

## Supplementary Online Content

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### **eMethods**

**eTable 1.** ASCEND Committee Members

**eTable 2.** Inclusion and Exclusion Criteria

**eTable 3.** Starting Dose of Daprodustat and Darbepoetin Alfa

**eTable 4.** Randomized Treatment Dose Adjustment Algorithm

**eTable 5.** Rescue Algorithm for Anemia Management

**eTable 6.** Baseline Characteristics for the ITT Population by Treatment Group and Dialysis Type

**eTable 7.** Hb Response by Baseline hsCRP Category

**eTable 8.** Adverse Events of Special Interest

**eTable 9.** Evaluation of Blood Pressure Parameters

**eTable 10.** Hemoglobin Efficacy and Cardiovascular Safety Results From ASCEND-ND, -D and -ID (ITT Population)

**eFigure 1.** Randomized Treatment Doses From Baseline Through Week 52

**eFigure 2.** Forest Plot of Adjusted Means From the Analysis of Post-randomization Hemoglobin (g/dL) Change From baseline to the Evaluation Period for Subgroups of Interest

**eFigure 3.** Forest Plot of Adjusted Mean Difference in Hemoglobin (g/dL) Change from Baseline to the Evaluation Period

**eFigure 4.** Parameters of Iron Metabolism

This supplementary material has been provided by the authors to give readers additional information about their work.

## **eMethods**

### **Iron Management**

Iron therapy was required if the serum ferritin was  $\leq 100$  ng/mL (to convert to  $\mu\text{g/L}$ , multiply by 1) and/or transferrin saturation was  $\leq 20\%$ . However, all iron (excluding multivitamins) was prohibited if the serum ferritin was  $>800$  ng/mL and transferrin saturation  $>20\%$ , or transferrin saturation  $>40\%$  (regardless of ferritin concentration). Investigators could stop administration of iron at a lower ferritin or transferrin saturation level according to local guidelines as long as patients remained iron replete.

A rescue algorithm was utilized to minimize inadequate Hb response to the assigned treatment for anemia (eTable 4).

### **Rationale for Hemoglobin Non-Inferiority Margin**

The hemoglobin (Hb) non-inferiority margin of  $-0.75$  g/dL (to convert to g/L, multiply by 10) is based on a combination of statistical reasoning, clinical judgment, and regulatory guidance, considering the following factors: 1) to be less than a Hb change that would result in a clinically meaningful difference to the patient, 2) the percentage of the efficacy of recombinant human erythropoietin (rhEPO) preserved by the margin, 3) to be greater than a change in Hb that could be due to variability and 4) precedent for margins used in past rhEPO dialysis and non-dialysis pivotal trial for comparative Hb efficacy assessments (Aranesp®, peginesatide, and Mircera®).

A Hb change of at least 1 g/dL has been commonly used in past rhEPO trials in anemic CKD patients, to define a clinically meaningful Hb response. The trials that have reported a meaningful change in one or more Health Related Quality of Life

domains have also reported Hb changes exceeding 2 g/dL [Leaf, 2009]. Leaf describes an analysis of NHS where a 1% increase in hematocrit (Hct) (between 30-42% [to convert to proportion of 1, multiply by 0.01]) was associated with a 0.6 change in SF-36 Physical Functioning score. Using an approximate conversion to Hb (Hct/3), a 1 g/dL change in Hb could be expected to translate to roughly a 1.8 change in Physical Functioning score, a change significantly lower than the 3-5 units considered clinically meaningful for SF-36 [Samsa, 1999]. On the basis of this, the proposed margin seems reasonable, representing a difference that is unlikely to result in a clinically meaningful change to the patient. Also, a change of this magnitude from the Hb targets in the daprodustat trials would not trigger a need for red blood cell transfusion in a patient.

Based on regulatory guidance for designing non-inferiority trials [FDA, 2010; CHMP, 2006] we have considered what fraction of the Hb effect, presumed between rhEPO and placebo, would be preserved by the proposed margin of -0.75 g/dL in the daprodustat trials. This assumes a 'putative placebo' estimate because it would not be ethical to include placebo in the planned trials. In estimating this effect there are two considerations: 1) the target Hb level permitted in the trial and 2) what would happen to Hb if placebo was included. Based on these two factors it is estimated that the difference in Hb after 1-year follow-up between rhEPO and 'putative placebo' would be in the range of 2-3 g/dL. Following the principles for preservation of comparator effect as laid out in the FDA guidance, more than 50% of the presumed rhEPO vs. 'putative placebo' effect would be preserved by the proposed margin (63% for 2 g/dL and 75% for 3 g/dL, calculated by:  $(2-0.75) \times 100$  and  $(3-0.75) \times 100$ ). However, the relevance of this approach to support the choice of margin is

challenged for the daprodustat trials because constancy assumptions for trial design and clinical practice with rhEPOs cannot be assumed.

To ensure that the margin is not too narrow we have also considered what change in Hb could occur by chance as a result of variability. In clinical practice there are many factors that can contribute to variability in Hb results; a change for a patient of less than or equal to 0.5 g/dL is generally not considered actionable because of this.

Variability can come from the collection and handling of the blood specimens, physiological factors associated with the patient at the time of sampling and due to variability in the assay. The proposed margin of -0.75 g/dL allows for these multiple sources of variability and protects against the trials resulting in a false negative conclusion. [Gaillard, 1986, Westgard, 2011].

Finally, in considering the margin, we looked at non-inferiority margins that have been used in past trials of rhEPOs for the comparative assessment of Hb efficacy in anemic CKD patients. A non-inferiority margin of -1.0 g/dL was used in the registration trials for Aranesp® and Peginesatide, and -0.75 g/dL was used in the registration trials for Mircera®.

Considering this information, we used a non-inferiority margin of -0.75 g/dL for comparative Hb efficacy across all daprodustat Phase 3 trials. Further, the more recent vadadustat development program also reported use of a Hb NI margin of – 0.75 g/dL [Chertow, 2021, Eckardt 2021].

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## Subgroup Analyses

The primary Hb endpoint was evaluated for a list of pre-specified subgroups.

Subgroup categories included those of regulatory or clinical interest or potential biological plausibility for different subgroup effects. The final list of subgroups used for analyses was included in the analysis plan and incorporated pre-specified changes prior to unblinding, from those originally identified during protocol development. The list of subgroups used for final analyses are provided below.

Category	Subgroups	Subgroup Analysis for Primary Hgb & Principal Secondary Efficacy Endpoint Note: Does not include 'Missing' categories.
<b>Key Subgroups of Regulatory/Clinical Interest or Potential Biological Plausibility for Different Subgroup Effects</b>		
Age at randomization (Grouping 1)	<65 years, 65-<75 years, ≥75 years	Yes
Gender (sex)	Female, Male	Yes
Ethnicity	Hispanic or Latino, Not Hispanic or Latino	Yes
High level race (self-identified)	American Indian or Alaskan Native Asian Black or African American Native Hawaiian or Other Pacific Islander White Mixed Race	Yes (Asian, Black or African American, and White will be used in subgroup analyses due to small sample size in other groups)
Region	Region 1: USA Region 2: Europe Region 3: Rest of World	Yes
Regions combined	USA, Non-USA	Yes
Dialysis type at randomization <sup>a</sup>	HD, PD (repeat using HD, HDF/HF, PD)	Yes (HD/PD only)
Dialysis start manner	Planned Start, Unplanned (Urgent) Start	Yes
Baseline Hb group <sup>b</sup>	<9 g/dL, 9-<10g/dL, 10-11g/dL, >11 g/dL, Missing	Yes (the last two subgroups will be combined into a single ≥10 g/dL group if there are <25 subjects in either group)
Baseline body mass index group <sup>c</sup>	<30 kg/m <sup>2</sup> , ≥30 kg/m <sup>2</sup> , Missing	Yes
Baseline weight quartiles <sup>c</sup>	Overall ITT Population Quartile 1: < xx kg Overall ITT Population Quartile 2: xx kg – < xx kg Overall ITT Population Quartile 3: xx kg – < xx kg Overall ITT Population Quartile 4: ≥ xx kg Missing	Yes

Category	Subgroups	Subgroup Analysis for Primary Hgb & Principal Secondary Efficacy Endpoint Note: Does not include 'Missing' categories.
Baseline hsCRP quartiles <sup>d</sup>	Overall ITT Population Quartile 1: < xx mg/L Overall ITT Population Quartile 2: xx mg/L – < xx mg/L Overall ITT Population Quartile 3: xx mg/L – < xx mg/L Overall ITT Population Quartile 4: ≥xx mg/L Missing	Yes
<b>Other Exploratory Subgroups where Biological Plausibility for Heterogeneous Effects Are Not Known or Anticipated</b>		
History of diabetes	No, Yes, Missing	Yes
History of stroke	No, Yes, Missing	Yes
History of MI	No, Yes, Missing	Yes
History of cancer	No, Yes, Missing	Yes
History of heart failure	No, Yes, Missing	Yes
History of thromboembolic events	No, Yes, Missing	Yes
Hospitalization within 6 months prior to screening	No, Yes, Missing	Yes
Transfusion within 6 months prior to screening	No, Yes, Missing	Yes

**NOTES:**

<sup>a</sup>Subjects who change dialysis modalities during the study will be counted in the subgroup corresponding to their dialysis modality at randomization.

<sup>b</sup>To convert to g/L, multiply by 10.

<sup>c</sup>Note: For subjects with in-clinic dialysis, post-dialysis value are used.

<sup>d</sup>To convert to mg/L, multiply by 10.

HD, hemodialysis; HDF, hemodiafiltration; HF, hemofiltration; hsCRP, high-sensitivity C-reactive protein; ITT, intent-to-treat; MI, myocardial infarction; PD, peritoneal dialysis

**eTable 1.** ASCEND Committee Members**Executive Steering Committee Members**

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**eTable 2.** Inclusion and exclusion criteria

<p><b>Inclusion criteria</b></p> <ul style="list-style-type: none"><li>- Between 18 and 99 years of age inclusive</li><li>- Planning to start chronic dialysis with the next 6 weeks from the date of the screening visit OR have started and received dialysis (as specific below) for end-stage renal disease for a maximum of ≤90 days immediately prior to randomization and is not expected to stop dialysis during the duration of the trial:<ul style="list-style-type: none"><li>○ Hemodialysis ≥2 times a week</li><li>○ Peritoneal dialysis ≥4 times a week including incremental schedule; patients on continuous ambulatory peritoneal dialysis (CAPD) and automated peritoneal dialysis (APD) are eligible</li></ul></li><li>- Hemoglobin concentration as measured by HemoCue (ranges inclusive) of 8-10.5 g/dL (5-6.5 mmol/L; 80-105 g/L) at screening and 8-11.0 g/dL (5-6.8 mmol/L; 80-110 g/L) at randomization</li><li>- Informed consent at screening</li></ul>
<p><b>Exclusion criteria</b></p> <ul style="list-style-type: none"><li>- Planned living-related or living-unrelated kidney transplant during the study</li><li>- Ferritin ≤100 ng/mL (≤100 µg/L) as screening or after IV iron supplementation</li><li>- Transferrin saturation ≤20% at screening or after IV iron supplementation</li><li>- Vitamin B12 below the lower limit of the reference range at screening or after vitamin B12 supplementation</li><li>- Folate &lt;2.0 ng/mL (&lt;4.5 nmol/L) at screening</li><li>- History of bone marrow aplasia or pure red cell aplasia</li><li>- Untreated pernicious anemia, thalassemia major, sickle cell disease, or myelodysplastic syndrome</li><li>- Evidence of actively bleeding gastric, duodenal, or esophageal ulcer disease OR clinically significant gastrointestinal bleeding ≤10 weeks prior to screening through to randomization (Day 1)</li><li>- Use of any ESA treatment within 8 weeks prior to screening except for limited use as part of dialysis initiation</li></ul>

- Limited use defined as <6 weeks of short-acting ESA (rhEPO or biosimilars; maximum of 20000 U total) or long-acting ESA (darbepoetin alfa [maximum of 100 µg total] or methoxy polyethylene glycol-epoetin beta [maximum of 125 µg total]) received before or after starting dialysis
- Myocardial infarction or acute coronary syndrome ≤10 weeks prior to screening through to randomization (Day 1)
- Stroke or transient ischemic attack ≤10 weeks prior to screening through to randomization (Day 1)
- Chronic Class IV heart failure, as defined by the New York Heart Association (NYHA) functional classification system
- Current uncontrolled hypertension as determined by the Investigator that would contraindicate the use of rhEPO
- Day 1 QTcB >500 msec, or >530 msec in patients with bundle branch block. There is no QTc exclusion for patients with a predominantly ventricular paced rhythm
- Liver disease (any one of the following):
  - ALT >2x ULN (screening only)
  - Bilirubin >1.5x ULN (screening only, isolated bilirubin >1.5x ULN acceptable if bilirubin is fractionated and direct bilirubin <35%)
  - Current unstable liver or biliary disease per investigator assessment (stable chronic liver disease, including asymptomatic gallstones, chronic hepatitis B or C, of Gilbert's syndrome, are acceptable if subject otherwise meets entry criteria)
- History of malignancy within 2 years prior to screening through to randomization (Day 1), or currently receiving treatment for cancer or complex kidney cyst >3cm
  - Exception is localized squamous cell or basal cell carcinoma of the skin that has been definitively treated ≥10 weeks prior to screening
- History of severe allergic reactions of anaphylactic reactions or hypersensitivity to excipients in the investigational product or darbepoetin alfa
- Use of strong CYP2C8 inhibitors or strong CYP2C8 inducers

- Use of other investigational agent or device prior to screening through to randomization (Day 1) (at screening, this exclusion applies to use of the investigational agent within 30 days or within five half-lives, whichever is longer)
- Any prior treatment with daprodustat for treatment duration of >30 days
- Females only: subject is pregnant, breastfeeding or is of reproductive potential and does not agree to follow one of the contraceptive options in the List of Highly Effective Methods for Avoiding Pregnancy listed in the study protocol
- Any other condition, clinical or laboratory abnormality, or examination finding that the investigator considers would put the subject at unacceptable risk, which may affect study compliance (such as intolerance to rhEPO) or prevent understanding of the aims of investigation procedures or possible consequences of the study

ALT, alanine transaminase; CYP, cytochrome P450; ESA, erythropoiesis-stimulating agent; Hb, hemoglobin; QTcB, corrected QT interval by Bazett; rhEPO, recombinant human erythropoietin; ULN, upper limit of normal.

**eTable 3.** Starting dose of daprodustat and darbepoetin alfa

Criteria	Starting dose
Daprodustat, oral	
Hb $\geq 8$ to $<9$ g/dL <sup>a</sup>	4 mg/day
Hb $\geq 9$ to $\leq 10$ g/dL <sup>a</sup>	2 mg/day
Hb $>10$ g/dL <sup>a</sup>	1 mg/day
Darbepoetin alfa, SC (PD) or IV (HD)	
	40 or 60 $\mu$ g every 2 or 4 weeks (based on weight)

<sup>a</sup>To convert to g/L, multiply by 10.

Hb, hemoglobin; HD, hemodialysis; IV, intravenous; PD, peritoneal dialysis; SC, subcutaneous.

Dose adjustments for both treatments were made by the Interactive Response Technology system to achieve and maintain Hb within the range of 10 to 11 g/dL based on the HemoCue Hb value.

**eTable 4.** Randomized treatment dose adjustment algorithm

HemoCue Hb at current study visit (g/dL) <sup>a</sup>	HemoCue Hb change since last study visit <sup>a</sup>	Randomized Treatment Dose Adjustment <sup>e</sup>
<7.5 <sup>b</sup>	Any change	Repeat Hb and average values <sup>6</sup> ; if confirmed, increase to the next higher dose step
7.5 to <9.5	Decreasing or no change or increasing by <0.5	Increase to the next higher dose step
7.5 to <9.5	Increasing by ≥0.5	Maintain dose
≥9.5 to <10 at two consecutive visits	Decreasing or no change	Increase to the next higher dose step
≥9.5 to ≤11.5	Any change	Maintain dose
>11 to ≤11.5 at two consecutive visits	Increasing or no change	Decrease to the next lower dose step
>11.5 to <12	Decreasing	Maintain dose
>11.5 to <12	Increasing or no change	Decrease to the next lower dose step
≥12 <sup>c</sup>	Any change	Repeat Hb and average values <sup>f</sup> ; if confirmed, temporary hold the dose and re-check Hb at next study visit; restart at one dose step lower when Hb <11.5 g/dL and provided it has been at least 2 weeks from the prior study visit
Any	>2 g/dL increase over 4 weeks (>1 g/dL increase over 2 weeks <sup>d</sup> )	Repeat Hb and average values <sup>f</sup> ; if confirmed, decrease to the next lower dose step
Any	>2 g/dL decrease over 4 weeks (>1 g/dL decrease over 2 weeks <sup>d</sup> )	Repeat Hb and average values <sup>f</sup> ; if confirmed, increase to the next higher dose step

Hb, hemoglobin.

<sup>a</sup>'Study visit' refers to scheduled study visits (every 4 weeks through Week 52). To convert to g/L, multiply by 10.

<sup>b</sup>This rule also applies to any mandated visit or an unscheduled visit, provided it has been at least 2 weeks from the prior study visit.

<sup>c</sup>This rule applies to any mandated or unscheduled visit.

<sup>d</sup>This rule applies to Weeks 2, 4, 6, and 8 visits only.

<sup>e</sup>Those receiving the highest dose of randomized treatment who require a dose increase will maintain the same dose, while those receiving the lowest dose of randomized treatment that require a dose decrease will have doses withheld.

<sup>f</sup>Repeat HemoCue Hb at the same study visit to confirm Hb (using the same sample) and take average.

**eTable 5.** Rescue algorithm for anemia management

<b>Evaluate Subject for Rescue if:</b> HemoCue Hb remains <9 g/dL <sup>a</sup> (at a scheduled visit, Week 4 onwards) despite three <sup>b</sup> consecutive dose increases above the starting dose of post-rescue <sup>c</sup> (where HemoCue Hb <9 g/dL <sup>a</sup> prior to each dose increase) OR HemoCue Hb is <7.5 g/dL <sup>a</sup> despite a dose increase at the prior study visit	
<b>Step 1:</b> <b>Initial Intervention</b>	While continuing randomized treatment (increase dose if HemoCue Hb <7.5 g/dL <sup>a</sup> ; otherwise maintain current dose), intervene with one or more of the following as dictated by clinical comorbidities <ul style="list-style-type: none"> <li>- Single course of IV iron up to 1000 mg (in addition to the iron management criteria)</li> <li>- Transfusion of up to two units of PRBC if clinically indicated</li> <li>- Allow additional 4 weeks on randomized treatment (this is a required choice; can be combined with either or both of the above)</li> </ul>
<b>Step 2:</b> <b>Rescue</b>	Check HemoCue Hb 4 weeks ±1 week from last study visit; earlier checks of Hb may be obtained to advise further intervention as clinically indicated. <b>Randomized treatment should be permanently discontinued and the subject should be rescued according to local clinical practice if either:</b> <ul style="list-style-type: none"> <li>- HemoCue Hb remains &lt;9 g/dL<sup>a</sup> despite initial intervention based on the average of two HemoCue Hb values<sup>d</sup></li> <li>OR</li> <li>- More than two units of PRBC were needed for transfusion (and was not related to acute bleeding)</li> </ul>

Hb, hemoglobin; PRBC, packed red blood cells.

<sup>a</sup>To convert to g/L, multiply by 10.

<sup>b</sup>Two consecutive dose increases if starting/post-rescue dose is daprodustat 12 mg or darbepoetin alfa 200 µg over 4 weeks; one dose increase if starting/post-rescue dose is daprodustat 16 mg or darbepoetin alfa 300 µg over 4 weeks; and no prior dose increase if starting/post-rescue dose is daprodustat 24 mg or darbepoetin alfa 400 µg over 4 weeks (top dose).

<sup>c</sup>For patients who previously are evaluated for rescue and who are able to continue in the trial, “post-rescue” dose is the dose of randomized treatment that a subject is receiving at the study visit after initial intervention.



<sup>d</sup>Repeat HemoCue Hb at the same study visit to confirm Hb (using the same sample); take average of two values.

**eTable 6.** Baseline characteristics for the ITT population by treatment group and dialysis type

	Daprodustat		Darbepoetin alfa	
	HD (n=126)	PD (n=31)	HD (n=126)	PD (n=29)
Age (y)	53.5 (45–65)	49.0 (46–57)	58.0 (48–69)	47.0 (43–60)
Sex, No. (%)				
Male	79 (63)	17 (55)	78 (62)	20 (69)
Female	47 (37)	14 (45)	48 (38)	9 (31)
Self-identified race, No. (%)				
African American/Black	13 (10)	3 (10)	11 (9)	2 (7)
American Indian or Alaska Native	2 (2)	3 (10)	1 (<1)	1 (3)
Asian	13 (10)	13 (42)	17 (13)	14 (48)
Native Hawaiian or other Pacific Islander	0 (0)	0 (0)	0 (0)	0 (0)
White	98 (78)	12 (39)	95 (75)	12 (41)
Mixed race <sup>a</sup>	0 (0)	0 (0)	2 (2)	0 (0)
Dialysis start manner, No. (%)				
Planned	85 (67)	24 (77)	83 (66)	24 (83)
Unplanned	41 (33)	7 (23)	43 (34)	5 (17)
Dialysis status at randomization, No. (%)				
Dialysis not initiated	6 (5)	2 (6)	3 (2)	1 (3)
On Dialysis	120 (95)	29 (94)	123 (98)	28 (97)
Baseline BMI, kg/m <sup>2b</sup>	26.1 (22.1–29.9)	26.5 (23.4–28.5)	26.6 (23.0–30.7)	26.0 (22.1–28.5)
Baseline weight, kg <sup>c</sup>	75.0 (62.2–88.0)	73.1 (63.6–79.4)	74.5 (63.6–88.5)	70.6 (62.0–80.0)
Cardiovascular disease history, No. (%)				
Heart failure	43 (34)	4 (13)	39 (31)	6 (21)
Myocardial infarction	23 (18)	2 (6)	30 (24)	4 (14)
Stroke	11 (9)	1 (3)	7 (6)	2 (7)
Stroke	7 (6)	0 (0)	9 (7)	0 (0)
Thromboembolic events, No. (%)	12 (10)	1 (3)	7 (6)	1 (3)
Diabetes, No. (%)	55 (44)	15 (48)	57 (45)	13 (45)
Cancer, No. (%)	3 (2)	0 (0)	3 (2)	1 (3)
Baseline post-dialysis blood pressure (mmHg) <sup>d</sup>				
Systolic	139.2 (125.0–150.3)	135.7 (128.7–148.3)	140.0 (120.0–155.0)	140.7 (127.3–151.3)
Diastolic	77.5 (69.0–84.0)	80.7 (73.3–90.0)	76.0 (70.0–85.0)	73.7 (69.7–82.7)
Mean arterial pressure	96.7 (86.7–105.2)	97.9 (92.6–107.3)	97.0 (85.0–107.2)	97.7 (88.9–105.4)
hsCRP (mg/dL) <sup>e</sup>	0.28 (0.13–0.66)	0.29 (0.11–0.61)	0.43 (0.15–0.91)	0.22 (0.12–0.67)
Hemoglobin (g/dL) <sup>f</sup>	9.3 (8.6–10.1)	9.6 (8.9–10.7)	9.5 (8.8–10.0)	9.9 (9.2–10.1)
Mean (SD)	9.4 (1.0)	9.7 (1.1)	9.5	9.7 (0.9)

Medication, No. (%)				
Diabetes medications	47 (37)	9 (29)	43 (34)	11 (38)
Insulin	32 (25)	8 (26)	33 (26)	9 (31)
ACE inhibitor or ARB	59 (47)	14 (45)	47 (37)	11 (38)
Beta Blocker	65 (52)	15 (48)	64 (51)	13 (45)
Statin	51 (40)	13 (42)	38 (30)	12 (41)
Aspirin	35 (28)	4 (13)	37 (29)	3 (10)
Vitamin K antagonist	2 (2)	1 (3)	2 (2)	1 (3)
Phosphate binders <sup>g</sup>				
Iron-based	2 (2)	0 (0)	3 (2)	0 (0)
Calcium-based	42 (33)	13 (42)	43 (34)	13 (45)
Non-calcium and non-iron based	9 (7)	3 (10)	9 (7)	4 (14)
Vitamin D	44 (35)	12 (39)	58 (46)	9 (31)
Calcimimetics	3 (2)	0 (0)	3 (2)	0 (0)
Oral iron, No. (%)	16 (13)	9 (29)	9 (7)	12 (41)
Intravenous iron, No. %	96 (76)	9 (29)	98 (78)	11 (38)
Standardized IV iron dose (mg/month) <sup>h</sup>	133.6 (18.6–260.9)	0.0 (0.0–87.0)	153.7 (43.5–326.1)	0.0 (0.0–105.6)
Mean (SD) standardized IV iron dose (mg/month)	187.2 (218.5)	45.7 (86.8)	204.9 (216.2)	72.5 (138.1)

Continuous variables are expressed as median (interquartile range), unless otherwise indicated. All baseline laboratory tests were performed by central laboratory except for hemoglobin, which uses central laboratory values if available, or a point of care HemoCue value if the central laboratory value is missing.

<sup>a</sup>Multiple high level race categories are selected.

<sup>b</sup>For patients with in-clinic dialysis, post-dialysis values are used. Data presented from daprodustat n=123 (HD), 31 (PD) and darbepoetin alfa n=124 (HD), 29 (PD).

<sup>c</sup>Data presented from daprodustat n=124 (HD), 31 (PD) and darbepoetin alfa n=124 (HD), 29 (PD).

<sup>d</sup>For patients with in-clinic dialysis, post-dialysis values are used. Data presented from daprodustat n=124 (HD), 31 (PD) and darbepoetin alfa n=125 (HD), 29 (PD).

<sup>e</sup>Data presented from daprodustat n=124 (HD), 31 (PD) and darbepoetin alfa n=123 (HD), 29 (PD). To convert to mg/L, multiply by 10.

<sup>f</sup>To convert to g/L, multiply by 10.

<sup>g</sup>Patients may have more than one type of phosphate binder use, so percentages may sum to more than 100%.

<sup>h</sup>Includes patients receiving no IV iron (0 mg).

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; HD, hemodialysis; hsCRP, high-sensitivity C-reactive protein; ITT, intent-to-treat; IV, intravenous; PD, peritoneal dialysis.

**eTable 7.** Hb response by baseline hsCRP category

hsCRP quartiles	Daprodustat n=155	Darbepoetin alfa n=152
<b>1: &lt;1.30 mg/L</b>		
Adjusted mean change from baseline (SE)	1.00 (0.166)	1.38 (0.174)
Adjusted mean treatment difference (95% CI)	-0.37 (-0.85, 0.10)	
<b>2: 1.30 mg/L – &lt;3.30 mg/L</b>		
Adjusted mean change from baseline (SE)	1.12 (0.162)	1.16 (0.172)
Adjusted mean treatment difference (95% CI)	-0.04 (-0.50, 0.42)	
<b>3: 3.30 mg/L – &lt;7.90 mg/L</b>		
Adjusted mean change from baseline (SE)	1.07 (0.177)	1.02 (0.171)
Adjusted mean treatment difference (95% CI)	0.05 (-0.43, 0.54)	
<b>4: ≥7.90 mg/L</b>		
Adjusted mean change from baseline (SE)	0.86 (0.183)	0.96 (0.171)
Adjusted mean treatment difference (95% CI)	-0.10 (-0.59, 0.40)	

ANCOVA, analysis of covariance; CI, confidence interval; hsCRP, high sensitivity C-reactive protein; SE, standard error.

Based on an ANCOVA model with terms for treatment, baseline hemoglobin, dialysis type, dialysis start manner, subgroup and treatment by subgroup interaction.

**eTable 8.** Adverse events of special interest

Preferred term	Daprodustat n=157		Darbepoetin alfa n=155	
	No. (%)	Rate/100 PY	No. (%)	Rate/100 PY
Death, MI, stroke, heart failure, thromboembolic events, thrombosis of vascular access	27 (17)	24.67	27 (17)	23.67
Thrombosis and/or tissue ischemia secondary to excessive erythropoiesis	0	0	1 (<1)	0.79
Cardiomyopathy	0	0	2 (1)	1.59
Pulmonary artery hypotension	1 (<1)	0.85	0	0
Cancer-related mortality of tumour progression and recurrence	1 (<1)	0.84	3 (2)	2.38
Esophageal and gastric erosions	1 (<1)	0.84	3 (2)	2.39
Proliferative retinopathy, macular edema, choroidal neovascularization	4 (3)	3.4	1 (<1)	0.79
Exacerbation of rheumatoid arthritis	0	0	0	0
Worsening of hypertension	38 (24)	38.36	29 (19)	26.48

AESI, adverse event of special interest; ESA, erythropoiesis-stimulating agent; HIF, hypoxia inducible factor; MI, myocardial infarction; PY, person-years.

AESIs were defined for daprodustat based on data from non-clinical and clinical studies, current information about HIF-associated pathophysiology, and identified risks for ESAs. A programmatic approach for identifying potential AESIs was implemented using a broad set of pre-defined terms of interest.

**eTable 9.** Evaluation of blood pressure parameters

	<b>Daprodustat (N=157)</b>	<b>Darbepoetin alfa (N=155)</b>
<b>On-treatment Post-dialysis BP (mmHg)</b>		
Systolic, No.	155	154
Baseline, mean (SD)	137.9 (19.37)	138.7 (22.98)
End of treatment, mean (SD)	134.8 (21.32)	135.3 (23.54)
Diastolic, No.	155	154
Baseline, mean (SD)	77.3 (11.86)	76.7 (12.52)
End of treatment, mean (SD)	77.5 (13.43)	75.3 (13.85)
<b>On-treatment BP Elevation Events<sup>a</sup></b>		
Patients with on-treatment post-dialysis BP, No.	155	154
Post-dialysis BP elevations, No. (%)	91 (58.7)	100 (64.9)
Model estimated elevation rate (daprodustat vs. darbepoetin alfa; two-sided 95 % CI) <sup>b</sup> One-sided p-value	1.01 (0.73, 1.39) 0.52	
Changes in on-treatment BP medications to end of treatment, No.	151	145
No change, No. (%)	64 (42)	72 (50)
At least one change, No. (%)	87 (58)	73 (50)
Increase, No. (%)	37 (25)	33 (23)
Decrease, No. (%)	72 (48)	65 (45)
Switch, No. (%)	23 (15)	20 (14)
<b>Treatment-emergent Worsening Hypertension Events</b>		
Worsening hypertension <sup>c</sup> , No.	157	155
No. (%)	38 (24)	29 (19)
Rate per 100 PY	38.36	26.48

BP, blood pressure; CI, confidence interval; DBP, diastolic blood pressure; PY, person-years; SBP, systolic blood pressure; SD, standard deviation.

<sup>a</sup>Defined as an increase in SBP of  $\geq 25$  mmHg from baseline or SBP  $\geq 180$  mmHg or an increase in DBP of  $\geq 15$  mmHg from baseline or DBP  $\geq 110$  mmHg.

<sup>b</sup>Model estimated elevation rates and CIs, ratio of model estimated elevation rates (daprodustat/darbepoetin alfa) and CI are based on a negative binomial model with treatment, dialysis type and dialysis start manner as covariates and the logarithm of time on-treatment as an offset variable. One-sided p-value based on test of null hypothesis: (daprodustat/darbepoetin alfa)  $\geq 1$  vs alternative: Ratio  $< 1$ .

<sup>c</sup>Based on adverse-event reporting.

**eTable 10.** Hemoglobin efficacy and cardiovascular safety results from ASCEND-ND, -D and -ID (ITT population)

	Daprodustat	ESA <sup>a</sup>	Treatment effect
<b>Adjusted mean (SE) change in Hb (g/dL) from baseline to Week 28–52 (g/dL)<sup>c</sup></b>			<b>Adjusted mean difference (95% CI)<sup>b,c</sup></b>
ASCEND-ND <sup>1</sup>	0.74 (0.02)	0.66 (0.02)	0.08 (0.03, 0.13)
ASCEND-D <sup>2</sup>	0.28 (0.02)	0.10 (0.02)	0.18 (0.12, 0.24)
ASCEND-ID	1.02 (0.09)	1.12 (0.09)	-0.10 (-0.34, 0.14)
<b>First occurrence of adjudicated MACE<sup>d</sup>, n/N (%)</b>			<b>HR (95% CI)<sup>e</sup></b>
ASCEND-ND <sup>1</sup>	378/1937 (19.5)	371/1935 (19.2)	1.03 (0.89, 1.19)
ASCEND-D <sup>2</sup>	374/1487 (25.2)	394/1477 (26.7)	0.93 (0.81, 1.07)
ASCEND-ID	19/157 (12.1)	15/155 (9.7)	N/A
<b>MACE rate per 100 PY (95% CI)</b>			<b>Absolute rate difference per 100 PY (95% CI)</b>
ASCEND-ND <sup>3</sup>	10.86 (9.80, 12.02)	10.63 (9.58, 11.77)	0.23 (-1.31, 1.77)
ASCEND-D <sup>3</sup>	11.07 (9.98, 12.26)	11.86 (10.72, 13.09)	-0.78 (-2.41, 0.84)
ASCEND-ID <sup>3</sup>	11.65 (7.02, 18.20)	9.24 (5.17, 15.24)	2.41 (-4.61, 9.43)

<sup>a</sup>Darbepoetin alfa in ASCEND-ID and -ND<sup>1</sup>, epoetin alfa or darbepoetin alfa in ASCEND-D<sup>2</sup>

<sup>b</sup>Non-inferiority was declared if the lower bound of the two-sided 95% CI exceeded -0.75 g/dL<sup>1, 2</sup>

<sup>c</sup>Based on an ANCOVA model: ASCEND-ND<sup>1</sup>, adjusted for baseline hemoglobin, treatment group, use or nonuse of an ESA and geographic region; ASCEND-D<sup>2</sup>, adjusted for baseline hemoglobin, treatment group, dialysis type and geographic region; ASCEND-ID, adjusted for baseline Hb, dialysis modality type, and dialysis start manner. To convert to g/L, multiply by 10.

<sup>d</sup>MACE was a composite outcome of death from any cause, non-fatal MI and non-fatal stroke, and time to first occurrence of adjudicated MACE was a co-primary endpoint in ASCEND-ND and -D<sup>1, 2</sup>

<sup>e</sup>Pre-specified non-inferiority margin of <1.25<sup>1, 2</sup>

ANCOVA, analysis of covariance; CI, confidence interval; ESA, erythropoiesis-stimulating agent; HR, hazard ratio; MACE, major adverse cardiovascular event; n, number of patients with event of interest; N, total number of patients; N/A, not available; PY, patient-year

## References

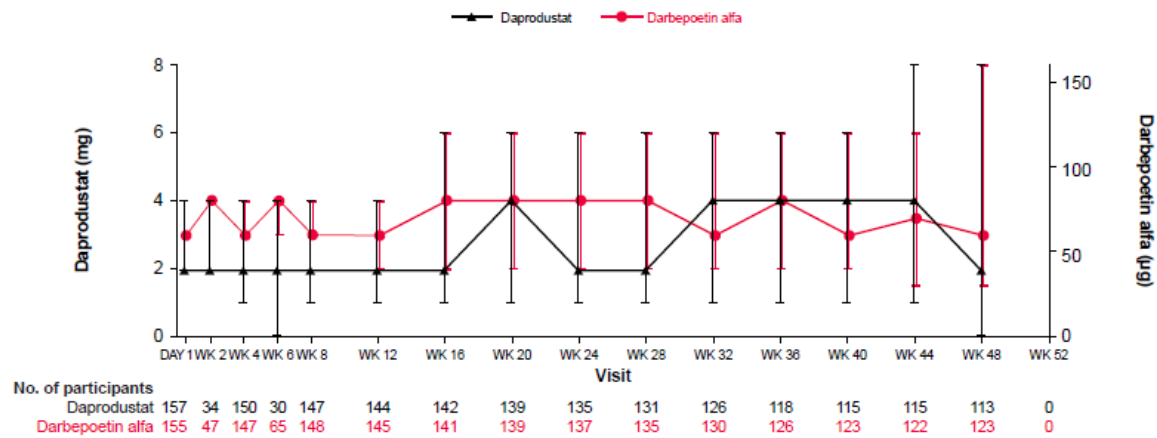
1. Singh AK, Carroll K, McMurray JJV, et al. Daprodustat for the Treatment of Anemia in Patients Not Undergoing Dialysis. *N Engl J Med*. 2021;385(25):2313-2324. doi:10.1056/NEJMoa2113380

2. Singh AK, Carroll K, Perkovic V, et al. Daprodustat for the Treatment of Anemia in Patients Undergoing Dialysis. *N Engl J Med*. 2021;385(25):2325-2335. doi:10.1056/NEJMoa2113379
3. Singh AK, Carroll K, Perkovic V, et al. ASCEND Program: Efficacy and Safety from ASCEND-D and -ND and Overall MACE Finding. presented at: American Society of Nephrology - Kidney Week; 2021; <https://www.asn-online.org/education/kidneyweek/2021/program-abstract.aspx?controlId=3639686>

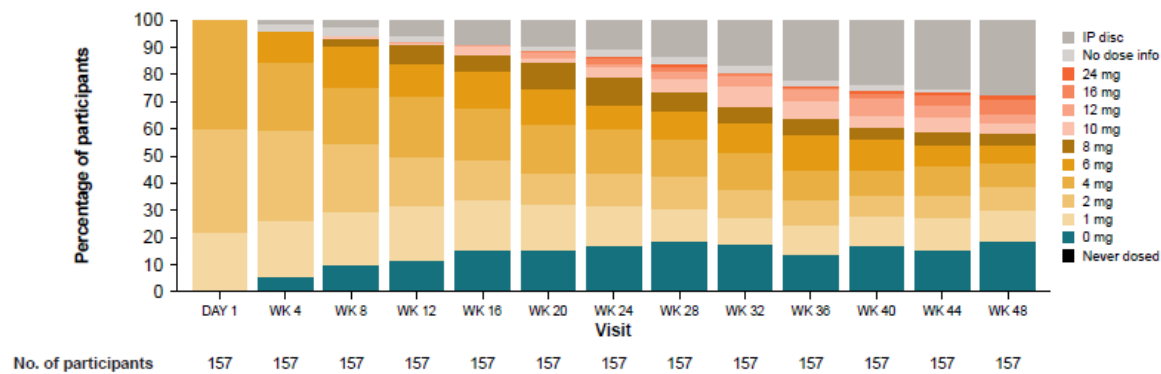


## eFigure 1. Randomized treatment doses from baseline through Week 52

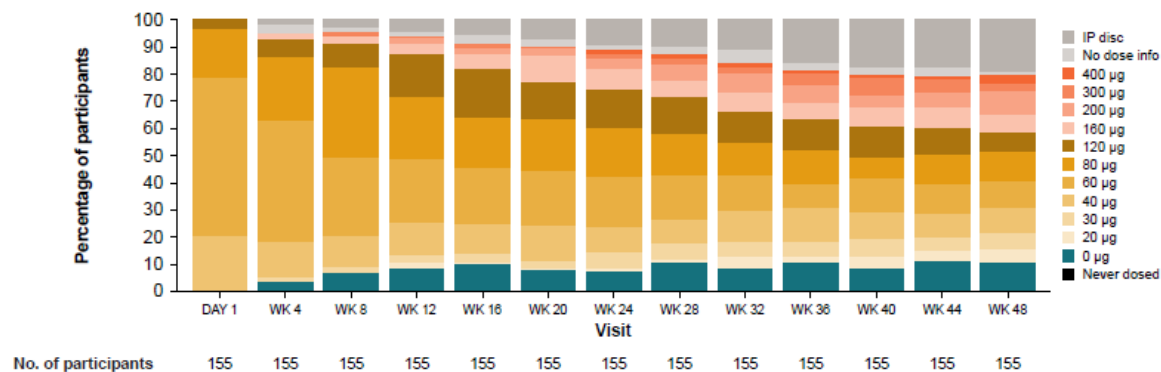
A. Median assigned dose by visit



B. Stacked bar chart for daprodustat



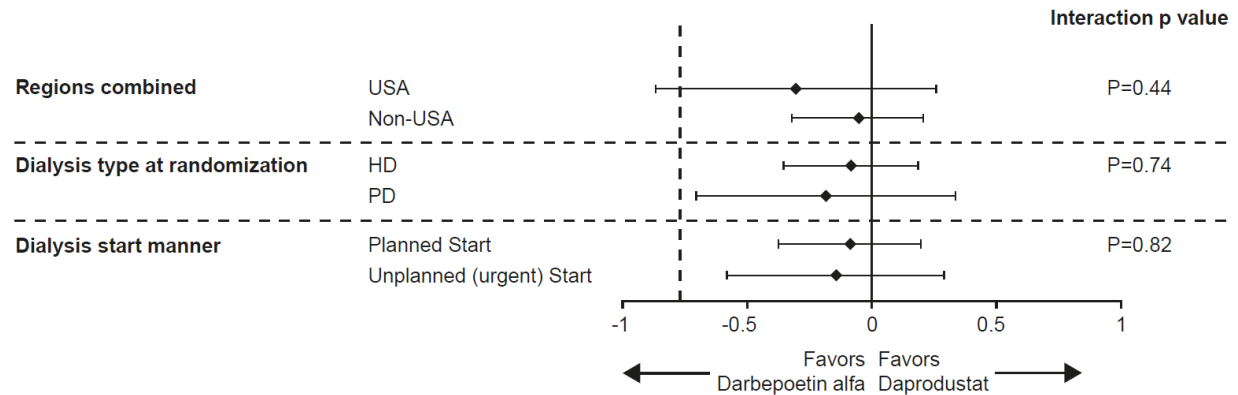
C. Stacked bar chart for darbepoetin alfa



A. Error bars indicate first and third quartiles.

B & C. IP discontinuation includes patients who were permanently IP discontinued, and patients who withdrew from the study. No dose information includes patients with missing data due to skipped visits, unavailable randomized treatment, or other reasons.

**eFigure 2.** Forest plot of adjusted means from the analysis of post-randomization hemoglobin (g/dL)<sup>a</sup> change from baseline to the evaluation period for subgroups of interest

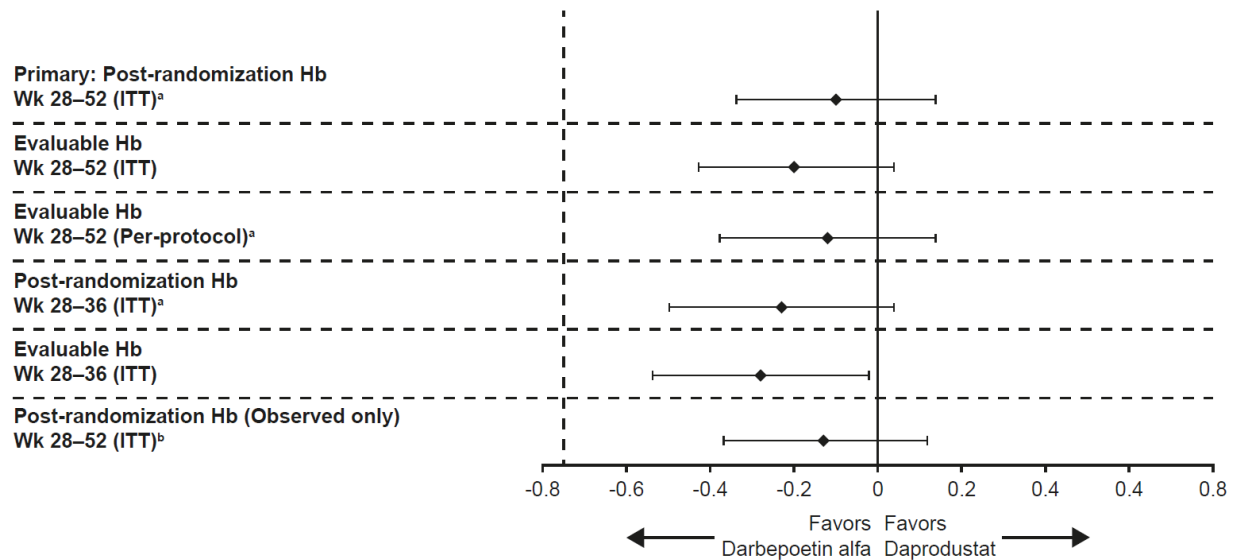


ANCOVA, analysis of covariance; HD, hemodialysis; PD, peritoneal dialysis

Based on an ANCOVA model with terms for treatment, baseline hemoglobin, dialysis type, dialysis start manner, subgroup and treatment by subgroup interaction. Vertical dashed line represents non-inferiority margin of -0.75 g/dL.

<sup>a</sup>To convert to g/L, multiply by 10.

**eFigure 3.** Forest plot of adjusted mean difference in hemoglobin (g/dL) change from baseline to the evaluation period



ESA, erythropoiesis-stimulating agent; Hb, hemoglobin; ITT, intent-to-treat; Wk, week.

Analyses based on an ANCOVA model adjusting for baseline Hb, dialysis modality type, and dialysis start manner.

<sup>a</sup>Analysis includes imputation.

<sup>b</sup>Analysis was conducted post-hoc.

Evaluable values were 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.

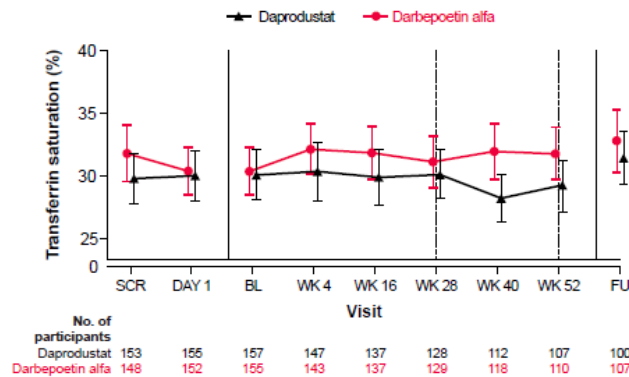
A supportive analysis was performed for the primary ITT analysis, repeating the analysis using an alternative evaluation period (Week 28 to 36).

Vertical dashed line represents non-inferiority margin of -0.75 g/dL.

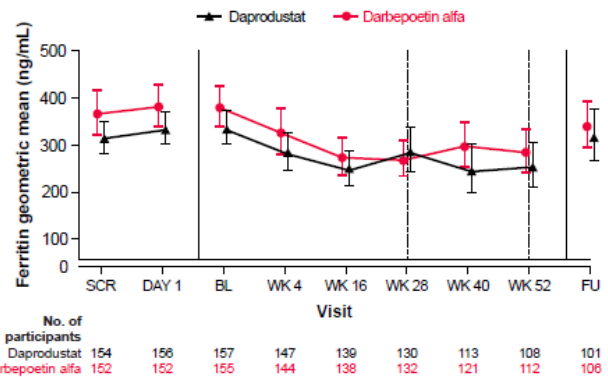
<sup>a</sup>To convert to g/L, multiply by 10.

## eFigure 4. Parameters of iron metabolism

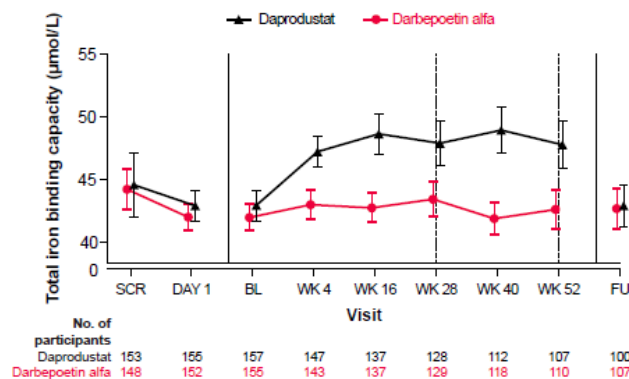
A. Transferrin saturation



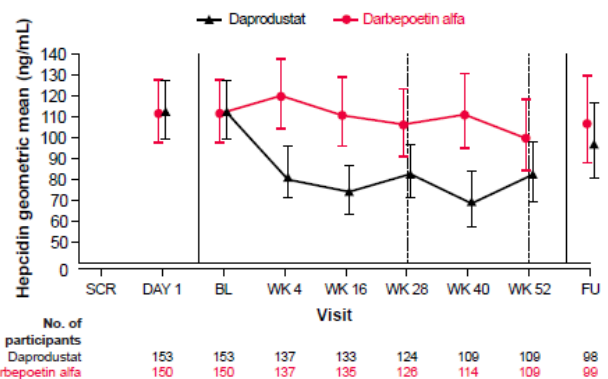
B. Ferritin



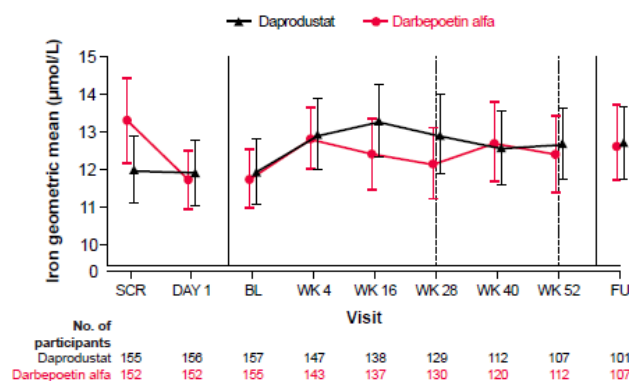
C. Total iron binding capacity



D. Hepcidin



E. Total iron



CI, confidence interval; FU, follow-up; SCR, screening; WK, week.

B. To convert to μg/L, multiply by 1.

Error bars indicate 95% CI.

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

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

Follow-up visit includes only post-treatment values.