2023 10:40 AM EST GUID: 20232115403		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
CMC (OPQ/ONDP) Discipline Primary Reviewer	Theodore Carver ONDP DNDPIII	Sections: 9	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Theodore Carver		Digitally signed by Theodore Carver Date: 2/1/2023 11:04 AM EST GUID: 20232116416		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Primary Reviewer	Sarabdeep Singh OB DBIX	Sections: 7.4, 7.6, 7.7, 17, 6	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.	
Signature: Sarabdeep Singh		Digitally signed by Sarabdeep Singh		
		Date: 2/1/2023 11:11 AM EST GUID: 202321161113		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Outcomes Assessment Reviewer Discipline Primary Reviewer	Selena Daniels ODES DCOA	Sections: Review Sections 3.1, 4, Review Sections 6.2.2, 6.4, Review Section 16.1	Based on my assessment of the application: ☐ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.	
Signature: Selena Daniels		Digitally signed by Selena Daniels		
		Date: 2/1/2023 11:17 AM EST GUID: 202321161759		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Outcomes Assessment Reviewer Discipline Tertiary Reviewer	David Reasner ODES DCOA	Sections: Review Sections 3.1, 4, Review Sections 6.2.2, 6.4, Review Section 16.1	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: David Reasner			Digitally signed by David Reasner	
			Date: 2/1/2023 11:38 AM EST GUID: 202321163847	

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
CMC (OPQ/ONDP) Discipline Secondary Reviewer	Theodore Carver ONDP DNDPIII	Sections: 9	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Theodore Carver			Digitally signed by Theodore Carver	
			Date: 2/1/2023 12:48 PM EST GUID: 202321174810	

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

TANYA M WROBLEWSKI
02/01/2023 10:43:01 AM

ANN T FARRELL
02/01/2023 10:51:52 AM

HYLTON V JOFFE
02/01/2023 01:49:26 PM

CLINICAL OUTCOME ASSESSMENT (COA) CONSULT REVIEW

COA Tracking ID:	C2022070
NDA#/Referenced IND for NDA:	NDA 216951/IND 101291
Applicant:	GlaskoSmithKline
Established Name/Trade Name:	Daprodustat (GSK1278863)
Indication:	Treatment of anemia due to chronic kidney disease in adults on dialysis
PDUFA Goal Date:	February 1, 2023
Review Division:	Division of Nonmalignant Hematology
Clinical Reviewer:	Justin Penzenstadler
Clinical Team Leader (TL)	Tanya Wroblewski
Regulatory Project Manager:	Caden Brennen
COA Reviewer:	Yasmin Choudhry, M.D.
COA TL:	Selena Daniels, PharmD, PhD
COA Division Director:	David Reasner, PhD
Instruments reviewed:	1. 36-item Short-Form Health Survey version 2.0 (SF-36 v2.0) Vitality Domain ☒ Patient-reported outcome (PRO)

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1. EXECUTIVE SUMMARY

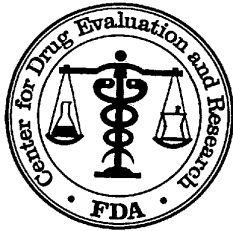
(b) (4)

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

SELENA R DANIELS
01/10/2023 08:17:10 AM

DAVID S REASNER
01/10/2023 10:55:16 AM



U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Science
Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDIES

IND/NDA Number: NDA 216951

Drug Name: GSK1278863A (Daprodustat)

Indication: Treatment of anemia due to chronic kidney disease in adult patients on dialysis and not on dialysis

Studies: Carcinogenicity Studies in Rats and Mice for 104 weeks

Applicant: Study Sponsor:
GlaxoSmithKline (GSK)
7333 Mississauga Road North
Mississauga, ON L5N 6L4
Canada

Testing Facility:  (b) (4)

Documents Reviewed: Electronic submission: Submitted on March 31 2022
Electronic data: Submitted on March 31 2022

Review Priority: Standard

Biometrics Division: Division of Biometrics - VI

Statistical Reviewer: Dr. Hepei Chen

Concurring Reviewer: Dr. Karl Lin

Medical Division: Division of Pharmacology Toxicology for Cardiology, Hematology, Endocrinology & Nephrology

Reviewing Pharmacologist: Dr. Bo Lee

Keywords: Carcinogenicity, Dose response

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1. Background

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were to determine the carcinogenic potential of GSK1278863A, a HIF-prolyl hydroxylase inhibitor, when given once daily by oral gavage for up to 104 consecutive weeks to Crl:CD (SD) rats, and once daily by oral gavage and subcutaneous injection of a cocktail formulation containing the three major circulating human metabolites for a minimum of 104 weeks to Crl:CD1 (ICR) mice, respectively.

In this review the phrase "dose response relationship" refers to the linear component (trend) of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as dose increases.

2. Rat Study

Two separate experiments, one in male rats and one in female rats were conducted. As indicated in Table 1, in each of these two experiments there were four treated groups, and one vehicle control group. Three hundred and fifty Crl:CD (SD) rats of each sex were assigned randomly in size of 70 rats per group. The dose levels for the four treated groups were 0.02, 0.2, 0.8, and 4 mg/kg/day for male rats, and 0.02, 0.2, 0.8, and 7 mg/kg/day for female rats, respectively. In this review these dose groups were referred to as the low (Group 2), mid (Group 3), mid-high (Group 4), and high (Group 5) dose groups, respectively. The rats in the vehicle control groups were administered with 1% (w/v) methyl cellulose (400 cP@ 2%) with 0.2% Tween 80 (v/v) with 5 mM NaOH in Ultra Pure Water, and handled for the same duration and in the same manner as the treated groups.

In view of the fact that the number of survivors had reached 15 in the high dose male rats (Group 5, 4 mg/kg/day) on Day 650, and had reached 20 in the Reference Item group on Days 675 and 695 for male and female rats, respectively, early study termination was initiated on Day 653 for Group 5 of male rats, Day 679 for Group 1 to Group 4 of male rats and on Day 698 for all female rats.

Table 1: Experimental Design in Rat Study

Group No.	No. of Toxicity Animals		Test Material	Dosage Level (mg/kg/day)	
	Male	Female		Male	Female
1	70	70	Vehicle control	0	0
2	70	70	GSK1278863A Low	0.02	0.02
3	70	70	GSK1278863A Mid	0.2	0.2
4	70	70	GSK1278863A Mid-High	0.8	0.8
5	70	70	GSK1278863A High	4	7

A viability check was performed near the start and end of each working day for main study animals. A necropsy was performed on those animals that died or were euthanized during the dosing period. Detailed examinations were performed on all Main Study animals weekly starting during the last week of the prestudy period and on the day of necropsy. All animals found dead during the study were subjected to necropsy, and tissue samples were preserved. Main study

animals were subjected to a complete necropsy examination, which included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

2.1. Sponsor's analyses

2.1.1. Survival analysis

In the sponsor's report, each animal survival time was measured by the number of study days until its death. The animal was classified as uncensored when death occurred within the experimental period and was not accidental. Otherwise, that is, when death was accidental, as it is the case for animals' nos. 3016 and 4524, and/or occurred during the terminal sacrifice period, the animal was classified as censored. The first day of the terminal sacrifice period was considered as the censoring time of all terminal animals.

For each sex, Kaplan-Meier product-limit method, applied on daily intervals, was used to estimate each group survival function for the dosing period and to generate the corresponding survival curves for males and females. At the 5% level, the significance of a dose-related trend in mortality across the reference item group (Group 1) and the four test item treated groups (Groups 2 to 5) was evaluated for each sex via Peto's two-sided test (Peto et al., 1980), using the corresponding arithmetic dose level scores. For the females, the doses are 0, 0.02, 0.1, 0.8, and 7 mg/kg/day. However, for the males, given that dosing stopped after Day 670 for Groups 1 to 4 and at Day 631 for Group 5, and given that the first day of sacrifice was Day 679 for Groups 1 to 4 and Day 653 for Group 5, the doses 0, 0.02, 0.1, 0.8, and 4 mg/kg/day were adjusted accordingly and, the adjusted doses 0, 0.0198, 0.0988, 0.7906, and 3.8712 mg/kg/day were used. In addition, Peto's two-tailed trend test was conducted, at the 5% significance level, to compare the mortality rates in the reference item group and in each of the test item treated group.

Sponsor's findings:

The sponsor's analysis showed that the numbers of rats surviving to their terminal necropsy were 20 (29%), 17 (24%), 17 (24%), 16 (23%), and 15 (21%) in the vehicle control, the low, mid, mid-high, and high dose groups for male rats, respectively, and 20 (29%), 21 (30%), 20 (29%), 27 (39%), and 30 (43%) for female rats respectively. For female rats, the trend and the pairwise comparisons test results were not found to be significant. For male rats, the trend test result revealed a significant increase in the mortality rate across doses, with p-value = 0.0025, and the pairwise comparison results revealed that the mortality rate in the high dose group (Group 5) is significantly higher than the one in the vehicle control group (Group 1), with p-value = 0.0131.

2.1.2. Tumor data analysis

In the sponsor's analysis, the statistical evaluation was done for each sex separately and was limited to all non-secondary neoplastic lesions found in study plan-required tissues/sites as well as to the combined hemangiosarcomas across whole body. Tumors listed under subcutis and hemolymphoreticular tissue were statistically analyzed using all study animals. Tumors listed

under tissues/sites that were not systematically examined for all animals were excluded from the statistical analysis.

For male rats, given that the last day of dosing was Day 670 for Groups 1 to 4 and Day 631 for Group 5, and given that the first day of terminal sacrifice was Day 679 for animals in Groups 1 to 4 and Day 653 for animals in Group 5, two separate analyses were considered. More precisely, the first one included all groups with a common experimental period of 652 days and with doses 0, 0.02, 0.1, and 0.8 mg/kg/day for Groups 1 to 4 respectively, and adjusted dose of 3.8712 mg/kg/day for Group 5. The second analysis included Groups 1 to 4, with a common experimental period of 678 days and with respective adjusted doses of 0, 0.0198, 0.0988, and 0.7906 mg/kg/day. For female rats, given that animals in all groups have been dosed during the common experimental period of 697 days, only one analysis, with all groups and respective doses 0, 0.02, 0.1, 0.8, and 7 mg/kg/day, was conducted. In addition to the overall trend test, pairwise groups comparisons were made using Peto's one-sided test to determine if the tumor rate in each of the test item treated group is significantly higher than the one in the reference item group.

Neoplastic lesions designated as palpable and listed under clitoral, preputial and mammary glands, and under skin and subcutis were statistically analyzed according to Peto's onset rate method for tumors observed in a "mortality independent" context (Peto et al., 1980), using all animals. The onset time of each palpable tumor was given by the first date of detection. The death time was used as the onset time for a lesion categorized as palpable and not detected in vivo. The death time of all animals dying after the end of the experimental period will be considered to be the first day following the experimental period.

For combinations of both palpable and non-palpable tumors, the animal death time was used in the analysis which was conducted according to the "mortality dependent" method described hereafter for non palpable tumors. Tumors categorized as non-palpable were analyzed using the "mortality dependent" method. In this context, each neoplastic finding was classified as the cause of death (designated by the study pathologist as definitely or probably fatal) or not the cause of death (designated by the study pathologist as definitely or probably incidental or undetermined). However, all non-palpable neoplasms found in an animal were automatically classified as incidental if the animal in question died after the experimental period. For combinations of neoplastic findings, if an animal had one of the related tumors classified as fatal, then the neoplastic finding defined by this combination was classified as fatal. Otherwise, it was classified as incidental. Neoplastic findings classified as fatal and incidental were processed using the death rate method and the prevalence method, respectively.

As per FDA's draft guidance (FDA's draft Guidance for industry, 2001), the processing of incidental tumors was done by creating a single separate interval for the scheduled terminal sacrifice period and by dividing the experimental period into the following fixed intervals: Days 0-350, Days 351-560, Days 561-652, and Day 653 and over for male rats first analysis, Days 0-350, Days 351-560, Days 561-678, and Day 679 and over for male rats second analysis, and Days 0-350, Days 351-560, Days 561- 697, and Day 698 and over for female rats analysis.

Using the derived outcomes from the processing of both fatal and incidental tumors, a test statistic was built to perform a global survival-adjusted trend test on tumor data observed in a “mortality dependent” context (Peto et al., 1980). For each dataset of interest within each sex, the significance of a linear dose-related increase in tumor occurrence rates across the reference item group and test item treated groups was evaluated using Peto's survival-adjusted one-tailed trend test. The related arithmetic dose level scores, multiplied by 10 to the power 4 for male rats and 2 for female rats, were used to perform the overall trend test.

As mentioned by Lin (Lin, 1997), the discrete permutation distribution was used to compute the corresponding p-value for each statistical test performed on a dataset containing 10 or less tumor occurrences. The interpretation of the results should be accomplished with the consideration of both historical control data and biological relevance. In fact, the evaluation of the carcinogenic potential of the test item should be undertaken in the light of an overall scientific judgment.

Adjustment for multiple testing:

In the sponsor’s report, according to the recommendations of Lin and Rahman (Lin and Rahman, 1998), the dose-related increase in tumor incidence should be considered significant if the p-value is less than or equal to 0.025 for rare tumors and less than or equal to 0.005 for common tumors (historical incidence of more than 1%); the increased tumor rate in a test item treated group when compared with the reference item group is considered to be significant when the p-value is less than or equal to 0.05 for a rare tumor, or is less than or equal to 0.01 for a common tumor.

Sponsor’s findings:

In the sponsor’s report, the overall trend results from the analysis of male rats data excluding Group 5, were not found to be significant (p-value > 0.05), whereas the overall trend results from the analysis of male rats data and female rats data including all groups, revealed significance (p-value ≤ 0.05) for the datasets listed in Table 2.

Table 2. Significant Findings (p-value ≤ 0.05) of the Overall Trend Results

(For $0.025 < p\text{-value} \leq 0.05$, the flag is +, for $0.005 < p\text{-value} \leq 0.025$, the flag is @, and for $p\text{-value} \leq 0.005$, the flag is @@)

Organ Name	Tumor Name	P-value	Flag
--- MALES (1st analysis)---			
Mammary gland	Fibroadenoma	<0.0001	@@
Spleen	Hemangiosarcoma	0.0208	@
Stomach	Adenoma	0.0208	@
--- FEMALES ---			
Mammary gland	Fibroadenoma	0.0202	@
Hemolymphoreticular tissue	Histiocytic sarcoma	0.0487	+

Based on the adjustment criteria for multiple testing described in the sponsor’s report, for the male rats first analysis including the high dose group (group 5), the results corresponding to fibroadenoma under mammary gland will be considered to be statistically significant regardless of this tumor type classification (rare or common), and the results corresponding to

hemangiosarcoma under spleen and to adenoma under stomach will be considered to be statistically significant only if these tumors are classified as rare. For female rats, the result corresponding to fibroadenoma under mammary gland will be considered to be statistically significant only if this tumor is classified as rare, and the result corresponding to histiocytic sarcoma under hemolymphoreticular tissue will not be considered to be statistically significant.

The pairwise comparisons results revealed significance ($p\text{-value} \leq 0.05$) for the datasets listed in Table 3. Based on the adjustment criteria for multiple testing described in the sponsor's report, for the male rats first analysis including the high dose group (group 5), mammary gland fibroadenoma incidence rate in Group 5 will be considered to be significantly higher than the one in Group 1 regardless of this tumor type classification (rare or common), and for male rats first and second analyses, thyroid gland c-cell adenoma incidence rate in Group 4 will be considered to be significantly higher than the one in Group 1 only if this tumor is classified as rare. For female rats, both mammary gland fibroadenoma incidence rate in Group 5 and uterus endometrial stromal polyp incidence rate in Group 3 will be considered to be significantly higher than the one in Group 1 only if these tumors are classified as rare.

Table 3. Significant Findings ($p\text{-value} \leq 0.05$) of the Pairwise Comparison Results

(For $0.01 < p\text{-value} \leq 0.05$, the flag is #, and for $p\text{-value} \leq 0.01$, the flag is ##)

Organ Name	Tumor Name	Comparison	P-Value	Flag
--- MALES (1st analysis)---				
Mammary gland	Fibroadenoma	1 vs 5	0.0021	##
Thyroid gland	C-cell adenoma	1 vs 4	0.0351	#
--- MALES (2nd analysis)--				
Thyroid gland	C-cell adenoma	1 vs 4	0.0352	#
--- FEMALES ---				
Mammary gland	Fibroadenoma	1 vs 5	0.0221	#
Uterus	Endometrial stromal polyp	1 vs 3	0.0412	#

2.2. Reviewer's analyses

To verify the sponsor's analyses and to perform additional analyses suggested by the reviewing toxicologist, this reviewer independently performed the survival and tumor data analyses using the data provided by the sponsor electronically.

2.2.1. Survival analysis

In the reviewer's analysis, the survival distributions of rats in all five groups (Groups 1, 2, 3, 4, and 5) were estimated using the Kaplan-Meier product limit method. The dose response relationship was tested across Groups 1, 2, 3, 4, and 5 using the likelihood ratio test, and the homogeneity of survival distributions was tested using the log-rank test. The Kaplan-Meier curves for survival rates are

given in Figures 1A and 1B in the appendix for all five groups in male and female rats, respectively. The intercurrent mortality data of all five groups and the results of the tests for dose response relationship and homogeneity of survivals for Groups 1, 2, 3, 4, and 5 are given in Tables 1A and 1B in the appendix for male and female rats, respectively.

Reviewer's findings:

The reviewer's analysis showed that the numbers of rats surviving to their terminal necropsy were 20 (29%), 17 (24%), 17 (24%), 16 (23%), and 15 (21%) in the vehicle control, the low, mid, mid-high, and high dose groups for male rats, respectively, and 20 (29%), 21 (30%), 20 (29%), 27 (39%), and 30 (43%) for female rats respectively. A statistically significant increasing trend in mortality was noted for male rats (p -value = 0.0040), with a corresponding statistically significant increase in the high dose group when compared to the vehicle control group (p -values = 0.0143). No other statistically significant dose response relationship and pairwise comparisons in mortality was noted for male and female rats.

2.2.2. Tumor data analysis

The tumor data were analyzed for dose response relationships across the vehicle control group, and low, mid, mid-high, and high dose groups, and pairwise comparisons of each of the four treated groups against the vehicle control group, using the Poly-k method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993).

In the ploy-k method, the adjustment for differences in mortality among treatment groups is made by modifying the number of animals at risk in the denominators in the calculations of overall tumor rates in the Cochran-Armitage test to reflect less-than-whole-animal contributions for animals that die without tumor before the end of the study (Bailer and Portier 1988). The modification is made by defining a new number of animals at risk for each treatment group. The number of animals at risk for the i -th treatment group R^*_i is defined as $R^*_i = \sum W_{ij}$ where w_{ij} is the weight for the j -th animal in the i -th treatment group, and the sum is over all animals in the group.

Bailer and Portier (1988) proposed the weight w_{ij} as follows:

$w_{ij} = 1$ to animals dying with the tumor, and

$w_{ij} = (t_{ij} / \text{tsacr})^3$ to animals dying without the tumor,

where t_{ij} is the time of death of the j -th animal in the i -th treatment group, and tsacr is the planned (or intended) time of terminal sacrifice. The above formulas imply that animals living up to the end of the planned terminal sacrifice date without developing any tumor will also be assigned $w_{ij} = 1$ since $t_{ij} = \text{tsacr}$. Also animals developed the tumor type being tested before the end of the study will be assigned as $w_{ij} = 1$.

Certain treatment groups of a study or the entire study may be terminated earlier than the planned (or intended) time of terminal sacrifice due to excessive mortalities. However, based on the principle of the Intention-to-treat (ITT) analysis in randomized trials, the tsacr should not be affected by the unplanned early terminations. The tsacr should always be equal to the planned (or intended) time of terminal sacrifice. For those animals that were sacrificed later than tsacr ,

regardless their actual terminal sacrifice time, tsacr was used as their time of terminal sacrifice in the analysis.

One critical point for Poly-k test is the choice of the appropriate value of k, which depends on the tumor incidence pattern with the increased dose. For long term 104 week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data.

Multiple testing adjustment:

For the adjustment of multiple testing, this reviewer used the methodologies suggested in the FDA guidance for statistical design and analysis of carcinogenicity studies (2001). For dose response relationship tests, the guidance suggests the use of test levels of $\alpha=0.005$ for common tumors and $\alpha=0.025$ for rare tumors for a submission with two species where both are two-years studies, in order to keep the false-positive rate at the nominal level of approximately 10%. For multiple pairwise comparisons of treated group with control, the guidance suggests the use of test levels of $\alpha=0.01$ for common tumors and $\alpha=0.05$ for rare tumors, in order to keep the false-positive rate at the nominal level of approximately 10% for both submissions with two or one species.

A rare tumor is defined as one in which the published spontaneous tumor rate is less than 1%. However, if the background information for the common or rare tumor is not available, the number of animals bearing tumors in the vehicle control group in the present study was used to determine the common or rare tumor status in the review report.

Reviewer's findings:

The tumor rates and the p-values of the tested tumor types are listed in Tables 2A and 2B in the appendix for male and female rats, respectively. The tumor types with p-values less than or equal to 0.05 for dose response relationship and/or pairwise comparisons of treated groups and vehicle control are reported in Table 4.

Based on the criteria of adjustment for multiple testing discussed above, for male rats, a statistically significant dose response relationship was noted in the incidence of fibroadenoma in mammary gland (p-value = 0.0005), with a corresponding statistically significant increase in the high dose group (Group 5) when compared to the vehicle control group (p-value = 0.0044), regardless of the tumor classification (rare or common). Statistically significant dose response relationships were also noted in the incidence of hemangiosarcoma in spleen, and adenoma in stomach (p-value = 0.0224 and 0.0224, respectively) without corresponding statistically significant pairwise comparisons if these tumors were considered to be rare tumors. No statistically significant findings were noted in tumor data for female rats.

Table 4: Summary Table of Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship and/or Pairwise Comparisons of Treated Groups and Vehicle control Group in Rats

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	4 mg P-HvsC
<i>Males</i>						
Gland, Mammary	Fibroadenoma	1/65 (33) 0.0005 \$	4/56 (30) 0.1489	0/63 (33) 1.0000	3/62 (29) 0.2587	8/63 (26) 0.0044 \$
Spleen	Hemangiosarcoma	0/70 (36) 0.0224 \$	0/70 (35) NC	0/70 (37) NC	0/70 (31) NC	2/70 (25) 0.1639
Stomach	Adenoma	0/70 (36) 0.0224 \$	0/70 (35) NC	0/70 (37) NC	0/70 (31) NC	2/70 (25) 0.1639
<i>Females</i>						
Gland, Mammary	Adenoma	14/70 (42) 0.4548	13/69 (45) 0.7516	17/70 (50) 0.5619	15/70 (49) 0.6930	16/70 (49) 0.6156
	Fibroadenoma	41/70 (52) 0.0466 @	48/69 (57) 0.3171	41/70 (57) 0.8549	50/70 (59) 0.2875	54/70 (61) 0.1266

* X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.

\$ = Statistically significant in rare tumor at 0.025 level and in common tumor at 0.005 level for test of dose response relationship or at 0.01 level for test of pairwise comparisons;

@ = Not statistically significant in common tumor at 0.005 level for test of dose response relationship;

3. Mouse Study

Two separate experiments, one in male mice and one in female mice were conducted. As indicated in Table 5, in the experiment there were four treated groups, and one vehicle control group. Three hundred Crl:CD1 (ICR) mice of each sex, respectively, were assigned randomly in size of 60 mice per group. Three of the four treated groups received once daily oral gavage at 0.2, 0.8, or 3 mg/kg/day of GSK1278863 for a minimum of 104 weeks for both male and female mice. These three treated groups were also dosed via once daily subcutaneous injection of a cocktail formulation containing the three major circulating human metabolites [2.0 mg/kg/day GSK2391220 (M2), 2.5 mg/kg/day GSK2506104 (M3) and 1.3 mg/kg/day GSK2531401 (M13)]. In this review these three treated groups were referred to as the low (Group 2), mid (Group 4), and high (Group 5) dose groups, respectively. The other treated group (Group 3) received 0.8 mg/kg/day GSK1278863 by once daily oral gavage + subcutaneous reference item by once daily subcutaneous injection without metabolites. The mice in the vehicle control group (Group 1) were administered once daily with oral gavage at 0 mg/kg/day of GSK1278863 and subcutaneous reference items [1% (w/v) methyl cellulose (400 cP@ 2%) / 0.2% Tween 80 (v/v) / 5 mM NaOH in Ultra Pure Water], and handled for the same duration and in the same manner as the treated groups.

The same clinical examinations, laboratory investigations and pathology procedures used in the rat study were performed in the mouse study.

Table 5: Experimental Design in Mouse Study

Group No.	No. of Toxicity Animals		Test Material	Dosage Level (mg/kg/day)	
	Male	Female		Male	Female
1	60	60	Vehicle control	0	0
2	60	60	GSK1278863A + M* Low	0.2	0.2
3	60	60	GSK1278863A	0.8	0.8
4	60	60	GSK1278863A + M* Mid	0.8	0.8
5	60	60	GSK1278863A+ M* High	3	3

* Metabolite cocktail consisted of GSK2391220 (M2), GSK2506104 (M3) and GSK2531401 (M13)

3.1. Sponsor's analyses

3.1.1. Survival analysis

In the sponsor's report, each animal survival time was measured by the number of study days until its death. The animal was classified as uncensored when death occurred within the experimental period and was not accidental. Otherwise, that is, when death was accidental, as it is the case for Animal No. 2532, and/or whenever death occurred during the terminal sacrifice period, the animal was classified as censored. The first day of the terminal sacrifice period, Day 729, was considered as the censoring time of all terminal animals.

For each sex, Kaplan-Meier product-limit method, applied on daily intervals, was used to estimate each group survival function for the dosing period, and to generate the corresponding survival curves. At the 5% level, the significance of an overall dose-related trend in mortality across the reference item group (Group 1) and the three test item treated groups having the same subcutaneous dose level (Groups 2, 4, and 5) was evaluated for each sex via Peto's two-sided test (Peto et al., 1980), using the corresponding oral dose level scores 0, 0.2, 0.8, and 3 mg/kg/day. In addition, Peto's two-tailed test was conducted, at the 5% significance level, to compare the mortality rates in the reference item group and in each of Groups 2, 4, and 5.

Sponsor's findings:

The sponsor's analysis showed that the numbers of mice surviving to their terminal necropsy were 34 (57%), 33 (55%), 28 (47%), 33 (55%), and 32 (53%) in the vehicle control group, the low, mid, and high dose groups, and Group 3 for male mice, respectively, and 29 (48%), 27 (45%), 36 (60%), 26 (43%), and 32 (53%) for female mice respectively. No statistically significant findings in mortality were noted for both male and female mice.

3.1.2. Tumor data analysis

Regarding the tumor data, the statistical evaluation was done for each sex separately and was limited to all non-secondary neoplastic lesions found in study plan-required tissues/sites as well as to the combined hemangiosarcomas across whole body. Tumors listed under subcutis and hemolymphoreticular tissue were statistically analyzed using all study animals.

Neoplastic lesions designated as palpable and listed under mammary glands, skin and subcutis were statistically analyzed according to Peto's onset rate method for tumors observed in a "mortality independent" context (Peto et al., 1980), using all animals. The onset time of each palpable tumor was given by the first date of detection. The death time was used as the onset time for a lesion categorized as palpable and not detected *in vivo*. The death time of all animals dying after the end of the experimental period will be considered to be the first day following the experimental period. For combinations of both palpable and non-palpable tumors, the animal death time was used in the analysis which was conducted according to the "mortality dependent" method described hereafter for non palpable tumors. Tumors categorized as non-palpable were analyzed using the "mortality dependent" method. In this context, each neoplastic finding was classified as the cause of death (designated by the study pathologist as definitely or probably fatal) or not the cause of death (designated by the study pathologist as definitely or probably incidental or undetermined). However, all non-palpable neoplasms found in an animal were automatically classified as incidental if the animal in question died after the experimental period. For combinations of neoplastic findings, if an animal had one of the related tumors classified as fatal, then the neoplastic finding defined by this combination was classified as fatal. Otherwise, it was classified as incidental.

Neoplastic findings classified as fatal and incidental were processed using the death rate method and the prevalence method, respectively. As per FDA's draft guidance (FDA's draft Guidance for industry, 2001), the processing of incidental tumors was done by creating a single separate interval for the scheduled terminal sacrifice period and by dividing the experimental period into the following fixed intervals: Days 0-364, Days 365-546, Days 547-644, and Days 645-728. Using the derived outcomes from the processing of both fatal and incidental tumors, a test statistic was built to perform a global survival-adjusted trend test on tumor data observed in a "mortality dependent" context (Peto et al., 1980). For each dataset of interest within each sex, the significance of a linear dose-related increase in tumor occurrence rates across Groups 1, 2, 4, and 5 was evaluated using Peto's survival-adjusted one-tailed trend test. For both sexes, the related oral dose level scores multiplied by 10 were used to perform the overall trend test. In addition to the overall trend test, pairwise group comparisons were made using Peto's one-sided test to determine if the tumor rate in each of Groups 2, 4, and 5 were significantly higher than the one in Group 1.

As mentioned by Lin (Lin, 1997), the discrete permutation distribution was used to compute the corresponding p-value for each statistical test performed on a dataset containing 10 or less tumor occurrences. The interpretation of the results should be accomplished with the consideration of both historical control data and biological relevance. In fact, the evaluation of the carcinogenic potential of the test item should be undertaken in the light of an overall scientific judgment.

Multiple testing adjustment:

According to the recommendations of Lin and Rahman (Lin and Rahman, 1998), the dose-related increase in tumor incidence should be considered significant if the p-value is less than or equal to 0.025 for rare tumors and less than or equal to 0.005 for common tumors (historical incidence of more than 1%); the increased tumor rate in a test item treated group when compared

with the reference item group is considered to be significant when the p-value is less than or equal to 0.05 for a rare tumor, or is less than or equal to 0.01 for a common tumor.

Sponsor's findings:

In the sponsor's report, based on the adjustment criteria for multiple testing described in the sponsor's report, no statistically significant overall trend was noted in tumor data for both male and female mice. The pairwise comparison results revealed significance (p-value $\leq$ 0.05) for the datasets listed in Table 6Table 3.

**Table 6: Significant Findings (p-value $\leq$ 0.05) of the Pairwise Comparison Results
Text Table 1 (For 0.01 < p-value $\leq$ 0.05, the flag is #)**

Organ Name	Tumor Name	Comparison	P-Value	Flag
--- MALES ---				
Liver	hepatocellular carcinoma	1 vs 3	0.0377	#
--- FEMALES ---				
Harderian gland	Adenoma	1 vs 3	0.0430	#

Based on the adjustment criteria for multiple testing described in the sponsor's report, Group 3 tumor incidence rates corresponding to hepatocellular carcinoma in male mice and to harderian gland adenoma in female mice will be considered to be significantly higher than the corresponding incidence rate in Group 1 only if these tumors are classified as rare.

3.2. Reviewer's analyses

Similar to the rat study, this reviewer independently performed survival and tumor data analyses of mouse data to verify sponsor's analyses. Data used in this reviewer's analyses were provided by the sponsor electronically.

For the analysis of both the survival data and the tumor data in mice, this reviewer used similar methodologies that were used for the analyses of the rat survival and tumor data.

3.2.1. Survival analysis

Reviewer's findings:

The reviewer's analysis showed that the numbers of mice surviving to their terminal necropsy were 34 (57%), 33 (55%), 28 (47%), 33 (55%), and 32 (53%) in the vehicle control group, the low, mid, and high dose groups, and Group 3 for male mice, respectively, and 29 (48%), 27 (45%), 36 (60%), 26 (43%), and 32 (53%) for female mice respectively. No statistically significant findings in mortality were noted for both male and female mice.

3.2.2. Tumor data analysis

Reviewer's findings:

The tumor rates and the p-values of the tested tumor types are listed in Tables 4A and 4B in the appendix for male and female mice, respectively. The tumor types with p-values less than or equal to 0.05 for dose response relationship and/or pairwise comparisons of treated groups and vehicle control are reported in Table 7.

Based on the criteria of adjustment for multiple testing discussed above, no statistically significant finding was noted in the reviewer's analysis for both male and female mice.

Table 7: Summary Table of Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship and/or Pairwise Comparisons of Treated Groups and Vehicle control Group in Mice

Organ name	Tumor name	Vehicle (VC)	Low (L)	Mid (M)	High (H)	Group 3 (G3)
		0 mg	0.2 mg	0.8 mg	3 mg	0 mg
		P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. G3
<i>Male</i>						
Liver	Hepatocellular Carcinoma	4/60 (47)	9/60 (46)	6/60 (48)	7/60 (51)	12/60 (50)
		0.4680	0.1075	0.3836	0.3116	0.0360 @
<i>Female</i>						
Gland, Harderian	Adenoma	4/60 (47)	7/60 (46)	7/60 (52)	2/60 (43)	12/60 (49)
		0.9013	0.2489	0.3240	0.8768	0.0325 @

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;

NC = Not calculable.

@ = Not statistically significant in common tumor at 0.01 level for test of pairwise comparisons;

4. Summary

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were to determine the carcinogenic potential of GSK1278863A, a HIF-prolyl hydroxylase inhibitor, when given once daily by oral gavage for up to 104 consecutive weeks to Crl:CD (SD) rats, and once daily by oral gavage and subcutaneous injection of a cocktail formulation containing the three major circulating human metabolites for up to 104 consecutive weeks to Crl:CD1 (ICR) mice, respectively.

Rat Study:

Two separate experiments, one in male rats and one in female rats were conducted. In each of these two experiments there were four treated groups, and one vehicle control group. Three hundred and fifty Crl:CD (SD) rats of each sex were assigned randomly in size of 70 rats per group. The dose levels for the four treated groups were 0.02, 0.2, 0.8, and 4 mg/kg/day for male rats, and 0.02, 0.2, 0.8, and 7 mg/kg/day for female rats, respectively.

The reviewer's analysis showed that the numbers of rats surviving to their terminal necropsy were 20 (29%), 17 (24%), 17 (24%), 16 (23%), and 15 (21%) in the vehicle control, the low, mid, mid-high, and high dose groups for male rats, respectively, and 20 (29%), 21 (30%), 20 (29%), 27 (39%), and 30 (43%) for female rats respectively. A statistically significant increasing trend in mortality was noted for male rats (p-value = 0.0040), with a corresponding statistically significant increase in the high dose group when compared to the vehicle control group (p-values = 0.0143). No other statistically significant dose response relationship and pairwise comparisons

in mortality was noted for male and female rats.

In the reviewer's analysis, for male rats, a statistically significant dose response relationship was noted in the incidence of fibroadenoma in mammary gland (p-value = 0.0005), with a corresponding statistically significant increase in the high dose group (Group 5) when compared to the vehicle control group (p-value = 0.0044), regardless of the tumor classification (rare or common). Statistically significant dose response relationships were also noted in the incidence of hemangiosarcoma in spleen, and adenoma in stomach (p-value = 0.0224 and 0.0224, respectively) without corresponding statistically significant pairwise comparisons if these tumors were considered to be rare tumors. No statistically significant findings were noted in tumor data for female rats.

Mouse Study:

Two separate experiments, one in male mice and one in female mice were conducted. In each of the experiment there were four treated groups, and one vehicle control group. Three hundred Crl:CD1 (ICR) mice of each sex, respectively, were assigned randomly in size of 60 mice per group. Three of the four treated groups received once daily oral gavage at 0.2, 0.8, or 3 mg/kg/day of GSK1278863 for a minimum of 104 weeks for both male and female mice. These three treated groups were also dosed via once daily subcutaneous injection of a cocktail formulation containing the three major circulating human metabolites [2.0 mg/kg/day GSK2391220 (M2), 2.5 mg/kg/day GSK2506104 (M3) and 1.3 mg/kg/day GSK2531401 (M13)]. The other treated group (Group 3) received 0.8 mg/kg/day GSK1278863 by once daily oral gavage + subcutaneous reference item by once daily subcutaneous injection without metabolites.

The reviewer's analysis showed that the numbers of mice surviving to their terminal necropsy were 34 (57%), 33 (55%), 28 (47%), 33 (55%), and 32 (53%) in the vehicle control group, the low, mid, and high dose groups, and Group 3 for male mice, respectively, and 29 (48%), 27 (45%), 36 (60%), 26 (43%), and 32 (53%) for female mice respectively. No statistically significant findings in mortality were noted for both male and female mice.

In the reviewer's analysis, no statistically significant finding was noted in the reviewer's analysis for both male and female mice.

Dr. Hepei Chen.
Mathematical Statistician

Concur: Dr. Karl Lin.
Team Leader, DBVI

Cc: Archival NDA 216951
Dr. Bo Lee

5. Appendix

Table 1A: Intercurrent Mortality Rate in Male Rats

Week / Type of Death	Vehicle Control		0.02 mg/kg/day Low		0.1 mg/kg/day Mid		0.8 mg/kg/day Mid-High		4 mg/kg/day High	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	10	14.29	9	12.86	8	11.43	14	20.00	20	28.57
53 - 78	15	35.71	15	34.29	17	35.71	20	48.57	22	60.00
79 - 91	17	60.00	22	65.71	21	65.71	14	68.57	10	74.29
92 - 98	8	71.43	7	75.71	6	74.29	6	77.14	3	78.57
Accidental Death	.	.	.	.	1	1.43	.	.	.	.
Terminal sacrifice	20	28.57	17	24.29	17	24.29	16	22.86	15	21.43
Total	70	.	70	.	70	.	70	.	70	.
Test	All Dose Groups		Vehicle vs. Low		Vehicle vs. Mid		Vehicle vs. Mid-high		Vehicle vs. High	
Dose-Response (Likelihood Ratio)	0.0040*		0.6059		0.8070		0.2641		0.0143*	
Homogeneity (Log-Rank)	0.0452		0.6004		0.8041		0.2582		0.0129	

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

Table 1B: Intercurrent Mortality Rate in Female Rats

Week / Type of Death	Vehicle Control		0.02 mg/kg/day Low		0.1 mg/kg/day Mid		0.8 mg/kg/day Mid		7 mg/kg/day High	
	No. of Death	Cum %	No. of Death	No. of Death	Cum %	No. of Death	Cum %	Cum %	No. of Death	Cum %
0 - 52	7	10.00	4	5.71	.	.	3	4.29	4	5.71
53 - 78	17	34.29	12	22.86	14	20.00	15	25.71	12	22.86
79 - 91	16	57.14	17	47.14	21	50.00	11	41.43	18	48.57
92 - 101	10	71.43	16	70.00	15	71.43	13	60.00	6	57.14
Accidental Death	.	.	.	.	.	.	1	1.43	.	.
Terminal sacrifice	20	28.57	21	30.00	20	28.57	27	38.57	30	42.86
Total	70	.	70	.	70	.	70	.	70	.
Test	All Dose Groups		Vehicle vs. Low		Vehicle vs. Mid		Vehicle vs. Mid-high		Vehicle vs. High	
Dose-Response (Likelihood Ratio)	0.1437		0.4366		0.4293		0.0742		0.0799	
Homogeneity (Log-Rank)	0.2759		0.4292		0.4223		0.0702		0.0770	

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

Table 2A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Rats

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	4 mg P-HvsC
Adipose Tissue	Hibernoma, Benign	1/5 (3)	0/6 (4)	0/7 (3)	0/4 (2)	0/3 (1)
		1.0000	1.0000	1.0000	1.0000	1.0000
Body Cavity, Nasal	Adenoma	0/70 (36)	1/70 (36)	0/70 (37)	0/70 (31)	0/70 (25)
		0.7818	0.5000	NC	NC	NC
	Squamous Cell Carcinoma	0/70 (36)	1/70 (36)	1/70 (38)	0/70 (31)	0/70 (25)
		0.7135	0.5000	0.5135	NC	NC
Bone, Femur	Hemangiosarcoma	1/70 (37)	0/70 (35)	0/70 (37)	0/70 (31)	0/70 (25)
		1.0000	1.0000	1.0000	1.0000	1.0000
Brain	Astrocytoma, Malignant	1/70 (36)	3/70 (37)	2/70 (38)	0/70 (31)	0/70 (25)
		0.9541	0.3177	0.5205	1.0000	1.0000
	Meningioma, Malignant	1/70 (37)	0/70 (35)	1/70 (37)	0/70 (31)	1/70 (26)
		0.3134	1.0000	NC	1.0000	0.6590
Gland, Adrenal	Cortical Adenoma	1/70 (36)	1/70 (36)	1/70 (37)	1/70 (32)	0/70 (25)
		0.7306	NC	0.7603	0.7234	1.0000
	Cortical Carcinoma	0/70 (36)	1/70 (36)	0/70 (37)	0/70 (31)	0/70 (25)
		0.7818	0.5000	NC	NC	NC
	Cortical Adenoma/ Cortical Carcinoma	1/70 (36)	2/70 (36)	1/70 (37)	1/70 (32)	0/70 (25)
		0.8128	0.5000	0.7603	0.7234	1.0000
	Pheochromocytoma, Benign	7/70 (37)	7/70 (38)	6/70 (39)	4/70 (33)	1/70 (25)
		0.9713	0.6372	0.7620	0.8666	0.9886
	Pheochromocytoma, Malignant	2/70 (36)	1/70 (36)	1/70 (37)	1/70 (32)	0/70 (25)
		0.8399	0.8803	0.8852	0.8575	1.0000
Pheochromocytoma, Benign/ Malignant	9/70 (39)	8/70 (38)	7/71 (40)	5/71 (34)	1/70 (25)	
	0.9864	0.6869	0.8148	0.8864	0.9958	
Pheochromocytoma, Complex, Benign	0/70 (36)	0/70 (35)	0/70 (37)	1/70 (32)	0/70 (25)	
	0.3455	NC	NC	0.4706	NC	
Gland, Mammary	Adenocarcinoma	2/65 (34)	0/56 (28)	0/63 (33)	0/62 (28)	0/63 (23)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Adenoma	1/65 (33)	0/56 (28)	3/63 (34)	1/62 (29)	1/63 (23)
		0.3949	1.0000	0.3181	0.7208	0.6571
	Adenocarcinoma/Adenoma	3/65 (34)	0/56 (28)	3/63 (34)	1/62 (29)	1/63 (23)
		0.6079	1.0000	NC	0.9221	0.8826
	Fibroadenoma	1/65 (33)	4/56 (30)	0/63 (33)	3/62 (29)	8/63 (26)
		0.0005	0.1489	1.0000	0.2587	0.0044
	Adenocarcinoma/Adenoma/ Fibroadenoma	4/65 (35)	4/56 (30)	3/63 (34)	4/62 (29)	9/63 (27)
		0.0046	0.5544	0.7738	0.5337	0.0372

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 2A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Rats (Continued)

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	4 mg P-HvsC
Gland, Parathyroid	Adenoma	2/69 (36)	1/64 (33)	1/64 (35)	0/66 (29)	1/67 (24)
		0.5329	0.8637	0.8751	1.0000	0.7914
Gland, Pituitary	Adenoma	22/70 (46)	23/70 (45)	27/70 (48)	22/70 (41)	17/68 (33)
		0.5037	0.4587	0.2708	0.3715	0.4620
	Carcinoma	1/70 (37)	0/70 (35)	1/70 (37)	1/70 (32)	1/68 (24)
		0.2349	1.0000	NC	0.7161	0.6361
Adenoma/ Carcinoma	23/70 (47)	23/70 (45)	28/70 (49)	23/70 (41)	18/68 (34)	
		0.4736	0.5000	0.2741	0.3240	0.4481
Gland, Preputial	Papilloma	1/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	0/70 (25)
		1.0000	1.0000	1.0000	1.0000	1.0000
Gland, Prostate	Polyp	0/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	1/70 (25)
		0.1524	NC	NC	NC	0.4098
Gland, Thyroid	C-Cell Adenoma	3/70 (37)	6/69 (38)	8/70 (39)	8/70 (34)	5/70 (28)
		0.3342	0.2534	0.1125	0.0707	0.2103
	C-Cell Carcinoma	6/70 (38)	1/69 (35)	3/70 (38)	1/70 (32)	1/70 (25)
		0.8357	0.9923	0.9235	0.9895	0.9772
C-Cell Adenoma/ C-Cell Carcinoma	9/70 (39)	7/69 (38)	11/70 (41)	9/70 (35)	6/70 (28)	
		0.5443	0.7831	0.4492	0.5019	0.6728
	Follicular Cell Adenoma	1/70 (36)	2/69 (35)	1/70 (37)	2/70 (32)	0/70 (25)
		0.7458	0.4893	0.7603	0.4553	1.0000
Hemolymphoretic ular Tissue	Histiocytic Sarcoma	3/70 (37)	2/70 (36)	2/70 (38)	2/70 (33)	0/70 (25)
		0.9000	0.8127	0.8293	0.7839	1.0000
	Leukemia, Granulocytic	1/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	0/70 (25)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Leukemia, Large Granular Lymph	2/70 (37)	1/70 (36)	3/70 (39)	1/70 (32)	0/70 (25)
		0.8715	0.8751	0.5253	0.8517	1.0000
Leukemia, Granulocytic/ Leukemia, Large Granular Lymph	3/70 (38)	1/70 (36)	3/70 (39)	1/70 (32)	0/70 (25)	
	0.9177	0.9358	0.6751	0.9195	1.0000	
	Lymphoma, Malignant	2/70 (37)	1/70 (36)	1/70 (37)	0/70 (31)	0/70 (25)
		0.9549	0.8751	0.8801	1.0000	1.0000
Kidney	Adenoma	1/70 (37)	0/70 (35)	3/70 (39)	1/70 (32)	3/70 (26)
		0.0517	1.0000	0.3277	0.7161	0.1866
	Liposarcoma	0/70 (36)	1/70 (36)	0/70 (37)	0/70 (31)	0/70 (25)
		0.7818	0.5000	NC	NC	NC

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

**Table 2A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Rats
(Continued)**

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	4 mg P-HvsC
Liver	Cholangioma	0/70 (36)	1/70 (36)	0/70 (37)	0/70 (31)	1/70 (25)
		0.2143	0.5000	NC	NC	0.4098
	Hemangioma	0/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	1/70 (25)
		0.1524	NC	NC	NC	0.4098
	Hepatocellular Adenoma	3/70 (36)	2/70 (36)	0/70 (37)	0/70 (31)	0/70 (25)
		0.9974	0.8215	1.0000	1.0000	1.0000
Lung	Bronchioloalveolar Adenoma	2/70 (36)	0/70 (35)	0/70 (37)	1/70 (32)	0/70 (25)
		0.7223	1.0000	1.0000	0.8575	1.0000
	Bronchioloalveolar Carcinoma	0/70 (36)	1/70 (36)	0/70 (37)	0/70 (31)	0/70 (25)
		0.7818	0.5000	NC	NC	NC
	Bronchioloalveolar Adenoma/ Bronchioloalveolar Carcinoma	2/70 (36)	1/70 (36)	0/70 (37)	1/70 (32)	0/70 (25)
		0.8102	0.8803	1.0000	0.8575	1.0000
	Hemangiosarcoma	1/70 (37)	0/70 (35)	0/70 (37)	0/70 (31)	0/70 (25)
		1.0000	1.0000	1.0000	1.0000	1.0000
Lymph Node, Mesenteric	Hemangioma	1/70 (36)	1/70 (36)	1/69 (37)	0/69 (31)	1/70 (25)
		0.4155	NC	0.7603	1.0000	0.6557
	Hemangiosarcoma	0/70 (36)	1/70 (36)	0/69 (36)	1/69 (31)	0/70 (25)
		0.4842	0.5000	NC	0.4627	NC
	Hemangioma/ Hemangiosarcoma	1/70 (36)	2/70 (36)	1/69 (37)	1/69 (31)	1/70 (25)
		0.4541	0.5000	0.7603	0.7151	0.6557
Meninges	Meningioma, Malignant	0/2 (0)	0/1 (0)	0/2 (1)	1/2 (1)	0/1 (1)
		0.6667	NC	NC	NC	NC
Muscle, Skeletal	Fibrosarcoma	1/69 (36)	0/70 (35)	1/68 (36)	0/68 (31)	0/70 (25)
		0.8118	1.0000	NC	1.0000	1.0000
	Hemangiosarcoma	1/69 (36)	0/70 (35)	0/68 (35)	0/68 (31)	0/70 (25)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Schwannoma, Malignant	0/69 (35)	0/70 (35)	0/68 (35)	1/68 (31)	0/70 (25)
		0.3478	NC	NC	0.4697	NC

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 2A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Rats (Continued)

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	4 mg P-HvsC
Pancreas	Adenoma	1/70 (37)	1/70 (36)	1/70 (37)	1/70 (32)	0/70 (25)
		0.7264	0.7466	NC	0.7161	1.0000
	Ductal Cell Adenocarcinoma	1/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	0/70 (25)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Islet Cell Adenoma	6/70 (37)	2/70 (36)	9/70 (39)	4/70 (33)	5/70 (26)
		0.2808	0.9696	0.3228	0.7958	0.5053
Islet Cell Carcinoma	3/70 (37)	3/70 (37)	2/70 (37)	2/70 (32)	0/70 (25)	
	0.9270	NC	0.8212	0.7732	1.0000	
Islet Cell Adenoma/ Islet Cell Carcinoma	9/70 (38)	5/70 (37)	11/70 (40)	6/70 (34)	5/70 (26)	
	0.5895	0.9240	0.4503	0.8209	0.7654	
Skin	Basal Cell Tumor, Benign	1/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	0/70 (25)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Basal Cell Tumor, Malignant	0/70 (36)	1/70 (36)	0/70 (37)	0/70 (31)	0/70 (25)
		0.7818	0.5000	NC	NC	NC
	Basal Cell Tumor, Benign/ Basal Cell Tumor, Malign	1/70 (36)	1/70 (36)	0/70 (37)	0/70 (31)	0/70 (25)
		0.9534	NC	1.0000	1.0000	1.0000
	Fibroma	1/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	0/70 (25)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Hair Follicle Tumor, Benign	1/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	0/70 (25)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Keratoacanthoma	9/70 (38)	3/70 (37)	6/70 (39)	1/70 (32)	3/70 (26)
		0.7104	0.9861	0.8865	0.9988	0.9429
	Squamous Cell Carcinoma	0/70 (36)	0/70 (35)	3/70 (38)	2/70 (32)	0/70 (25)
		0.6006	NC	0.1301	0.2177	NC
	Keratoacanthoma/Squamous Cell Carcinoma	9/70 (38)	3/70 (37)	9/70 (41)	3/70 (32)	3/70 (26)
		0.7917	0.9861	0.6745	0.9741	0.9429
	Melanoma, Malignant	0/70 (36)	0/70 (35)	1/70 (37)	0/70 (31)	0/70 (25)
		0.5671	NC	0.5068	NC	NC
Papilloma	2/70 (36)	3/70 (37)	3/70 (38)	5/70 (34)	2/70 (26)	
	0.4337	0.5132	0.5260	0.1911	0.5616	
Sebaceous Cell Adenoma	0/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	1/70 (25)	
	0.1524	NC	NC	NC	0.4098	
Small Intestine, Duodenum	Adenocarcinoma	0/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	1/70 (25)
		0.1524	NC	NC	NC	0.4098

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 2A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Rats (Continued)

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	4 mg P-HvsC
Small Intestine, Jejunum	Leiomyosarcoma	0/69 (36)	0/70 (35)	1/70 (37)	0/70 (31)	0/70 (25)
		0.5671	NC	0.5068	NC	NC
Spleen	Hemangiosarcoma	0/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	2/70 (25)
		0.0224	NC	NC	NC	0.1639
Stomach	Adenoma	0/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	2/70 (25)
		0.0224	NC	NC	NC	0.1639
	Papilloma	0/70 (36)	1/70 (36)	0/70 (37)	0/70 (31)	0/70 (25)
		0.7818	0.5000	NC	NC	NC
	Polyp	0/70 (36)	0/70 (35)	0/70 (37)	1/70 (32)	0/70 (25)
		0.3455	NC	NC	0.4706	NC
Subcutis	Fibroma	9/16 (12)	9/17 (13)	9/17 (13)	9/15 (12)	6/10 (7)
		0.2370	0.7769	0.7769	NC	0.5250
	Fibrosarcoma	0/16 (8)	3/17 (12)	2/17 (11)	3/15 (11)	3/10 (7)
		0.0616	0.1930	0.3216	0.1703	0.0769
	Fibroma/ Fibrosarcoma	9/16 (12)	12/17 (15)	11/17 (14)	12/16 (14)	9/10 (10)
		0.1955	0.5570	0.5957	0.4217	0.3684
	Lipoma	3/16 (10)	1/17 (11)	1/17 (10)	3/15 (10)	0/10 (5)
		0.7317	0.9649	0.9567	NC	1.0000
	Liposarcoma	0/16 (8)	1/17 (11)	0/17 (10)	0/15 (9)	0/10 (5)
		0.8140	0.5789	NC	NC	NC
	Lipoma/ Liposarcoma	3/16 (10)	2/17 (11)	1/17 (10)	3/15 (10)	0/10 (5)
		0.7940	0.8741	0.9567	NC	1.0000
	Sarcoma	1/16 (9)	0/17 (10)	0/17 (10)	0/15 (9)	0/10 (5)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Schwannoma, Benign	0/16 (8)	0/17 (10)	3/17 (11)	0/15 (9)	1/10 (5)
		0.2605	NC	0.1703	NC	0.3846
Schwannoma, Malignant	0/16 (8)	0/17 (10)	0/17 (10)	1/15 (10)	0/10 (5)	
	0.3488	NC	NC	0.5556	NC	
Schwannoma, Benign/ Schwannoma, Malignant	0/16 (8)	0/17 (10)	3/17 (11)	1/15 (10)	1/10 (5)	
	0.1999	NC	0.1703	0.5556	0.3846	

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

**Table 2A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Rats
(Continued)**

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	4 mg P-HvsC
Tail	Liposarcoma	1/4 (3)	0/2 (2)	0/4 (3)	0/3 (2)	0/1 (1)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Papilloma	1/4 (3)	0/2 (2)	0/4 (3)	0/3 (2)	0/1 (1)
		1.0000	1.0000	1.0000	1.0000	1.0000
Squamous Cell Carcinoma	0/4 (2)	0/2 (2)	1/4 (4)	0/3 (2)	0/1 (1)	
		0.6364	NC	0.6667	NC	NC
Testis	Hemangioma	0/70 (36)	0/70 (35)	0/70 (37)	0/70 (31)	1/70 (25)
		0.1524	NC	NC	NC	0.4098
	Interstitial (Leydig) Cell Ade	1/70 (36)	2/70 (36)	1/70 (37)	1/70 (32)	0/70 (25)
		0.8128	0.5000	0.7603	0.7234	1.0000
Thymus	Thymoma, Benign	0/70 (36)	0/69 (35)	1/70 (37)	0/70 (31)	0/69 (24)
		0.5644	NC	0.5068	NC	NC
Urinary Bladder	Papilloma	1/70 (36)	0/70 (35)	0/69 (36)	0/70 (31)	0/70 (25)
		1.0000	1.0000	1.0000	1.0000	1.0000
Whole Body	Hemangioma	1/70 (36)	1/70 (36)	1/70 (37)	0/70 (31)	2/70 (25)
		0.1393	NC	0.7603	1.0000	0.364
	Hemangiosarcoma	1/70 (37)	1/70 (36)	0/70 (37)	1/70 (32)	2/70 (25)
		0.0921	0.7466	1.0000	0.7161	0.3543
	Hemangioma/ Hemangiosarcoma	2/70 (37)	2/70 (36)	1/70 (37)	1/70 (32)	4/70 (26)
		0.0376	0.6823	0.8801	0.8517	0.1857

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 2B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Rats

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	7 mg P-HvsC
Adipose Tissue	Liposarcoma	0/3 (2)	0/5 (2)	0/2 (1)	1/6 (4)	0/8 (6)
		0.6667	NC	NC	0.6667	NC
Body Cavity, Nasal	Adenoma	0/70 (38)	0/70 (43)	0/70 (44)	1/70 (45)	0/70 (44)
		0.4159	NC	NC	0.5422	NC
	Squamous Cell Carcinoma	0/70 (38)	0/70 (43)	0/70 (44)	1/70 (45)	1/70 (44)
		0.1284	NC	NC	0.5422	0.5366
Bone, Femur	Hemangiosarcoma	1/70 (39)	0/70 (43)	0/70 (44)	0/70 (44)	0/69 (43)
		1.0000	1.0000	1.0000	1.0000	1.0000
Brain	Astrocytoma, Malignant	1/70 (39)	0/69 (42)	1/70 (45)	1/70 (45)	1/70 (44)
		0.3632	1.0000	0.7874	0.7874	0.7823
	Granular Cell Tumor, Malignant	0/70 (38)	0/69 (42)	0/70 (44)	0/70 (44)	1/70 (44)
		0.2075	NC	NC	NC	0.5366
	Meningioma, Malignant	0/70 (38)	0/69 (42)	0/70 (44)	0/70 (44)	1/70 (45)
	0.2113	NC	NC	NC	0.5422	
	Oligodendroglioma, Malignant	0/70 (38)	1/69 (43)	0/70 (44)	0/70 (44)	1/70 (45)
		0.3021	0.5309	NC	NC	0.5422
Cervix	Endometrial Stromal Polyp	0/70 (38)	0/69 (42)	0/70 (44)	0/70 (44)	1/70 (44)
		0.2075	NC	NC	NC	0.5366
	Sarcoma	1/70 (39)	0/69 (42)	0/70 (44)	1/70 (45)	0/70 (44)
		0.6600	1.0000	1.0000	0.7874	1.0000
Eye	Melanoma, Benign	0/70 (38)	0/70 (43)	0/70 (44)	1/69 (44)	0/70 (44)
		0.4131	NC	NC	0.5366	NC
Gland, Adrenal	Cortical Adenoma	0/70 (38)	2/70 (43)	4/70 (46)	1/70 (45)	2/70 (45)
		0.4668	0.2787	0.0846	0.5422	0.2909
	Pheochromocytoma, Benign	2/70 (38)	2/70 (43)	2/70 (44)	0/70 (44)	3/70 (44)
		0.2652	0.7376	0.7456	1.0000	0.5701
	Pheochromocytoma, Malignant	0/70 (38)	1/70 (43)	2/70 (44)	0/70 (44)	2/70 (44)
		0.2037	0.5309	0.2849	NC	0.2849
	Pheochromocytoma, Benign/ Pheochromocytoma, Malignant	2/70 (38)	3/70 (43)	4/70 (45)	0/70 (44)	5/70 (45)
		0.1393	0.5592	0.4221	1.0000	0.2923

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 2B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Rats (Continued)

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	7 mg P-HvsC
Gland, Clitoral	Adenocarcinoma	1/69 (38)	0/70 (43)	2/70 (45)	0/70 (44)	1/70 (44)
		0.4850	1.0000	0.5639	1.0000	0.7883
	Papilloma	0/69 (37)	1/70 (43)	0/70 (44)	0/70 (44)	0/70 (44)
		0.8255	0.5375	NC	NC	NC
	Squamous Cell Carcinoma	0/69 (37)	0/70 (43)	0/70 (44)	1/70 (45)	0/70 (44)
		0.4178	NC	NC	0.5488	NC
Gland, Mammary	Adenocarcinoma	9/70 (43)	4/69 (44)	8/70 (48)	2/70 (45)	10/70 (49)
		0.1317	0.9692	0.7853	0.9971	0.6263
	Adenoma	14/70 (42)	13/69 (45)	17/70 (50)	15/70 (49)	16/70 (49)
		0.4548	0.7516	0.5619	0.6930	0.6156
	Fibroadenoma	41/70 (52)	48/69 (57)	41/70 (57)	50/70 (59)	54/70 (61)
		0.0466	0.3171	0.8549	0.2875	0.1266
	Adenoma /Fibroadenoma	43/70 (54)	51/69 (59)	45/70 (59)	50/70 (59)	57/70 (63)
		0.0395	0.2372	0.7436	0.3207	0.0813
	Adenocarcinoma/Adenoma	23/70 (48)	17/69 (49)	25/70 (55)	17/70 (51)	26/70 (57)
		0.2811	0.9371	0.6728	0.9540	0.6671
	Adenosquamous Carcinoma	0/70 (38)	2/69 (43)	2/70 (45)	1/70 (45)	1/70 (44)
		0.5464	0.2787	0.2909	0.5422	0.5366
Gland, Parathyroid Adenoma		0/67 (35)	0/67 (41)	0/64 (40)	1/68 (43)	0/63 (40)
		0.4171	NC	NC	0.5513	NC
Gland, Pituitary	Adenoma	51/70 (62)	44/70 (60)	38/70 (59)	39/70 (61)	41/70 (58)
		0.5973	0.9203	0.9928	0.9940	0.9570
	Carcinoma	0/70 (38)	1/70 (43)	1/70 (44)	1/70 (45)	0/70 (44)
		0.6655	0.5309	0.5366	0.5422	NC
	Adenoma/ Carcinoma	51/70 (62)	45/70 (60)	39/70 (59)	40/70 (61)	41/70 (58)
		0.6650	0.8850	0.9879	0.9901	0.9570

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 2B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Rats (Continued)

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	7 mg P-HvsC
Gland, Thyroid	C-Cell Adenoma	5/70 (39)	3/70 (43)	4/70 (46)	5/70 (46)	4/70 (45)
		0.5327	0.8971	0.8337	0.7316	0.8248
	C-Cell Carcinoma	2/70 (38)	1/70 (43)	2/70 (45)	0/70 (44)	2/70 (44)
		0.4123	0.9011	0.7532	1.0000	0.7456
	C-Cell Adenoma/ C-Cell Carcinoma	7/70 (40)	4/70 (44)	6/70 (47)	5/70 (46)	6/70 (46)
		0.4733	0.9291	0.8209	0.8843	0.8098
	Follicular Cell Adenoma	1/70 (38)	1/70 (43)	0/70 (44)	2/70 (45)	0/70 (44)
0.7094		0.7830	1.0000	0.5639	1.0000	
Follicular Cell Carcinoma	0/70 (38)	1/70 (43)	0/70 (44)	0/70 (44)	0/70 (44)	
	0.8216	0.5309	NC	NC	NC	
Follicular Cell Adenoma/ Follicular Cell Carcinoma	1/70 (38)	2/70 (43)	0/70 (44)	2/70 (45)	0/70 (44)	
	0.8013	0.5468	1.0000	0.5639	1.0000	
Hemolymphoretic ular Tissue	Histiocytic Sarcoma	1/70 (38)	0/70 (43)	1/70 (44)	0/70 (44)	3/70 (45)
		0.0510	1.0000	0.7883	1.0000	0.3745
	Leukemia, Large Granular Lymph	0/70 (38)	0/70 (43)	0/70 (44)	1/70 (45)	1/70 (44)
		0.1284	NC	NC	0.5422	0.5366
Lymphoma, Malignant	0/70 (38)	1/70 (43)	1/70 (44)	2/70 (46)	0/70 (44)	
	0.6605	0.5309	0.5366	0.2969	NC	
Kidney	Adenoma	0/70 (38)	1/70 (44)	1/70 (44)	0/70 (44)	0/70 (44)
		0.7809	0.5366	0.5366	NC	NC
	Liposarcoma	0/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	1/70 (44)
		0.2066	NC	NC	NC	0.5366
Larynx	Fibrosarcoma	0/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	1/70 (44)
		0.2066	NC	NC	NC	0.5366
Liver	Hepatocellular Adenoma	1/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	0/70 (44)
		1.0000	1.0000	1.0000	1.0000	1.0000
Lung	Bronchioloalveolar Adenoma	0/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	1/70 (44)
		0.2066	NC	NC	NC	0.5366
	Epithelioma	0/70 (38)	0/70 (43)	0/70 (44)	1/70 (45)	0/70 (44)
		0.4159	NC	NC	0.5422	NC
Lymph Node	Hemangioma	0/17 (9)	1/21 (13)	0/17 (10)	0/16 (10)	0/19 (12)
		0.8333	0.5909	NC	NC	NC

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 2B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Rats (Continued)

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	7 mg P-HvsC
Lymph Node, Mesenteric	Hemangioma	0/70 (38)	1/70 (43)	0/70 (44)	0/70 (44)	0/70 (44)
		0.8216	0.5309	NC	NC	NC
	Hemangiosarcoma	0/70 (38)	1/70 (43)	0/70 (44)	0/70 (44)	0/70 (44)
		0.8216	0.5309	NC	NC	NC
	Hemangioma/ Hemangiosarcoma	0/70 (38)	2/70 (43)	0/70 (44)	0/70 (44)	0/70 (44)
		0.8965	0.2787	NC	NC	NC
Neuroendocrine Cell Tumor, Ben	0/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	1/70 (44)	
		0.2066	NC	NC	NC	0.5366
Muscle, Skeletal	Fibrosarcoma	0/70 (38)	0/70 (43)	0/70 (44)	1/70 (45)	0/70 (44)
		0.4159	NC	NC	0.5422	NC
	Hemangiosarcoma	0/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	1/70 (44)
		0.2066	NC	NC	NC	0.5366
Ovary	Granulosa Cell Tumor, Benign	0/70 (38)	1/70 (43)	0/70 (44)	0/70 (44)	1/70 (44)
		0.2972	0.5309	NC	NC	0.5366
	Mesothelioma, Malignant	1/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	0/70 (44)
		1.0000	1.0000	1.0000	1.0000	1.0000
Pancreas	Adenocarcinoma	0/70 (38)	0/70 (43)	0/70 (44)	1/70 (45)	0/70 (44)
		0.4159	NC	NC	0.5422	NC
	Adenocarcinoma/ Adenoma	0/70 (38)	1/70 (43)	2/70 (44)	1/70 (45)	0/70 (44)
		0.7462	0.5309	0.2849	0.5422	NC
	Adenoma	0/70 (38)	1/70 (43)	2/70 (44)	0/70 (44)	0/70 (44)
		0.8339	0.5309	0.2849	NC	NC
	Islet Cell Adenoma	0/70 (38)	3/70 (43)	3/70 (45)	2/70 (45)	1/70 (44)
		0.7014	0.1446	0.1544	0.2909	0.5366
	Islet Cell Carcinoma	0/70 (38)	1/70 (43)	0/70 (44)	1/70 (45)	0/70 (44)
		0.5849	0.5309	NC	0.5422	NC
Islet Cell Adenoma/ Islet Cell Carcinoma	0/70 (38)	4/70 (44)	3/70 (45)	3/70 (45)	1/70 (44)	
		0.7656	0.0776	0.1544	0.1544	0.5366

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 2B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Rats (Continued)

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	7 mg P-HvsC
Skin	Basal Cell Tumor, Malignant	1/70 (39)	0/69 (42)	0/70 (44)	0/70 (44)	0/70 (44)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Keratoacanthoma	2/70 (38)	1/69 (42)	0/70 (44)	2/70 (45)	2/70 (44)
		0.2692	0.8973	1.0000	0.7532	0.7456
	Squamous Cell Carcinoma	1/70 (38)	0/69 (42)	0/70 (44)	0/70 (44)	0/70 (44)
		1.0000	1.0000	1.0000	1.0000	1.0000
Keratoacanthoma/ Squamous Cell Carcinoma	3/70 (39)	1/69 (42)	0/70 (44)	2/70 (45)	2/70 (44)	
Melanoma, Benign	0/70 (38)	0/69 (42)	1/70 (44)	0/70 (44)	0/70 (44)	
	0.6226	NC	0.5366	NC	NC	
Small Intestine, Jejunum	Adenocarcinoma	0/69 (38)	1/70 (43)	0/70 (44)	0/69 (44)	0/70 (44)
		0.8216	0.5309	NC	NC	NC
	Leiomyoma	0/69 (38)	0/70 (43)	1/70 (44)	2/69 (45)	1/70 (44)
		0.2180	NC	0.5366	0.2909	0.5366
	Leiomyosarcoma	0/69 (38)	1/70 (43)	0/70 (44)	0/69 (44)	0/70 (44)
		0.8216	0.5309	NC	NC	NC
Leiomyoma/ Leiomyosarcoma	0/69 (38)	1/70 (43)	1/70 (44)	2/69 (45)	1/70 (44)	
0.3317	0.5309	0.5366	0.2909	0.5366		
Spleen	Fibrosarcoma	0/70 (38)	0/70 (43)	1/70 (44)	0/70 (44)	0/70 (44)
		0.6197	NC	0.5366	NC	NC
	Hemangioma	1/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	0/70 (44)
		1.0000	1.0000	1.0000	1.0000	1.0000
Stomach	Adenoma	0/70 (38)	0/70 (43)	0/70 (44)	1/70 (45)	0/70 (44)
		0.4159	NC	NC	0.5422	NC
	Papilloma	0/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	1/70 (44)
		0.2066	NC	NC	NC	0.5366

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

**Table 2B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Rats
(Continued)**

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)	
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	7 mg P-HvsC	
Subcutis	Fibroma	3/10 (8) 0.6047	4/13 (9) 0.5806	8/13 (12) 0.2049	4/12 (8) 0.5000	5/14 (11) 0.5506	
	Fibrosarcoma	2/10 (7) 0.1901	1/13 (9) 0.9375	1/13 (10) 0.9485	2/12 (8) 0.7692	3/14 (10) 0.6855	
	Fibroma/ Fibrosarcoma	5/10 (9) 0.4040	5/13 (9) NC	9/13 (12) 0.3189	6/12 (9) 0.5000	8/14 (12) 0.4721	
	Hemangiosarcoma	0/10 (6) 0.2381	0/13 (9) NC	0/13 (10) NC	0/12 (7) NC	1/14 (10) 0.6250	
	Lipoma	3/10 (7) 0.6779	4/13 (10) 0.7318	0/13 (10) 1.0000	2/12 (8) 0.8998	2/14 (10) 0.9400	
	Liposarcoma	0/10 (6) 0.6341	0/13 (9) NC	1/13 (10) 0.6250	0/12 (7) NC	0/14 (9) NC	
	Lipoma/ Liposarcoma	3/10 (7) 0.7507	4/13 (10) 0.7318	1/13 (10) 0.9853	2/12 (8) 0.8998	2/14 (10) 0.9400	
	Osteosarcoma	1/10 (7) 1.0000	0/13 (9) 1.0000	0/13 (10) 1.0000	0/12 (7) 1.0000	0/14 (9) 1.0000	
	Schwannoma, Benign	0/10 (6) 0.3954	1/13 (9) 0.6000	1/13 (10) 0.6250	0/12 (7) NC	1/14 (9) 0.6000	
	Schwannoma, Malignant	0/10 (6) 0.6759	1/13 (9) 0.6000	1/13 (10) 0.6250	1/12 (7) 0.5385	0/14 (9) NC	
	Schwannoma, Benign/ Schwannoma, Malignant	0/10 (6) 0.5351	2/13 (10) 0.3750	2/14 (11) 0.4044	1/12 (7) 0.5385	1/14 (9) 0.6000	
	Thymus	Thymoma, Benign	0/69 (37) 0.8061	0/70 (43) NC	3/70 (45) 0.1602	0/70 (44) NC	0/69 (43) NC
		Thymoma, Malignant	0/69 (37) 0.1269	0/70 (43) NC	0/70 (44) NC	1/70 (45) 0.5488	1/69 (43) 0.5375
		Thymoma, Benign/ Thymoma, Malignant	0/69 (37) 0.3919	0/70 (43) NC	3/70 (45) 0.1602	1/70 (45) 0.5488	1/69 (43) 0.5375

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 2B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Rats (Continued)

Organ name	Tumor name	Vehicle (C)	Low (L)	Mid (M)	Mid-High (MH)	High (H)
		0 mg P-Trend	0.02 mg LP-LvsC	0.1 mg P-MvsC	0.8 mg P-MHvsC	7 mg P-HvsC
Uterus	Adenocarcinoma	0/70 (38)	0/70 (43)	1/70 (44)	0/70 (44)	0/70 (44)
		0.6197	NC	0.5366	NC	NC
	Endometrial Stromal Polyp	1/70 (38)	2/70 (43)	7/70 (46)	2/70 (45)	6/70 (45)
		0.1114	0.5468	0.0526	0.5639	0.0855
	Endometrial Stromal Sarcoma	0/70 (38)	1/70 (43)	0/70 (44)	0/70 (44)	0/70 (44)
		0.8216	0.5309	NC	NC	NC
	Endometrial Stromal Polyp/ Endometrial Stromal Sarcoma	1/70 (38)	3/70 (44)	7/70 (46)	2/70 (45)	6/70 (45)
		0.1426	0.3654	0.0526	0.5639	0.0855
	Granular Cell Tumor, Benign	0/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	1/70 (44)
		0.2066	NC	NC	NC	0.5366
Leiomyosarcoma	0/70 (38)	0/70 (43)	0/70 (44)	0/70 (44)	1/70 (44)	
	0.2066	NC	NC	NC	0.5366	
Polyp	1/70 (38)	0/70 (43)	1/70 (44)	0/70 (44)	0/70 (44)	
	0.8565	1.0000	0.7883	1.0000	1.0000	
Whole Body	Hemangioma	1/70 (38)	2/70 (43)	0/70 (44)	0/70 (44)	0/70 (44)
		0.9757	0.5468	1.0000	1.0000	1.0000
	Hemangiosarcoma	1/70 (39)	1/70 (43)	0/70 (44)	0/70 (44)	2/70 (44)
		0.1798	0.7769	1.0000	1.0000	0.5457
	Hemangioma/ Hemangiosarcoma	2/70 (39)	3/70 (43)	0/70 (44)	0/70 (44)	2/70 (44)
		0.4370	0.5468	1.0000	1.0000	0.7364

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 3A: Intercurrent Mortality Rate in Male Mice

Week / Type of Death	Vehicle Control		0.2 mg/kg/day + M Low		0.8 mg/kg/day + M Mid		3 mg/kg/day + M High		0.8 mg/kg/day Group 3	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	4	6.67	5	8.33	3	5.00	1	1.67	4	6.67
53 - 78	8	20.00	5	16.67	3	10.00	2	5.00	4	13.33
79 - 91	8	33.33	12	36.67	12	30.00	17	33.33	5	21.67
92 - 104	6	43.33	5	45.00	14	53.33	7	45.00	15	46.67
Terminal sacrifice	34	56.67	33	55.00	28	46.67	33	55.00	32	53.33
Total	60		60		60		60		60	
Test	All Dose Groups		Vehicle Control vs. Low		Vehicle Control vs. Mid		Vehicle Control vs. High		Vehicle Control vs. Group 3	
Dose-Response (Likelihood Ratio)	0.7781		0.8515		0.4938		0.9040		0.9832	
Homogeneity (Log-Rank)	0.8802		0.8509		0.4916		0.9032		0.9831	

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

Table 3B: Intercurrent Mortality Rate in Female Mice

Week / Type of Death	Vehicle Control		0.2 mg/kg/day + M Low		0.8 mg/kg/day + M Mid		3 mg/kg/day + M High		0.8 mg/kg/day Group 3	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	4	6.67	4	6.67	1	1.67	3	5.00	2	3.33
53 - 78	7	18.33	6	16.67	5	10.00	11	23.33	5	11.67
79 - 91	8	31.67	8	30.00	7	21.67	11	41.67	10	28.33
92 - 104	12	51.67	14	53.33	11	40.00	9	56.67	11	46.67
Accidental Death			1	1.67						
Terminal sacrifice	29	48.33	27	45.00	36	60.00	26	43.33	32	53.33
Total	60		60		60		60		60	
Test	All Dose Groups		Vehicle Control vs. Low		Vehicle Control vs. Mid		Vehicle Control vs. High		Vehicle Control vs. Group 3	
Dose-Response (Likelihood Ratio)	0.3024		0.7997		0.1873		0.4047		0.5986	
Homogeneity (Log-Rank)	0.1934		0.7979		0.1841		0.4014		0.5968	

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

Table 4A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Mice

Organ name	Tumor name	Vehicle (VC)	Low (L)	Mid (M)	High (H)	Group 3 (G3)
		0 mg P - Trend	0.2 mg P - VC vs. L	0.8 mg P - VC vs. M	3 mg P - VC vs. H	0.8 mg P - VC vs. G3
Adipose Tissue	Hemangioma	0/1 (1) 0.9487	2/4 (4) 0.6000	0/5 (4) NC	0/6 (4) NC	0/2 (1) NC
Body Cavity, Nasal	Adenoma	1/60 (46) 1.0000	0/60 (46) 1.0000	0/60 (48) 1.0000	0/60 (49) 1.0000	0/60 (49) 1.0000
	Hemangioma	0/60 (46) 0.7566	1/60 (46) 0.5000	0/60 (48) NC	0/60 (49) NC	0/60 (49) NC
Bone Marrow	Hemangiosarcoma	3/60 (47) 0.9058	0/60 (46) 1.0000	2/60 (48) 0.8258	0/60 (49) 1.0000	0/60 (49) 1.0000
Bone, Femur	Osteosarcoma	0/59 (46) 0.2593	0/60 (46) NC	0/60 (48) NC	1/60 (49) 0.5158	0/59 (48) NC
Bone, Sternum	Osteosarcoma	1/60 (46) 1.0000	0/60 (46) 1.0000	0/60 (48) 1.0000	0/60 (49) 1.0000	0/60 (49) 1.0000
Epididymis	Fibrosarcoma	0/60 (46) NC	0/60 (46) NC	0/60 (48) NC	0/60 (49) NC	1/60 (49) 0.5158
	Hemangiosarcoma	0/60 (46) 0.7579	1/60 (47) 0.5054	0/60 (48) NC	0/60 (49) NC	0/60 (49) NC
	Interstitial (Leydig) Cell Ade	1/60 (46) 0.4523	0/60 (46) 1.0000	0/60 (48) 1.0000	1/60 (49) 0.7682	1/60 (49) 0.7682
Gallbladder	Adenoma	3/59 (46) 0.6532	2/59 (45) 0.8126	3/59 (47) 0.6718	2/60 (49) 0.8383	6/59 (49) 0.2757
Gland, Adrenal	Pheochromocytoma, Benign	1/60 (46) 0.9433	2/60 (46) 0.5000	0/60 (48) 1.0000	0/60 (49) 1.0000	2/60 (49) 0.5239
	Pheochromocytoma, Malignant	0/60 (46) 0.1986	0/60 (46) NC	1/60 (48) 0.5106	1/60 (49) 0.5158	0/60 (49) NC
	Pheochromocytoma, Benign/ Pheochromocytoma, Malignant	1/60 (45) 0.6346	2/60 (44) 0.4915	1/60 (46) 0.7582	1/60 (48) 0.7686	2/60 (48) 0.5245
	Subcapsular Adrenal Tumor, Benign	4/60 (46) 0.3305	2/60 (46) 0.8984	1/60 (48) 0.9750	4/60 (50) 0.6887	5/60 (49) 0.5406
	Subcapsular Adrenal Tumor, Malignant	0/60 (46) 0.3825	0/60 (46) NC	4/60 (48) 0.0638	1/60 (49) 0.5158	3/60 (49) 0.1331
	Subcapsular Adrenal Tumor, Benign/ Malignant	4/60 (45) 0.2774	2/60 (45) 0.8986	5/60 (47) 0.5280	5/60 (49) 0.5546	8/60 (48) 0.2102

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;

NC = Not calculable.

**Table 4A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Mice
(Continued)**

Organ name	Tumor name	Vehicle (VC)	Low (L)	Mid (M)	High (H)	Group 3 (G3)
		0 mg P - Trend	0.2 mg P - VC vs. L	0.8 mg P - VC vs. M	3 mg P - VC vs. H	0.8 mg P - VC vs. G3
Gland, Harderian	Adenocarcinoma	1/60 (46)	0/60 (46)	1/60 (48)	1/60 (49)	0/60 (49)
		0.4106	1.0000	0.7632	0.7682	1.0000
	Adenoma	8/60 (48)	14/60 (47)	10/60 (50)	10/60 (50)	9/60 (49)
		0.6220	0.1014	0.4351	0.4351	0.5188
	Adenocarcinoma/Adenoma	9/60 (47)	14/60 (46)	11/60 (49)	11/60 (49)	9/60 (48)
		0.5808	0.1537	0.4423	0.4423	0.6221
Gland, Pituitary	Adenoma, Pars Distalis	1/60 (46)	1/60 (46)	1/59 (48)	1/60 (49)	2/58 (48)
		0.5355	NC	0.7632	0.7682	0.5161
	Carcinoma, Pars Distalis	0/60 (46)	0/60 (46)	0/59 (48)	1/60 (49)	0/58 (47)
		0.2593	NC	NC	0.5158	NC
	Adenoma, Pars Distalis/ Carcinoma, Pars Distalis	1/60 (45)	1/60 (45)	1/59 (47)	2/60 (48)	2/58 (46)
		0.2792	NC	0.7635	0.5245	0.5083
Gland, Seminal Vesicle	Adenocarcinoma	0/60 (46)	0/60 (46)	0/60 (48)	2/60 (49)	0/60 (49)
		0.0662	NC	NC	0.2634	NC
	Adenoma	0/60 (46)	0/60 (46)	0/60 (48)	0/60 (49)	2/60 (49)
		NC	NC	NC	NC	0.2634
	Granular Cell Tumor, Malignant	1/60 (47)	0/60 (46)	0/60 (48)	0/60 (49)	0/60 (49)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Leiomyosarcoma	1/60 (46)	1/60 (46)	1/60 (48)	0/60 (49)	0/60 (49)
		0.8417	NC	0.7632	1.0000	1.0000
	Osteosarcoma	0/60 (46)	0/60 (46)	0/60 (48)	1/60 (49)	0/60 (49)
		0.2593	NC	NC	0.5158	NC
	Sarcoma	0/60 (46)	0/60 (46)	1/60 (48)	0/60 (49)	0/60 (49)
		0.5132	NC	0.5106	NC	NC
Gland, Thyroid	Follicular Cell Adenoma	0/60 (46)	0/60 (46)	0/60 (48)	1/60 (49)	0/60 (49)
		0.2593	NC	NC	0.5158	NC
Hemolymphoretic ular Tissue	Histiocytic Sarcoma	0/60 (46)	1/60 (46)	1/60 (48)	0/60 (49)	0/60 (49)
		0.6401	0.5000	0.5106	NC	NC
	Lymphoma, Malignant	4/60 (48)	8/60 (47)	3/60 (49)	2/60 (49)	10/60 (49)
		0.9458	0.1674	0.7907	0.9027	0.0794
Kidney	Lipoma	1/60 (47)	1/60 (46)	0/60 (48)	0/60 (49)	0/60 (49)
		0.9398	0.7473	1.0000	1.0000	1.0000
	Tubular Cell Carcinoma	1/60 (46)	0/60 (46)	0/60 (48)	0/60 (49)	2/60 (49)
		1.0000	1.0000	1.0000	1.0000	0.5239

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

**Table 4A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Mice
(Continued)**

Organ name	Tumor name	Vehicle (VC)	Low (L)	Mid (M)	High (H)	Group 3 (G3)
		0 mg P - Trend	0.2 mg P - VC vs. L	0.8 mg P - VC vs. M	3 mg P - VC vs. H	0.8 mg P - VC vs. G3
Large Intestine, Cecum	Leiomyosarcoma	0/60 (46)	0/60 (46)	0/60 (48)	0/60 (49)	1/60 (49)
		NC	NC	NC	NC	0.5158
Larynx	Squamous Cell Carcinoma	0/60 (46) 0.8226	2/60 (46) 0.2473	0/60 (48) NC	0/60 (49) NC	0/60 (49) NC
Liver	Hemangiosarcoma	2/60 (46) 0.3526	3/60 (46) 0.5000	0/60 (48) 1.0000	3/60 (50) 0.5399	2/60 (50) 0.7224
	Hepatocellular Adenoma	7/60 (47) 0.6773	3/60 (46) 0.9513	2/60 (48) 0.9860	4/60 (49) 0.9131	9/60 (49) 0.4283
	Hepatocellular Carcinoma	4/60 (47) 0.4680	9/60 (46) 0.1075	6/60 (48) 0.3836	7/60 (51) 0.3116	12/60 (50) 0.0360
	Hepatocellular Adenoma/ Hepatocellular Carcinoma	11/60 (46) 0.6097	12/60 (46) 0.5000	8/60 (47) 0.8603	11/60 (50) 0.6795	21/60 (49) 0.0408
Lung	Adenosquamous Carcinoma	0/60 (46) 0.2632	0/60 (46) NC	0/60 (48) NC	1/60 (50) 0.5208	0/60 (49) NC
	Bronchioloalveolar Adenoma	10/60 (48) 0.7916	13/60 (48) 0.3166	12/60 (49) 0.4260	9/60 (50) 0.7290	11/60 (49) 0.5214
	Bronchioloalveolar Carcinoma	19/60 (51) 0.3769	15/60 (49) 0.8191	19/60 (53) 0.6378	20/60 (54) 0.5892	21/60 (53) 0.4816
	Bronchioloalveolar Adenoma/ Carcinoma	29/60 (51) 0.6885	28/60 (50) 0.6133	31/60 (54) 0.5559	29/60 (55) 0.7331	32/60 (52) 0.3889
Lymph Node, Mesenteric	Hemangiosarcoma	0/60 (46) 0.7566	1/60 (46) 0.5000	0/60 (48) NC	0/60 (49) NC	0/59 (49) NC
Muscle, Skeletal	Hemangiosarcoma	0/59 (46) 0.7540	1/60 (46) 0.5000	0/59 (47) NC	0/59 (48) NC	0/59 (48) NC
Pancreas	Hemangiosarcoma	0/60 (46) 0.2632	0/60 (46) NC	0/60 (48) NC	1/60 (50) 0.5208	0/60 (49) NC
Site, Injection	Fibrosarcoma	0/60 (46) 0.5158	0/60 (46) NC	2/60 (48) 0.2581	0/60 (49) NC	1/60 (49) 0.5158
Skin	Basal Cell Tumor, Benign	1/60 (46) 1.0000	0/60 (46) 1.0000	0/60 (48) 1.0000	0/60 (49) 1.0000	1/60 (49) 0.7682
	Papilloma	0/60 (46) 0.7566	1/60 (46) 0.5000	0/60 (48) NC	0/60 (49) NC	0/60 (49) NC
	Sebaceous Cell Adenoma	1/60 (46) 1.0000	0/60 (46) 1.0000	0/60 (48) 1.0000	0/60 (49) 1.0000	1/60 (49) 0.7682

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

**Table 4A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Mice
(Continued)**

Organ name	Tumor name	Vehicle (VC)	Low (L)	Mid (M)	High (H)	Group 3 (G3)
		0 mg	0.2 mg	0.8 mg	3 mg	0.8 mg
		P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. G3
Small Intestine, Duodenum	Adenocarcinoma	1/60 (46)	0/60 (46)	1/58 (46)	0/60 (49)	0/60 (49)
		0.7593	1.0000	NC	1.0000	1.0000
	Adenoma	1/60 (47)	2/60 (46)	0/58 (46)	0/60 (49)	1/60 (49)
		0.9395	0.4918	1.0000	1.0000	0.7629
		Adenocarcinoma/Adenoma	2/60 (45)	2/60 (44)	1/58 (45)	0/60 (48)
	0.9515	0.6833	0.8792	1.0000	0.8906	
Small Intestine, Jejunum	Adenocarcinoma	1/58 (45)	0/60 (46)	0/60 (48)	1/59 (48)	0/60 (49)
		0.4485	1.0000	1.0000	0.7686	1.0000
Spinal Cord, Lumbar	Astrocytoma, Malignant	1/60 (47)	0/60 (46)	0/60 (48)	0/60 (49)	0/60 (49)
		1.0000	1.0000	1.0000	1.0000	1.0000
Spleen	Hemangiosarcoma	1/60 (46)	1/60 (46)	3/60 (48)	1/60 (49)	0/60 (49)
		0.5519	NC	0.3247	0.7682	1.0000
Stomach	Adenoma	0/60 (46)	1/60 (46)	1/60 (48)	0/60 (49)	0/60 (49)
		0.6401	0.5000	0.5106	NC	NC
	Papilloma	0/60 (46)	0/60 (46)	1/60 (48)	0/60 (49)	1/60 (49)
		0.5132	NC	0.5106	NC	0.5158
	Squamous Cell Carcinoma	1/60 (46)	0/60 (46)	1/60 (48)	0/60 (49)	1/60 (49)
	0.7644	1.0000	0.7632	1.0000	0.7682	
Subcutis	Hemangioma	1/3 (3)	0/2 (2)	0/1 (1)	0/1 (1)	0/2 (2)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Hemangiosarcoma	1/3 (3)	2/2 (2)	1/1 (1)	0/1 (1)	1/2 (2)
		0.6571	0.3000	0.5000	1.0000	0.7000
	Hemangioma/Hemangiosarcoma	2/3 (3)	2/2 (2)	1/1 (1)	0/1 (1)	1/2 (2)
		0.8571	0.6000	0.7500	1.0000	0.9000
	Myxosarcoma	1/3 (3)	0/2 (2)	0/1 (1)	0/1 (1)	0/2 (2)
1.0000		1.0000	1.0000	1.0000	1.0000	
Testis	Interstitial (Leydig) Cell Ade	1/60 (46)	5/60 (46)	0/60 (48)	1/60 (49)	0/60 (49)
		0.8652	0.1016	1.0000	0.7682	1.0000
Whole body	Hemangioma	1/60 (45)	3/60 (44)	0/60 (46)	0/60 (48)	0/60 (47)
		0.9622	0.2997	1.0000	1.0000	1.0000
	Hemangiosarcoma	6/60 (46)	5/60 (46)	5/60 (46)	4/60 (49)	3/60 (49)
		0.7613	0.7389	0.7389	0.8663	0.9346
	Hemangioma/Hemangiosarcoma	7/60 (46)	8/60 (46)	5/60 (46)	4/60 (49)	3/60 (49)
		0.9076	0.5000	0.8231	0.9191	0.9639
	Osteosarcoma	1/60 (45)	0/60 (44)	0/60 (46)	2/60 (48)	0/60 (47)
		0.1688	1.0000	1.0000	0.5245	1.0000

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;

NC = Not calculable.

Table 4B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Mice

Organ name	Tumor name	Vehicle (VC)	Low (L)	Mid (M)	High (H)	Group 3 (G3)
		0 mg P - Trend	0.2 mg P - VC vs. L	0.8 mg P - VC vs. M	3 mg P - VC vs. H	0.8 mg P - VC vs. G3
Adipose Tissue	Hemangioma	0/10 (8) 0.4615	0/8 (6) NC	1/6 (5) 0.3846	0/9 (7) NC	0/5 (4) NC
Bone Marrow	Hemangiosarcoma	2/60 (47) 0.5478	0/60 (45) 1.0000	0/60 (51) 1.0000	1/60 (43) 0.8620	0/60 (48) 1.0000
Bone, Femur	Osteosarcoma	1/60 (47) 0.4054	0/59 (44) 1.0000	0/60 (51) 1.0000	1/60 (42) 0.7240	1/60 (48) 0.7579
Bone, Sternum	Osteosarcoma	1/60 (47) 1.0000	0/60 (45) 1.0000	0/60 (51) 1.0000	0/60 (42) 1.0000	0/60 (48) 1.0000
Brain	Meningioma, Benign	0/60 (47) 0.2312	0/60 (45) NC	0/60 (51) NC	1/60 (43) 0.4778	0/60 (48) NC
Cervix	Leiomyosarcoma	0/59 (46) 0.2320	0/56 (42) NC	0/60 (51) NC	1/60 (42) 0.4773	0/60 (48) NC
	Squamous Cell Carcinoma	0/59 (46) 0.5138	0/56 (42) NC	1/60 (51) 0.5258	0/60 (42) NC	0/60 (48) NC
Gallbladder	Adenoma	1/60 (47) 0.0396	1/60 (45) 0.7418	2/59 (50) 0.5234	4/58 (42) 0.1472	2/59 (47) 0.5000
Gland, Adrenal	Subcapsular Adrenal Tumor, Ben	0/60 (47) 0.7190	2/60 (45) 0.2365	2/60 (51) 0.2683	0/60 (42) NC	0/60 (48) NC
	Subcapsular Adrenal Tumor, Mal	0/60 (47) 0.7220	2/60 (45) 0.2365	1/60 (51) 0.5204	0/60 (42) NC	1/60 (48) 0.5053
Gland, Harderian	Adenocarcinoma	0/60 (47) 0.5027	0/60 (45) NC	1/60 (51) 0.5204	0/60 (42) NC	0/60 (48) NC
	Adenoma	4/60 (47) 0.9013	7/60 (46) 0.2489	7/60 (52) 0.3240	2/60 (43) 0.8768	12/60 (49) 0.0325
	Adenocarcinoma/Adenoma	4/60 (45) 0.8985	7/60 (45) 0.2608	8/60 (51) 0.2449	2/60 (41) 0.8761	12/60 (48) 0.0358
Gland, Mammary	Adenocarcinoma	1/60 (47) 0.4151	3/59 (46) 0.3002	2/60 (51) 0.5309	2/60 (43) 0.4663	1/60 (48) 0.7579

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;

NC = Not calculable.

**Table 4B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Mice
(Continued)**

Organ name	Tumor name	Vehicle (VC)	Low (L)	Mid (M)	High (H)	Group 3 (G3)
		0 mg	0.2 mg	0.8 mg	3 mg	0.8 mg
		P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. G3
Gland, Pituitary	Adenoma, Pars Distalis	1/59 (47)	2/60 (45)	6/60 (51)	2/60 (43)	1/60 (48)
		0.4342	0.4835	0.0696	0.4663	0.7579
	Carcinoma, Pars Distalis	1/59 (47)	0/60 (45)	0/60 (51)	0/60 (42)	1/60 (49)
		1.0000	1.0000	1.0000	1.0000	0.7629
	Adenoma, Pars Distalis/ Carcinoma, Pars Distalis	2/59 (46)	2/60 (44)	6/60 (50)	2/60 (42)	2/60 (47)
0.5314		0.6747	0.1627	0.6566	0.6998	
Craniopharyngioma, Malignant	0/59 (46)	0/60 (45)	1/60 (51)	0/60 (42)	0/60 (48)	
	0.5054	NC	0.5258	NC	NC	
Heart	Hemangiosarcoma	0/60 (47)	1/60 (45)	0/60 (51)	0/60 (42)	0/60 (48)
		0.7459	0.4891	NC	NC	NC
Hemolymphoretic ular Tissue	Histiocytic Sarcoma	4/60 (48)	3/60 (46)	3/60 (51)	3/60 (43)	2/60 (49)
		0.5337	0.7645	0.8064	0.7347	0.9027
	Lymphoma, Malignant	21/60 (52)	17/60 (49)	15/60 (53)	15/60 (46)	18/60 (51)
		0.7099	0.7867	0.9347	0.8430	0.7689
Kidney	Tubular Cell Adenoma	0/60 (47)	0/60 (45)	1/60 (51)	0/60 (42)	0/60 (48)
		0.5027	NC	0.5204	NC	NC
Liver	Hemangiosarcoma	2/60 (47)	0/60 (45)	1/60 (51)	1/60 (43)	0/60 (48)
		0.5422	1.0000	0.8934	0.8620	1.0000
	Hepatocellular Carcinoma	0/60 (47)	1/60 (46)	0/60 (51)	1/60 (42)	0/60 (48)
		0.2868	0.4946	NC	0.4719	NC
Lung	Bronchioloalveolar Adenoma	8/60 (48)	5/60 (46)	12/60 (52)	12/60 (45)	8/60 (50)
		0.0532	0.8673	0.2919	0.1788	0.6415
	Bronchioloalveolar Carcinoma	9/60 (48)	12/60 (47)	11/60 (52)	7/60 (45)	9/60 (49)
		0.8065	0.2917	0.4809	0.7518	0.6214
	Bronchioloalveolar Adenoma/ Carcinoma	17/60 (48)	17/60 (48)	23/60 (52)	19/60 (47)	17/60 (50)
		0.3171	NC	0.2439	0.3853	0.6404
Lymph Node	Hemangiosarcoma	0/22 (16)	0/21 (16)	1/10 (8)	0/18 (11)	0/14 (12)
		0.3725	NC	0.3333	NC	NC
Lymph Node, Mesenteric	Hemangioma	0/60 (47)	1/59 (45)	1/59 (50)	0/59 (42)	0/60 (48)
		0.6118	0.4891	0.5155	NC	NC
	Hemangiosarcoma	0/60 (47)	0/59 (45)	1/59 (50)	0/59 (42)	0/60 (48)
		0.5000	NC	0.5155	NC	NC
	Hemangioma/Hemangiosarcoma	0/60 (45)	1/59 (43)	2/59 (49)	0/59 (41)	0/60 (47)
		0.6210	0.4886	0.2690	NC	NC

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;

NC = Not calculable.

Table 4B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Mice (Continued)

Organ name	Tumor name	Vehicle (VC)	Low (L)	Mid (M)	High (H)	Group 3 (G3)
		0 mg P - Trend	0.2 mg P - VC vs. L	0.8 mg P - VC vs. M	3 mg P - VC vs. H	0.8 mg P - VC vs. G3
Muscle, Skeletal	Hemangiosarcoma	0/60 (47) NC	0/58 (44) NC	0/59 (50) NC	0/60 (42) NC	1/60 (49) 0.5104
Ovary	Cystadenoma	2/59 (46) 0.3648	1/60 (45) 0.8750	2/60 (51) 0.7295	2/60 (42) 0.6566	1/60 (48) 0.8868
	Granulosa Cell Tumor, Benign	2/59 (46) 0.5426	0/60 (45) 1.0000	0/60 (51) 1.0000	1/60 (42) 0.8617	0/60 (48) 1.0000
	Granulosa Cell Tumor, Malignant	0/59 (46) 0.2324	0/60 (45) NC	0/60 (51) NC	1/60 (43) 0.4831	0/60 (48) NC
	Granulosa Cell Tumor, Benign/ Malignant	2/59 (44) 0.2276	0/60 (44) 1.0000	0/60 (49) 1.0000	2/60 (41) 0.6648	0/60 (47) 1.0000
	Hemangiosarcoma	0/59 (46) 0.4811	0/60 (45) NC	2/60 (51) 0.2738	0/60 (42) NC	0/60 (48) NC
	Luteoma	0/59 (46) 0.6174	1/60 (45) 0.4945	1/60 (51) 0.5258	0/60 (42) NC	1/60 (48) 0.5106
	Mixed Sex Cord Stromal Tumor,	0/59 (46) 0.7225	2/60 (45) 0.2418	2/60 (51) 0.2738	0/60 (42) NC	2/60 (48) 0.2581
Pancreas	Islet Cell Adenoma	0/59 (46) 0.2283	0/60 (45) NC	0/60 (51) NC	1/60 (42) 0.4773	2/60 (49) 0.2634
Site, Injection	Fibrosarcoma	0/60 (47) 0.5027	0/60 (45) NC	1/60 (51) 0.5204	0/60 (42) NC	1/60 (48) 0.5053
	Mast Cell Tumor, Malignant	0/60 (47) 0.2312	0/60 (45) NC	0/60 (51) NC	1/60 (43) 0.4778	0/60 (48) NC
Skin	Papilloma	0/60 (47) NC	0/59 (44) NC	0/60 (51) NC	0/60 (42) NC	1/59 (48) 0.5053
Small Intestine, Duodenum	Adenocarcinoma	0/60 (47) 0.5027	0/60 (45) NC	1/60 (51) 0.5204	0/60 (42) NC	0/60 (48) NC
	Adenoma	0/60 (47) NC	0/60 (45) NC	0/60 (51) NC	0/60 (42) NC	1/60 (48) 0.5053
Small Intestine, Jejunum	Adenocarcinoma	1/59 (46) 0.4054	0/60 (45) 1.0000	0/60 (51) 1.0000	1/60 (42) 0.7296	1/60 (48) 0.7632
Spleen	Hemangiosarcoma	2/60 (47) 0.7061	2/60 (46) 0.6834	2/60 (51) 0.7217	1/60 (43) 0.8620	0/60 (48) 1.0000
Stomach	Adenoma	0/60 (47) 0.5027	0/60 (45) NC	1/60 (51) 0.5204	0/60 (42) NC	0/60 (48) NC

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

**Table 4B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Mice
(Continued)**

Organ name	Tumor name	Vehicle (VC)	Low (L)	Mid (M)	High (H)	Group 3 (G3)	
		0 mg P - Trend	0.2 mg P - VC vs. L	0.8 mg P - VC vs. M	3 mg P - VC vs. H	0.8 mg P - VC vs. G3	
Ureter	Transitional Cell Carcinoma	0/2 (1) 0.8571	1/2 (2) 0.6667	0/2 (2) NC	0/2 (2) NC	0/2 (2) NC	
Uterus	Adenocarcinoma	1/59 (46) 0.1309	0/60 (45) 1.0000	0/60 (51) 1.0000	2/60 (42) 0.4655	0/60 (48) 1.0000	
	Endometrial Stromal Sarcoma	0/59 (46) 0.2900	1/60 (46) 0.5000	0/60 (51) NC	1/60 (42) 0.4773	0/60 (48) NC	
	Polyp, Endometrial Stromal	1/59 (46) 0.7478	4/60 (46) 0.1805	2/60 (51) 0.5390	1/60 (43) 0.7357	0/60 (48) 1.0000	
	Endometrial Stromal Sarcoma/ Polyp, Endometrial Stromal	1/59 (44) 0.6041	5/60 (46) 0.1119	2/60 (49) 0.5407	2/60 (42) 0.4824	0/60 (47) 1.0000	
	Granular Cell Tumor, Benign	0/59 (46) NC	0/60 (45) NC	0/60 (51) NC	0/60 (42) NC	1/60 (49) 0.5158	
	Hemangioma	0/59 (46) 0.6162	1/60 (46) 0.5000	1/60 (51) 0.5258	0/60 (42) NC	1/60 (48) 0.5106	
	Hemangiosarcoma	3/59 (46) 0.4491	3/60 (46) NC	3/60 (52) 0.7178	3/60 (43) 0.6289	2/60 (48) 0.8323	
	Hemangioma/Hemangiosarcoma	3/59 (44) 0.5408	4/60 (45) 0.5127	4/60 (51) 0.5828	3/60 (42) 0.6394	3/60 (47) 0.6927	
	Leiomyoma	1/59 (46) 0.4904	1/60 (45) 0.7473	1/60 (51) 0.7777	1/60 (42) 0.7296	2/60 (48) 0.5161	
	Leiomyosarcoma	3/59 (46) 0.7284	1/60 (45) 0.9389	4/60 (52) 0.5689	1/60 (42) 0.9300	3/60 (49) 0.6911	
	Leiomyoma/Leiomyosarcoma	4/59 (45) 0.6831	2/60 (44) 0.8935	5/60 (50) 0.5674	2/60 (41) 0.8761	5/60 (47) 0.5280	
	Polyp, Glandular	6/59 (46) 0.6726	2/60 (45) 0.9686	5/60 (51) 0.7946	3/60 (43) 0.9046	3/60 (48) 0.9304	
	Vagina	Leiomyosarcoma	0/59 (46) 0.6174	1/58 (45) 0.4945	1/60 (51) 0.5258	0/60 (42) NC	0/58 (46) NC
		Squamous Cell Carcinoma	0/59 (46) NC	0/58 (45) NC	0/60 (51) NC	0/60 (42) NC	1/58 (46) 0.5000

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

**Table 4B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Mice
(Continued)**

Organ name	Tumor name	Vehicle (VC)	Low (L)	Mid (M)	High (H)	Group 3 (G3)
		0 mg P - Trend	0.2 mg P - VC vs. L	0.8 mg P - VC vs. M	3 mg P - VC vs. H	0.8 mg P - VC vs. G3
Whole body	Hemangioma	0/60 (45)	2/60 (44)	3/60 (50)	0/60 (41)	1/60 (47)
		0.7576	0.2416	0.1416	NC	0.5109
	Hemangiosarcoma	7/60 (46)	4/60 (45)	7/60 (51)	5/60 (43)	4/60 (48)
		0.5585	0.8946	0.6911	0.7890	0.9136
	Hemangioma/Hemangiosarcoma	7/60 (46)	6/60 (45)	10/60 (52)	5/60 (43)	5/60 (48)
		0.6905	0.7101	0.4005	0.7890	0.8427
	Osteosarcoma	2/60 (45)	0/60 (44)	0/60 (49)	1/60 (41)	3/60 (47)
		0.5441	1.0000	1.0000	0.8613	0.5208

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Figure 1A: Kaplan-Meier Survival Functions for Male Rats

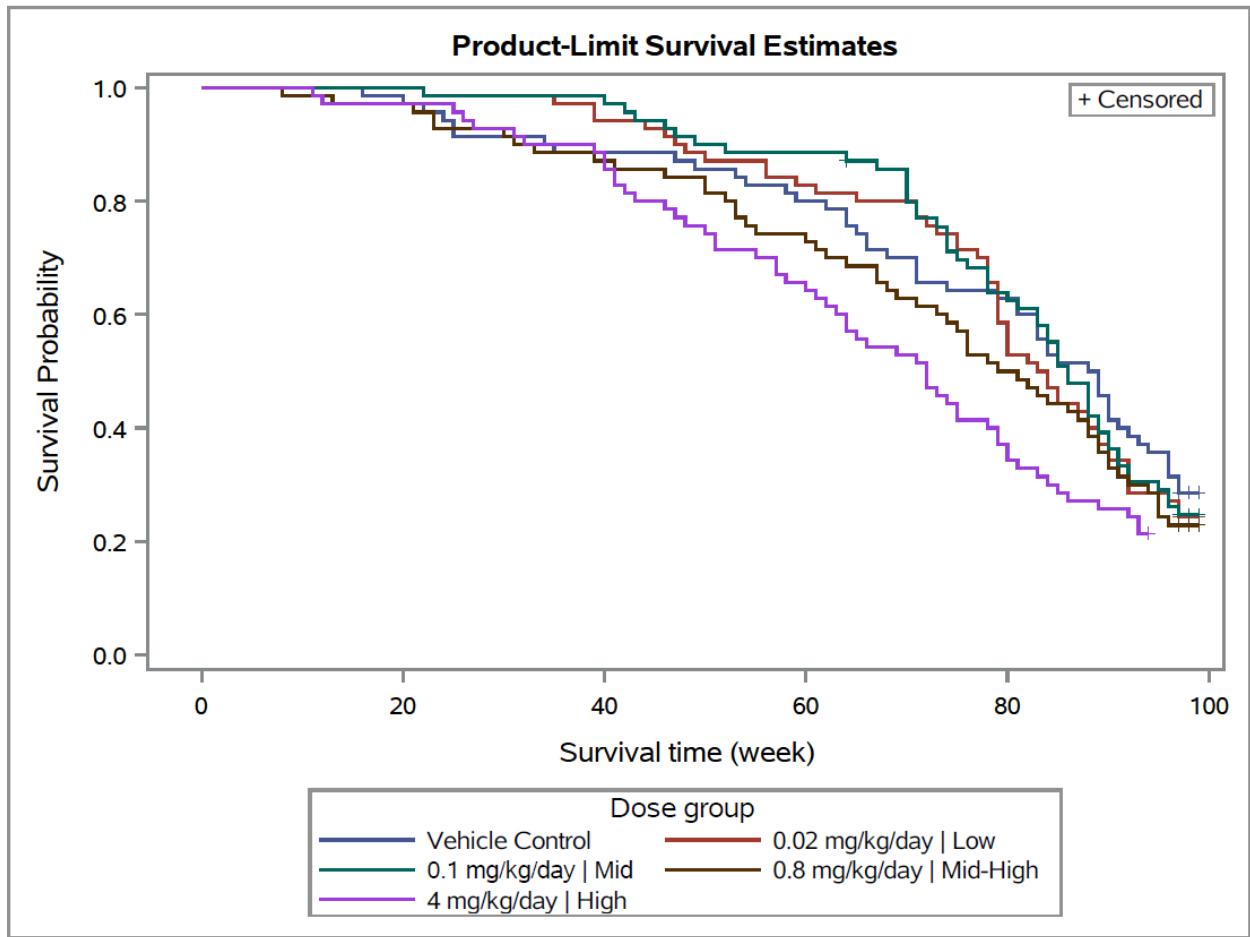


Figure 1B: Kaplan-Meier Survival Functions for Female Rats

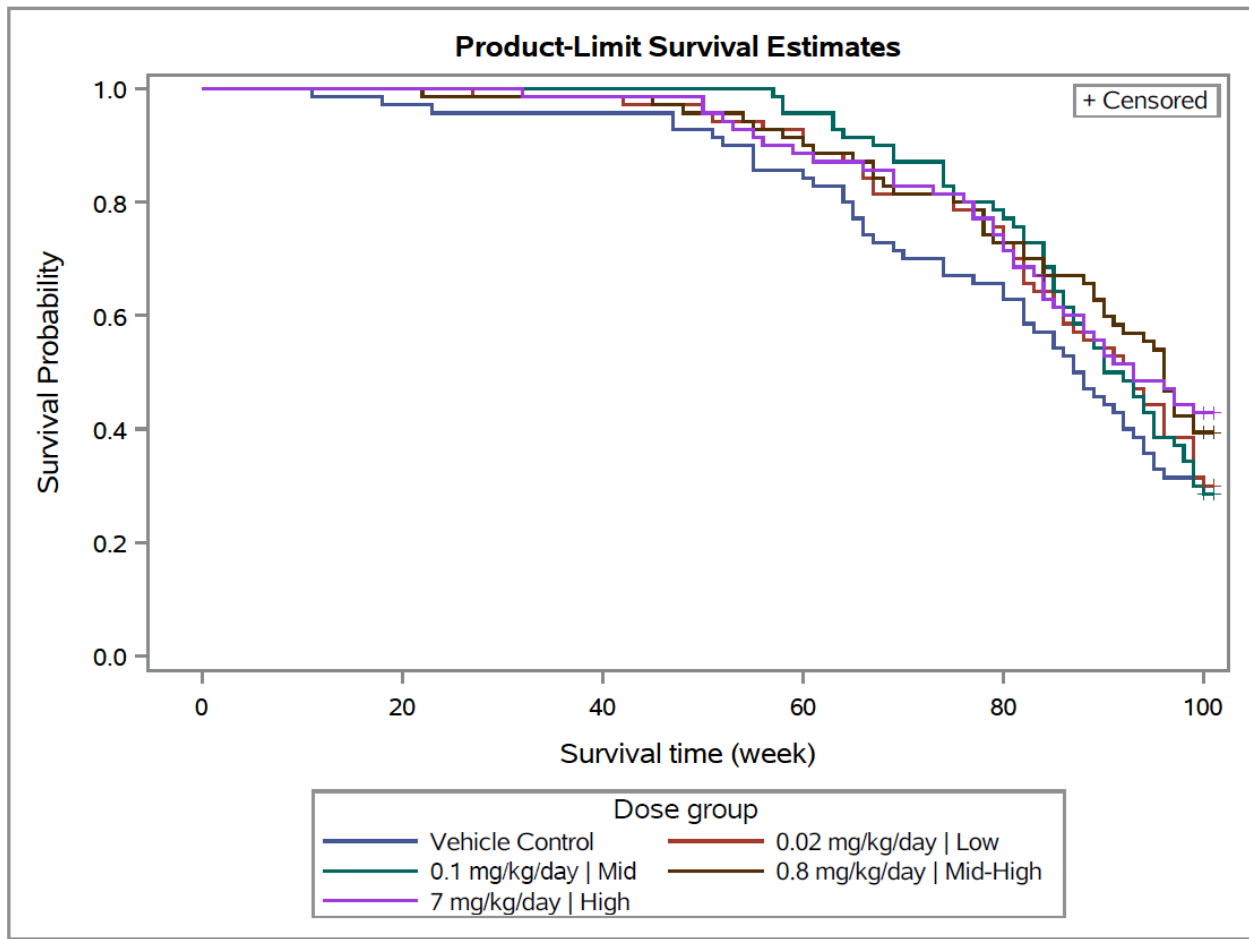


Figure 2A: Kaplan-Meier Survival Functions for Male Mice

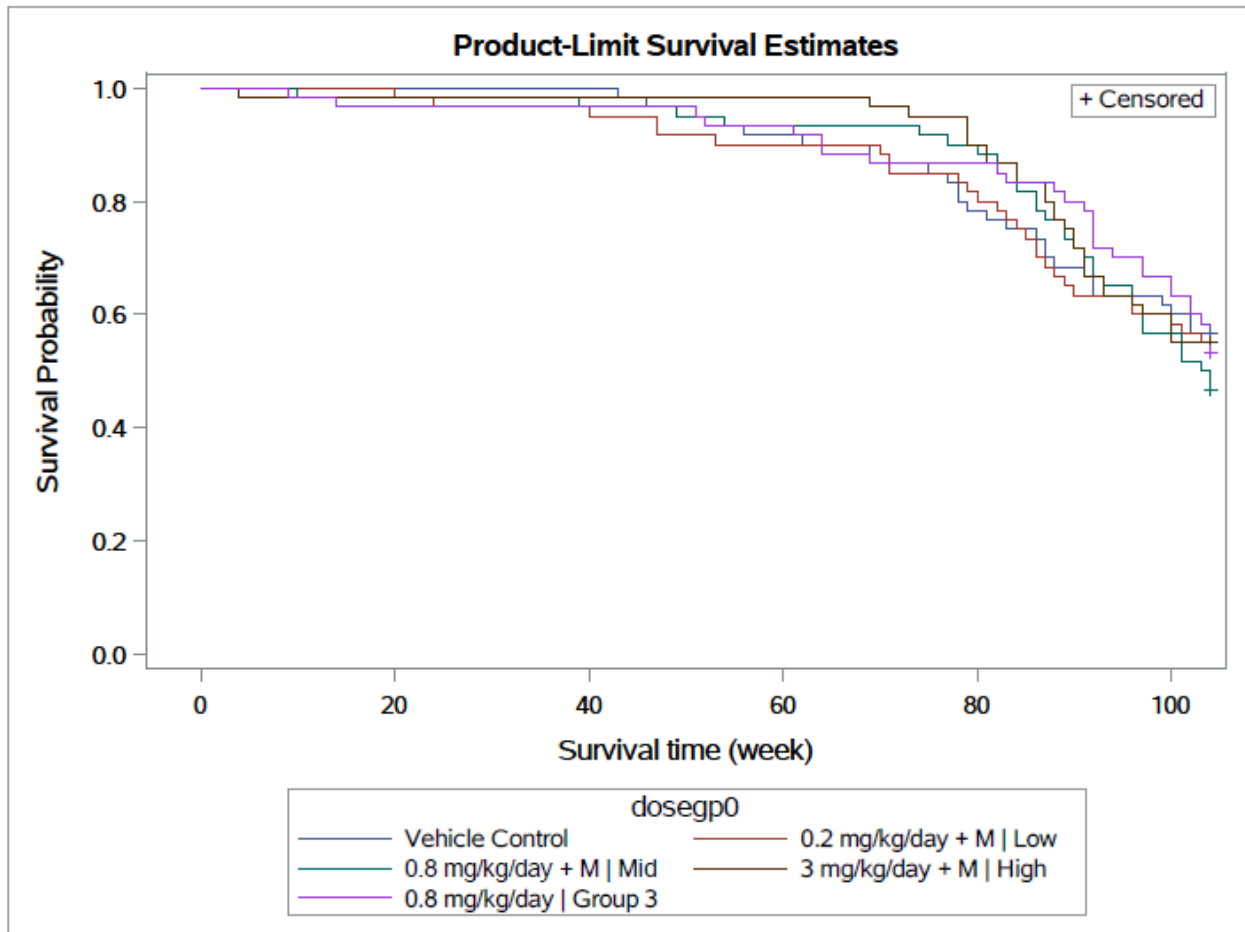
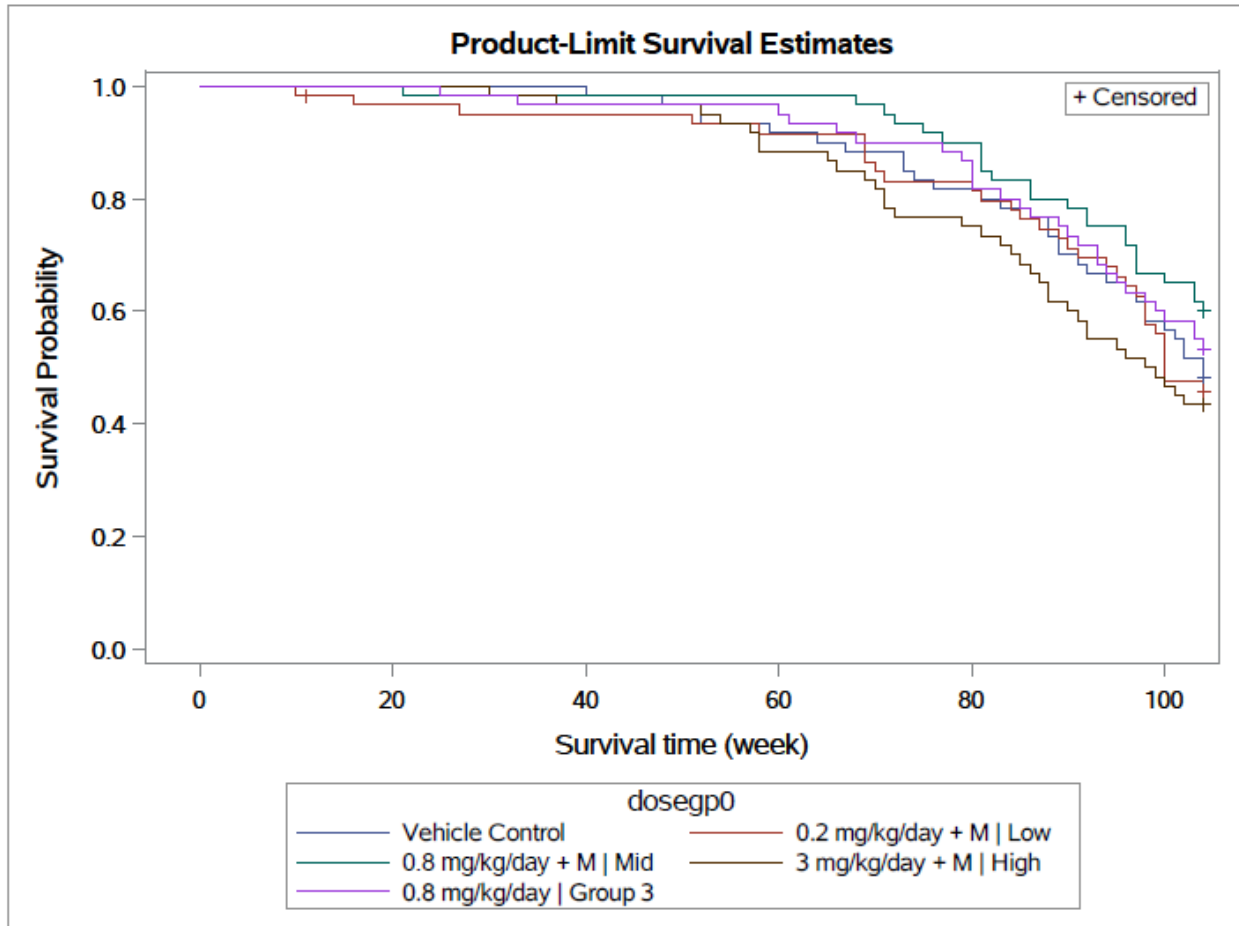


Figure 2B: Kaplan-Meier Survival Functions for Female Mice



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