Hyporesponsiveness to Darbepoetin Alfa in Patients With Heart Failure and Anemia in the RED-HF Study (Reduction of Events by Darbepoetin Alfa in Heart Failure)

Clinical and Prognostic Associations

BACKGROUND: A poor response to erythropoiesis-stimulating agents such as darbepoetin alfa has been associated with adverse outcomes in patients with diabetes mellitus, chronic kidney disease, and anemia; whether this is also true in heart failure is unclear.

METHODS AND RESULTS: We performed a post hoc analysis of the RED-HF trial (Reduction of Events by Darbepoetin Alfa in Heart Failure), in which 1008 patients with systolic heart failure and anemia (hemoglobin level, 9.0–12.0 g/dL) were randomized to darbepoetin alfa. We examined the relationship between the hematopoietic response to darbepoetin alfa and the incidence of all-cause death or first heart failure hospitalization during a follow-up of 28 months. For the purposes of the present study, patients in the lowest quartile of hemoglobin change after 4 weeks were considered nonresponders. The median initial hemoglobin change in nonresponders (n=252) was –0.25 g/dL and +1.00 g/dL in the remainder of patients (n=756). Worse renal function, lower sodium levels, and less use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers were independently associated with nonresponse. Although a low endogenous erythropoietin level helped to differentiate responders from nonresponders, its predictive value in a multivariable model was poor (C statistic=0.69). Nonresponders had a higher rate of all-cause death or first heart failure hospitalization (hazard ratio, 1.25; 95% confidence interval, 1.02–1.54) and a higher risk of all-cause mortality (hazard ratio, 1.30; 95% confidence interval, 1.04–1.63) than responders.

CONCLUSIONS: A poor response to darbepoetin alfa was associated with worse outcomes in heart failure patients with anemia. Patients with a poor response were difficult to identify using clinical and biochemical biomarkers.

CLINICAL TRIAL REGISTRATION: URL: https://www.clinicaltrials.gov. Unique identifier: NCT00358215.

Peter van der Meer, MD, PhD
Niels Grote Beverborg, MD
Marc A. Pfeffer, MD, PhD
Kurt Olson, MS
Inder S. Anand, MD, PhD
B. Daan Westenbrink, MD, PhD
John J.V. McMurray, MD
Karl Swedberg, MD, PhD
James B. Young, MD
Scott D. Solomon, MD
Dirk J. van Veldhuisen, MD, PhD

Correspondence to: Peter van der Meer, MD, PhD, Department of Cardiology, University Medical Center Groningen, Hanzeplein 1, PO Box 30.001, 9700 RB Groningen, the Netherlands. E-mail p.van.der.meer@umcg.nl

Key Words: anemia ■ darbepoetin alfa ■ erythropoietin ■ heart failure

WHAT IS NEW?

- Anemia is present in a large proportion of patients with heart failure, and the RED-HF trial (Reduction of Events by Darbepoetin Alfa in Heart Failure) showed that treatment with darbepoetin alfa did not result in improved clinical outcome in these patients.
- Our subanalysis of the RED-HF trial showed for the first time that approximately one quarter of heart failure patients do not exhibit an initial increase in hemoglobin in response to treatment with darbepoetin alfa and that this lack of response identifies patients at higher risk for heart failure hospitalization and all-cause mortality.

WHAT ARE THE CLINICAL IMPLICATIONS?

- Erythropoiesis-stimulating agents are not recommended in heart failure patients without chronic kidney disease. However, a large proportion of heart failure patients, approximately three quarters of the subjects in our study, have concomitant chronic kidney disease.
- Our findings may help to identify which patients do not respond to treatment with erythropoiesis-stimulating agents. Subsequently, discontinuation of the use of these agents should be considered.
- Our results highlight the need for the assessment of the cause of anemia.

Anemia is common in heart failure (HF) and associated with worse symptoms and unfavorable prognosis.[1–3] However, treatment of anemia with erythropoiesis-stimulating agents (ESAs), such as darbepoetin alfa (DA), did not improve clinical outcome in HF patients in the RED-HF trial (Reduction of Events by Darbepoetin Alfa in Heart Failure).[4] Moreover, use of DA was associated with more thromboembolic events and strokes in RED-HF. Consequently, ESAs are not recommended in HF patients.[5] In contrast, ESAs are widely used to treat anemia in patients with chronic kidney disease (CKD) even though a survival benefit has not been shown in the largest trial to date.[6] In CKD, a poor hematopoietic response to DA, leading to subsequent use of higher doses of ESA therapy, is prevalent and associated with worse prognosis.[7] Because the syndromes of HF and CKD show large overlap, impaired hematopoietic response might be of importance in HF patients as well. Furthermore, HF patients frequently have high levels of endogenous erythropoietin, which might indicate hyporesponsiveness of the bone marrow.[8–10] We assessed the hematopoietic response to DA in anemic HF patients in RED-HF trial.

METHODS

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Study Design and Patients

The RED-HF trial was a double-blind, placebo-controlled trial that randomized a total of 2278 patients from June 23, 2007, to May 4, 2012, at 453 sites in 33 countries.[4] The ethics permission was obtained for each study site, and each patient provided written informed consent. Patients with HF in New York Heart Association functional class II, III, or IV and left ventricular ejection fraction ≤40%, with a hemoglobin level between 9.0 and 12.0 g/dL who were receiving guideline-recommended HF therapy were eligible to be enrolled in the RED-HF trial. Key exclusion criteria included iron depletion (transferrin saturation, <15%) and evidence of bleeding or other correctable causes of anemia. The design of the study, including outcome measures, has been comprehensively described before.[11]

In this study, of those randomized (1136 in the DA group and 1142 in the placebo group), patients who did not receive the first 2 doses of the study drug during the first 4 weeks (n=52 and n=53, respectively), those who reached the primary composite end point in the first 4 weeks (n=35 and n=29, respectively), or those in whom the change in hemoglobin after 4 weeks was unknown (n=88 and n=73, respectively) were excluded. This left a total of 2033 subjects for these analyses: 1008 in the DA group and 1025 in the placebo group.

Hematopoietic Response

The hematopoietic response to DA was assessed as the percentage change in hemoglobin level between baseline and week 5 (after the 2 weight-based doses of DA). We observed that patients in the lowest quartile of response did not respond at all to DA; these were considered to be nonresponders, whereas subjects in the upper three quartiles were considered as responders.

Statistical Analysis

Values are presented as means±SD, median and interquartile range, or number and percentage when appropriate. Differences between baseline variables were assessed using ANOVA, *t* tests, and χ^2 tests when appropriate. A stepwise multivariable linear regression with $P<0.2$ as stay criteria and $P<0.1$ as entry criteria was performed to test whether baseline variables were associated with nonresponsiveness. Additionally, the incremental value of endogenous erythropoietin on predicting response was assessed by calculating the net reclassification improvement, integrated discrimination improvement, and the Harrell C statistic. Kaplan–Meier curves were produced and cut off when <5% of patients were at risk. Prognosis was assessed using a Cox proportional hazards model, with adjustment for 14 baseline covariates. All tests are 2 sided, and a *P* value of <0.05 was considered statistically significant.

RESULTS

Patients

Nonresponders (n=252) had an initial median reduction in hemoglobin of 0.2 g/dL (interquartile range, –0.6 to 0.0) and hemoglobin levels in this group remained lower throughout the trial despite higher average monthly doses of DA. No differences existed in levels of iron, ferritin, transferrin saturation, or cotreatment with oral or intravenous iron or red cell transfusions between responder and nonresponders. For results, see Tables I and II in the Data Supplement and Figure I in the Data Supplement.

Predictors of a Poor Hematopoietic Response

The results of the analysis of predictors of hematopoietic response are summarized in the Table and in Table II in the Data Supplement. Female sex, higher baseline hemoglobin or blood urea nitrogen, low serum sodium, and the absence of treatment with an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker were most strongly associated with a poor hematopoietic response, although the overall predictive value of the model was poor with a Harrell C statistic of 0.68. C-reactive protein and iron status were not associated with hemoglobin response. Adding endogenous erythropoietin level to the model resulted in significantly improved reclassification (P<0.01) but not discrimination.

Outcomes

The primary end point occurred in 137 (54.4%) nonresponders and in 361 (47.8%) responders (Figure). In a multivariable model adjusting for 14 baseline variables associated with outcome, patients with a poor initial response were at higher risk of a primary event (hazard ratio, 1.25; 95% confidence interval, 1.02–1.54) and all-cause mortality (hazard ratio, 1.30; 95% confidence interval, 1.04–1.63; Table IV in the Data Supplement). Regarding adverse events, no differences were observed between nonresponders and responders in total number of events, cerebrovascular disorders, or embolic and thrombotic events although a higher rate of cardiac failure (rate per 100 subject-years: 24.1 versus 18.5; P=0.03) was observed in the nonresponders (Table V in the Data Supplement). We found no significant interactions between tertiles of baseline endogenous erythropoietin levels and treatment with DA on outcome.

Table. Predictors of a Poor Response

Baseline Variables	Multivariable*	
	Odds Ratio (95% CI)	*P* Value
Demography		
Male vs female	0.625 (0.442–0.885)	<0.01
BMI, kg/m^2	1.019 (0.991–1.048)	0.19
Baseline medical history		
Atrial fibrillation/flutter	1.430 (1.017–2.009)	0.04
Stroke	1.489 (0.868–2.555)	0.15
Laboratory values		
Hemoglobin, g/dL	1.368 (1.077–1.737)	0.01
Reticulocytes, %	0.830 (0.696–0.991)	0.04
TIBC, µg/dL	1.003 (1.000–1.006)	0.05
BUN, mg/dL	1.011 (1.002–1.021)	0.01
Total bilirubin, mg/dL	1.650 (1.062–2.563)	0.03
Sodium, mEq/L	0.948 (0.911–0.988)	0.01
Vitamin B_{12}, pg/mL	1.000 (1.000–1.000)	0.03
Cholesterol, mg/dL	0.998 (0.994–1.001)	0.18
Medications		
ACEi and ARB	0.572 (0.366–0.895)	0.01

ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BUN, blood urea nitrogen; CI, confidence interval; and TIBC, total iron-binding capacity.

*Stepwise multivariable linear regression (with P<0.2 as stay criteria and P<0.1 as entry criteria).

DISCUSSION

The main finding of the present study was that approximately one quarter of HF patients do not exhibit an initial increase in hemoglobin in response to treatment with an ESA, a finding similar to that in anemic patients with CKD and type 2 diabetes mellitus enrolled in TREAT (Trial to Reduce Cardiovascular Events with Aranesp Therapy).[6] Importantly, this lack of response identifies patients at higher risk for HF hospitalization and all-cause mortality, independent of other prognostic factors. No increased rates of cerebrovascular disorders or thromboembolic events are observed in nonresponders, despite the use of higher doses of DA.

This is the first study in patients with HF evaluating hyporesponsiveness to an ESA in a large randomized controlled trial. Previously, TREAT showed similar results as the current study, including increased rates of cardiovascular events and all-cause mortality associated with a poor response.[7] Several factors could underlie a poor hematopoietic response to ESAs. Nonresponders might have a lower physiological set-point for their hemoglobin level, but our findings seem to suggest the opposite; nonresponders have high levels of endogenous erythropoietin at baseline which remained relatively high after 6 months of treatment, whereas responders had lower levels at baseline and showed a larger decrease at 6 months. A poor response can also occur in a wide range of disorders often concomitantly present in HF. These include malnutrition; iron, folate, or vitamin B_{12} deficiency; secondary hyperparathyroidism; hemolysis;

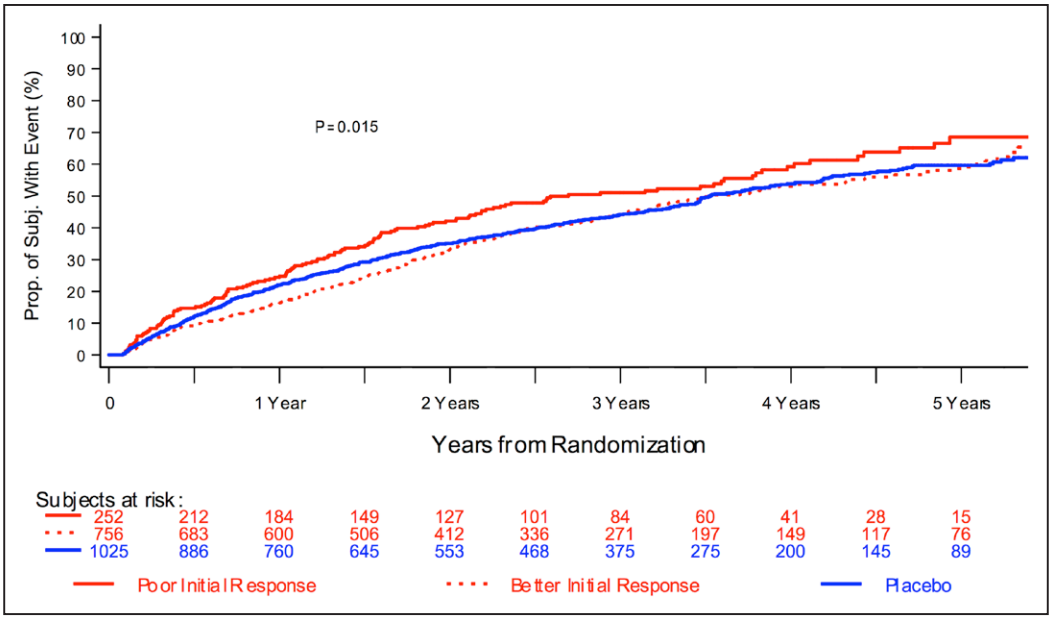


Figure. Rates of the primary composite end point for those with a poor compared with a better initial hematopoietic response.

Kaplan–Meier plot on the primary composite end point of all-cause death or first hospitalization for worsening heart failure of patients with a poor initial response compared with those with a better initial response and placebo. The *P* value for difference between poor compared with a better initial hematopoietic response is noted.

or in response to certain drugs such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers.[12] In our study, only a few parameters were moderately associated with the hematopoietic response to DA. Surprisingly, indices of iron homeostasis at baseline were not associated with a poor response, and iron supplementation rates, both oral and intravenous, did not differ between groups. Follow-up data on iron indices during ESA treatment could possibly give more insight into the pathophysiology of nonresponse.

Hemodilution plays a major role in the pathophysiology of anemia in HF patients and has previously been proposed to be one of the main reasons for the neutral effect of DA in the RED-HF study.[10,13] An indicator of hemodilution is a low serum sodium, which indeed showed an association with a poor response to ESAs in our study. Clinical signs of congestion and levels of NT-proBNP (N-terminal pro-B-type natriuretic peptide) were unfortunately not available. Another cause of anemia in patients with HF is chronic blood loss associated with the use of antiplatelets and oral anticoagulants. Although the use of antiplatelets did not differ significantly between response groups, the rate of oral anticoagulants in the nonresponders was almost 50% higher compared with responders (31.3% versus 23.0%; *P*=0.01). Taken together, these results highlight not only the need for the assessment of the underlying cause of anemia, specifically hemodilution and chronic blood loss, but also iron deficiency as this is relatively easily corrected with intravenous iron.

Because we could not identify hyporesponders with the use of other parameters except for the actual response to DA, we could not compare hyporesponsive patients who received DA to patients who received placebo with an expected poor response. Additionally, because the dose of DA was adjusted to the hematologic response of the patient, hyporesponsiveness to DA was accompanied by higher doses used. Therefore, effects of these 2 could not be assessed separately. Finally, although being the largest trial with ESA in HF, the number of studied subjects is limited.

Despite the overall neutral results of the RED-HF trial in HF,[4] as well as the TREAT trial,[6] the findings of the current subanalysis on hyporesponsiveness may help to identify which patients do not respond to ESA, which could subsequently lead to discontinuation of such agents. Importantly, the most recently published HF guidelines do not recommend the use of ESA outside the CKD population; however, approximately three quarters of the subjects of our study had concomitant CKD.[5] Other therapies for anemia have also failed in improving prognosis. Management of anemia in HF should, therefore, focus on the underlying cause. Potential future therapies for anemia in HF include hepcidin antagonists, hypoxia-inducible factor stabilizers, and intravenous iron.

CONCLUSIONS

In HF patients with anemia, a poor initial hemoglobin response to DA is associated with adverse outcomes.

Patients with a poor initial response are, however, difficult to identify using the available clinical and biochemical biomarkers.

SOURCES OF FUNDING

The RED-HF trial was funded by Amgen, Inc.

DISCLOSURES

Dr van der Meer received consultancy fees and an unrestricted grant from Vifor Pharma. Dr Pfeffer received consultancy fees and travel fees from Amgen, Inc. K. Olson is an employee from Amgen, Inc. Dr Anand received travel fees and RED-HF board membership fees from Amgen, Inc. Dr Westenbrink received consulting and speaker fees from Boehringer Ingelheim and Bayer and a travel grant from Novartis. Dr McMurray received travel fees and RED-HF board membership fees from Amgen, Inc. Dr Swedberg received consultancy fees, travel fees, and a grant from Amgen, Inc. Dr Young received board membership fees from Amgen, Inc. Dr Solomon received consultancy fees, travel fees, and a grant from Amgen, Inc. Dr van Veldhuisen received consultancy fees and board membership fees from Vifor Pharma and Amgen Inc. The other author reports no conflicts.

AFFILIATIONS

From the Department of Cardiology, University Medical Center Groningen, University of Groningen, the Netherlands (P.v.d.M., N.G.B., B.D.W., D.J.v.V.); Cardiovascular Division, Brigham and Women's Hospital, Boston, MA (M.A.P., S.D.S.), Amgen, Thousand Oaks, CA (K.O.); Department of Medicine, University of Minnesota Medical School and VA Medical Center, Minneapolis (I.S.A.); BHF Cardiovascular Research Centre, University of Glasgow, United Kingdom (J.J.V.M.); Department of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden (K.S.); and Department of Medicine, Cleveland Clinic, OH (J.B.Y.).

FOOTNOTES

Received April 4, 2017; accepted November 30, 2017.

The Data Supplement is available at http://circheartfailure.ahajournals.org/lookup/suppl/doi:10.1161/CIRCHEARTFAILURE.117.004431/-/DC1

Circ Heart Fail is available at http://circheartfailure.ahajournals.org.

REFERENCES

1. Anand I, McMurray JJ, Whitmore J, Warren M, Pham A, McCamish MA, Burton PB. Anemia and its relationship to clinical outcome in heart failure. *Circulation*. 2004;110:149–154. doi: 10.1161/01.CIR.0000134279.79571.73.
2. van Veldhuisen DJ, Anker SD, Ponikowski P, Macdougall IC. Anemia and iron deficiency in heart failure: mechanisms and therapeutic approaches. *Nat Rev Cardiol*. 2011;8:485–493.
3. van der Meer P, Voors AA, Lipsic E, van Gilst WH, van Veldhuisen DJ. Erythropoietin in cardiovascular diseases. *Eur Heart J*. 2004;25:285–291.
4. Swedberg K, Young JB, Anand IS, Cheng S, Desai AS, Diaz R, Maggioni AP, McMurray JJ, O'Connor C, Pfeffer MA, Solomon SD, Sun Y, Tendera M, van Veldhuisen DJ; RED-HF Committees; RED-HF Investigators. Treatment of anemia with darbepoetin alfa in systolic heart failure. *N Engl J Med*. 2013;368:1210–1219. doi: 10.1056/NEJMoa1214865.
5. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P; Authors/Task Force Members. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016;37:2129–2200. doi: 10.1093/eurheartj/ehw128.
6. Pfeffer MA, Burdmann EA, Chen CY, Cooper ME, de Zeeuw D, Eckardt KU, Feyzi JM, Ivanovich P, Kewalramani R, Levey AS, Lewis EF, McGill JB, McMurray JJ, Parfrey P, Parving HH, Remuzzi G, Singh AK, Solomon SD, Toto R; TREAT Investigators. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med*. 2009;361:2019–2032. doi: 10.1056/NEJMoa0907845.
7. Solomon SD, Uno H, Lewis EF, Eckardt KU, Lin J, Burdmann EA, de Zeeuw D, Ivanovich P, Levey AS, Parfrey P, Remuzzi G, Singh AK, Toto R, Huang F, Rossert J, McMurray JJ, Pfeffer MA; Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) Investigators. Erythropoietic response and outcomes in kidney disease and type 2 diabetes. *N Engl J Med*. 2010;363:1146–1155. doi: 10.1056/NEJMoa1005109.
8. van Der Meer P, Voors AA, Lipsic E, Smilde TDJ, van Gilst WH, van Veldhuisen DJ. Prognostic value of plasma erythropoietin on mortality in patients with chronic heart failure. *J Am Coll Cardiol*. 2004;44:63–67.
9. Belonje AM, Voors AA, van der Meer P, van Gilst WH, Jaarsma T, van Veldhuisen DJ. Endogenous erythropoietin and outcome in heart failure. *Circulation*. 2010;121:245–251. doi: 10.1161/CIRCULATIONAHA.108.844662.
10. Westenbrink BD, Visser FW, Voors AA, Smilde TD, Lipsic E, Navis G, Hillege HL, van Gilst WH, van Veldhuisen DJ. Anaemia in chronic heart failure is not only related to impaired renal perfusion and blunted erythropoietin production, but to fluid retention as well. *Eur Heart J*. 2007;28:166–171. doi: 10.1093/eurheartj/ehl419.
11. McMurray JJ, Anand IS, Diaz R, Maggioni AP, O'Connor C, Pfeffer MA, Polu KR, Solomon SD, Sun Y, Swedberg K, Tendera M, van Veldhuisen DJ, Wasserman SM, Young JB; RED-HF Committees and Investigators. Design of the Reduction of Events with Darbepoetin alfa in Heart Failure (RED-HF): a phase III, anaemia correction, morbidity-mortality trial. *Eur J Heart Fail*. 2009;11:795–801. doi: 10.1093/eurjhf/hfp098.
12. Johnson DW, Pollock CA, Macdougall IC. Erythropoiesis-stimulating agent hyporesponsiveness. *Nephrology (Carlton)*. 2007;12:321–330. doi: 10.1111/j.1440-1797.2007.00810.x.
13. Prakash ES. Haemodilution is a mechanism of anaemia in patients with heart failure. *Eur J Heart Fail*. 2013;15:1074. doi: 10.1093/eurjhf/hft120.

Circulation
Heart Failure

Hyporesponsiveness to Darbepoetin Alfa in Patients With Heart Failure and Anemia in the RED-HF Study (Reduction of Events by Darbepoetin Alfa in Heart Failure): Clinical and Prognostic Associations

Peter van der Meer, Niels Grote Beverborg, Marc A. Pfeffer, Kurt Olson, Inder S. Anand, B. Daan Westenbrink, John J.V. McMurray, Karl Swedberg, James B. Young, Scott D. Solomon and Dirk J. van Veldhuisen

Circ Heart Fail. 2018;11:
doi: 10.1161/CIRCHEARTFAILURE.117.004431
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1941-3289. Online ISSN: 1941-3297

Supplemental Material

Supplemental Methods 1. Dosing algorithm

Study Drug Dosing Algorithm

Investigational product, for both treatment groups, was administered subcutaneously every two weeks or monthly. The treatment assignment, including dose and frequency, was managed by the interactive voice response system (IVRS).

Darbepoetin alfa Treatment Group

Darbepoetin alfa was administered in dose strengths of 20, 30, 40, 50, 60, 80, 100, 130, 150, 200, 250, 300, 400, 500, and 600 µg. The starting dose of darbepoetin alfa was 0.75 µg/kg and administered every 2 weeks. Doses of darbepoetin alfa were adjusted to achieve and maintain the target hemoglobin of 13 g/dL (see table). The darbepoetin alfa dose did not exceed 300 µg for subjects receiving darbepoetin alfa every two weeks or 600 µg for subjects on monthly dosing. If a subject was receiving the lowest darbepoetin alfa dose on the 2 week dose schedule and required a dose decrease, the subject was switched to monthly dosing. If a subject required a dose decrease of darbepoetin alfa while receiving the lowest monthly dose, the subject was switched to placebo.

Placebo Treatment Group

The treatment assignment blinding was maintained by IVRS and the dose volume and frequency mirrored the darbepoetin alfa treatment group.

Dose Adjustment Table

Hb (g/dl)	Hb rate of rise (g/dl/2 weeks)	Dose Adjustment*
< 13.0	< 0.5	Increase to next higher dose strength
	≥ 0.5 to < 1.0	Maintain dose
	≥ 1.0	Decrease to next lower dose strength
13.0 to < 14.5	< 1.0	Maintain dose
	≥ 1.0	Decrease to next lower dose strength
≥ 14.5	Any	Administer placebo until Hb value is below 14.0 g/dl, then resume darbepoetin alfa at next lower dose strength

*Available dose strengths in the trial: 20, 30, 40, 50, 60, 80, 100, 130, 150, 200, 250, 300, 400, 500, and 600 µg.

Supplemental Table 1. Baseline characteristics

Variable	Non-responders	Responders			P-value
	Q1 (N = 252)	Q2 (N = 253)	Q3 (N =251)	Q4 (N = 252)	
Change in hemoglobin (at 28 days)					
Percent change	<1.3	1.3 to 6.7	6.7 to 11.7	>11.7	N/A
Median – g/dL (interquartile range)	-0.2 (-0.6 – 0.0)	0.4 (0.3 – 0.6)	1.0 (0.8 – 1.1)	1.8 (1.5 – 2.4)	N/A
Demography					
Age (years)	70.9 ± 11.3	70.0 ± 12.8	70.7 ± 10.3	68.6 ± 12.2	0.10
Males, n (%)	136 (54)	157 (62)	152 (61)	149 (59)	0.28
Race, n (%)					0.02
White	181 (72)	175 (69)	182 (73)	154 (61)	
Black	11 (4)	25 (10)	18 (7)	23 (9)	
Other	60 (24)	53 (21)	51 (20)	75 (30)	
BMI (kg/m^2)	27.6 ± 5.9	27.7 ± 6.5	26.7 ± 5.1	26.5 ± 4.9	0.03
Systolic blood pressure (mmHg)	121.8 ± 18.6	119.8 ± 17.9	122.0 ± 18.2	120.4 ± 16.5	0.45
Diastolic blood pressure (mmHg)	69.7 ± 11.2	69.6 ± 11.4	68.8 ± 11.0	70.5 ± 10.2	0.37
Heart rate (bpm)	71.9 ± 10.6	71.9 ± 10.7	70.6 ± 10.4	72.5 ± 11.9	0.27
Baseline medical history					
Smoking status, n (%)					0.21
Current	5 (2)	13 (5)	9 (4)	13 (5)	
Former	86 (34)	104 (41)	93 (37)	90 (36)	
Never	161 (64)	136 (54)	149 (59)	149 (59)	
Hypertension, n (%)	189 (75)	174 (69)	182 (72)	193 (77)	0.21
Diabetes mellitus, n (%)	119 (47)	107 (42)	111 (44)	117 (46)	0.68
Atrial fibrillation/flutter, n (%)	105 (42)	79 (31)	77 (31)	70 (28)	<0.01
Myocardial infarction, n (%)	141 (56)	132 (52)	138 (55)	133 (53)	0.81
Stroke, n (%)	27 (11)	21 (8)	21 (8)	13 (4)	0.15
Deep vein thrombosis, n (%)	8 (3)	11 (4)	10 (4)	10 (4)	0.92
Laboratory values					

Serum erythropoietin					
Baseline	15.2 (9.7 – 27.5)	15.6 (10.5 – 27.9)	13.2 (9.1 – 21.1)	13.3 (8.8 – 23.3)	0.03
6 months	12.3 (7.8 – 20.6)	12.3 (6.9 – 23.1)	8.8 (5.0 – 16.0)	8.1 (5.1 – 15.8)	0.02
Hematological					
Hematocrit (%)	34.7 ± 2.8	34.6 ± 2.9	34.1 ± 2.9	34.4 ± 4.1	0.19
Hemoglobin, g/dL	11.1 ± 0.7	11.1 ± 0.7	10.9 ± 0.7	10.9 ± 0.7	<0.01
Red Blood Cells (10^6/uL)	3.9 ± 0.5	3.9 ± 0.5	3.8 ± 0.5	3.8 ± 0.5	0.01
Reticulocytes (%)	2.23 ± 0.97	2.26 ± 0.91	2.18 ± 0.78	2.41 ± 1.19	0.08
MCV (fL)	90.3 ± 7.9	90.2 ± 8.2	91.5 ± 7.3	91.0 ± 7.3	0.20
Iron (ug/dL)	69 (56 – 92)	66 (54 – 83)	69 (56 – 88)	72 (56 – 92)	<0.01
Ferritin (ng/mL)	91 (42 – 189)	102 (45 – 167)	97 (60 – 185)	114 (61 – 225)	0.43
TSAT (%)	27.3 ± 12.1	25.4 ± 9.0	26.9 ± 9.9	28.3 ± 12.2	0.03
TIBC (ug/dL)	294 ± 57	281 ± 51	281 ± 55	287 ± 63	0.03
Kidney parameters					
Creatinine (mg/dL)	1.4 (1.1 – 1.9)	1.4 (1.0 – 1.8)	1.5 (1.2 – 1.9)	1.2 (0.9 – 1.7)	<0.01
eGFR (mL/min/1.73m^2)	47.3 ± 20.7	52.5 ± 23.8	45.7 ± 17.5	55.5 ± 23.0	<0.01
BUN (mg/dL)	31 (31 – 42)	27 (19 – 41)	31 (21 – 41)	24 (18 – 36)	<0.01
Liver parameters					
Albumin (g/dL)	3.86 ± 0.42	3.84 ± 0.42	3.85 ± 0.38	3.84 ± 0.49	0.94
Alkaline Phosphatase (U/L)	87 (66 – 112)	79 (64 – 100)	77 (61 – 101)	80 (64 – 110)	0.12
ALT (U/L)	18 (13 – 23)	16 (13 – 23)	16 (13 – 22)	17 (13 – 24)	0.39
AST (U/L)	22 (18 – 28)	21 (17 – 26)	21 (17 – 25)	21 (18 – 26)	0.24
Electrolytes					
Sodium (mEq/L)	139.1 ± 4.1	139.7 ± 3.8	139.7 ± 3.8	140.3 ± 3.9	0.01
Potassium (mEq/L)	4.59 ± 0.58	4.60 ± 0.56	4.65 ± 0.59	4.59 ± 0.59	0.59
Vitamins					
Folate (ng/mL)	10.1 (6.1 – 19.6)	10.5 (6.2 – 25.6)	10.5 (6.2 – 24.6)	10.6 (6.4 – 27.6)	0.91
Vitamin B12 (pg/mL)	413 (297 – 639)	395 (300 – 616)	374 (276 – 568)	420 (279 – 762)	<0.01
Other					
Glucose (mg/dL)	119 (97 – 164)	107 (95 – 145)	112 (95 – 154)	110 (94 – 146)	0.17
HbA1C (%)	6.2 (5.6 – 7.2)	6.0 (5.5 – 6.9)	6.0 (5.6 – 6.9)	6.0 (5.5 – 7.0)	0.37

C-reactive protein (mg/dL)	3.09 (1.06 – 8.18)	3.62 (1.32 – 7.30)	2.55 (1.14 – 6.92)	2.06 (0.75 – 5.42)	<0.01
Medications					
Iron, n (%)					
Oral	74 (29)	73 (29)	102 (41)	90 (36)	0.01
Intravenous	7 (3)	5 (2)	4 (2)	6 (2)	0.82
ACEi and/or ARB, n (%)	210 (83)	225 (89)	220 (88)	233 (93)	0.02
Statin, n (%)	147 (58)	154 (61)	165 (66)	131 (52)	0.02
Antiplatetets, n (%)	161 (64)	163 (64)	169 (67)	177 (70)	0.41
Oral anticoagulants, n (%)	79 (31.3)	68 (26.9)	59 (23.5)	50 (19.8)	0.02

Legend. Q = quartile, N/A = not applicable, BMI = body-mass index, MCV = mean corpuscular volume, TSAT = transferrin saturation, TIBC = total iron binding capacity, eGFR = estimated glomerular filtration rate, BUN = blood urea nitrogen, ALT = alanine transaminase, AST = aspartate transaminase, HbA1C = glycated hemoglobin, ACEi = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker.

Supplemental Table 2. Baseline characteristics

Variable	Non-responders (N = 252)	Responders (N = 756)	P-value
Change in hemoglobin (at 28 days)			
Percent change	<1.3	1.3 – 69	N/A
Median – g/dL (interquartile range)	-0.2 (-0.6 – 0.0)	1.00 (0.60 – 1.50)	N/A
Demography			
Age (years)	70.9 ± 11.3	69.8 ± 11.8	0.19
Males, n (%)	136 (54)	458 (61)	0.07
Race, n (%)			0.07
White	181 (72)	511 (67)	
Black	11 (4)	66 (9)	
Other	60(24)	179 (24)	
BMI (kg/m^2)	27.6 ± 5.9	27.0 ± 5.6	0.13
Systolic blood pressure (mmHg)	121.8 ± 18.6	120.7 ± 17.5	0.43
Diastolic blood pressure (mmHg)	69.7 ± 11.2	69.6 ± 10.9	0.95
Heart rate (bpm)	71.9 ± 10.6	71.7 ± 11.0	0.74
Baseline medical history			
Smoking status, n (%)			0.07
Current	5 (2)	35 (5)	
Former	86 (34)	287 (38)	
Never	161 (64)	434 (57)	
Hypertension, n (%)	189 (75)	549 (73)	0.46
Diabetes mellitus, n (%)	119 (47)	335 (44)	0.42
Atrial fibrillation/flutter, n (%)	105 (42)	226 (30)	<0.01
Myocardial infarction, n (%)	141 (56)	403 (53)	0.47
Stroke, n (%)	27 (11)	55 (7.3)	0.08
Deep vein thrombosis, n (%)	8 (3)	31 (4)	0.51
Laboratory values			
Serum erythropoietin			
Baseline	15.2 (9.7 – 27.5)	14.0 (9.4 – 24.0)	0.17

6 months	12.3 (7.8 – 20.6)	9.2 (5.4 – 17.4)	<0.01
Hematological			
Hematocrit (%)	34.7 ± 2.8	34.4 ± 3.3	0.18
Hemoglobin, g/dL	11.1 ± 0.7	11.0 ± 0.7	0.01
Red Blood Cells (10^6/uL)	3.9 ± 0.5	3.8 ± 0.5	0.07
Reticulocytes (%)	2.23 ± 0.97	2.28 ± 0.97	0.51
MCV (fL)	90.3 ± 7.9	90.9 ± 7.6	0.29
Iron (ug/dL)	69 (56 – 92)	68 (55 – 88)	0.09
Ferritin (ng/mL)	91 (42 – 189)	103 (55 – 189)	0.44
TSAT (%)	27.3 ± 12.1	26.9 ± 10.5	0.61
TIBC (ug/dL)	294 ± 57	283 ± 56	<0.01
Kidney parameters			
Creatinine (mg/dL)	1.4 (1.1 – 1.9)	1.3 (1.0 – 1.8)	0.10
eGFR (mL/min/1.73m^2)	47.3 ± 20.7	51.2 ± 22.0	0.01
BUN (mg/dL)	31 (31 – 42)	27 (19 – 40)	<0.01
Liver parameters			
Albumin (g/dL)	3.86 ± 0.42	3.84 ± 0.43	0.53
Alkaline Phosphatase (U/L)	87 (66 – 112)	79 (63 – 102)	0.06
ALT (U/L)	18 (13 – 23)	17 (13 – 23)	1.00
AST (U/L)	22 (18 – 28)	21 (17 – 26)	0.07
Electrolytes			
Sodium (mEq/L)	139.1 ± 4.1	139.9 ± 3.9	<0.01
Potassium (mEq/L)	4.59 ± 0.58	4.61 ± 0.58	0.64
Vitamins			
Folate (ng/mL)	10.1 (6.1 – 19.6)	10.5 (6.3 – 26.2)	0.84
Vitamin B12 (pg/mL)	413 (297 – 639)	394 (287 – 638)	0.16
Other			
Glucose (mg/dL)	119 (97 – 164)	110 (95 – 146)	0.05
HbA1C (%)	6.2 (5.6 – 7.2)	6.0 (5.5 – 6.9)	0.11
C-reactive protein (mg/dL)	3.09 (1.06 – 8.18)	2.77 (1.12 – 6.41)	0.15
Medications			
Iron, n (%)			

Oral	74 (29)	265 (35)	0.10
Intravenous	7 (3)	15 (2)	0.46
ACEi and/or ARB, n (%)	210 (83)	678 (90)	<0.01
Statin, n (%)	147 (58)	450 (60)	0.74
Antiplatetets, n (%)	161 (64)	509 (67)	0.32
Oral anticoagulants, n (%)	79 (31.3)	177 (23)	0.01

Legend. Q = quartile, N/A = not applicable, BMI = body-mass index, MCV = mean corpuscular volume, TSAT = transferrin saturation, eGFR = estimated glomerular filtration rate, BUN = blood urea nitrogen, ALT = alanine transaminase, AST = aspartate transaminase, HbA1C = glycated hemoglobin, ACEi = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker.

Supplemental Table 3. The incremental value of endogenous erythropoietin on predicting response

	Response (yes vs. no)		
	Reference Model *	Reference model with hyporesponsiveness measure	
Endogenous erythropoietin			
Discrimination			
C-statistic	0.68	0.69	0.40
Reclassification			
IDI	Reference	0.018 (0.007, 0.029)	<0.01
NRI	Reference	0.337 (0.175, 0.499)	<0.01

Legend.

* Reference model: Males vs. female, BMI (kg/m2), Atrial fibrillation/flutter, Stroke (yes vs. No), Hemoglobin (g/dL), Reticulocytes (%), TIBC (ug/dL), BUN (mg/dL), Total Bilirubin (mg/dL), Sodium (mEq/L), Vitamin B12 (pg/mL), Cholesterol (mg/dL), ACEi and/or ARB (yes vs. No).

IDI = Integrated Discrimination Improvement, NRI = Net Reclassification Improvement

Supplemental Table 4. Rate of End Points and Adjusted Hazard Ratios

	Placebo (N = 1025)	Non-responders (N = 252)	Responders (N = 756)	Unadjusted Hazard Ratio * (95% CI)	Adjusted Hazard Ratio † (95% CI)
	Rate per 100 patient-yr (95% CI)				
Primary Composite	19.8 (18.1 – 21.7)	24.4 (20.5 – 28.9)	19.3 (17.3 – 21.3)	1.27 (1.05 – 1.55)	1.25 (1.02 – 1.54)
CV death / hosp. wors. HF	17.9 (16.3 – 19.7)	21.9 (18.2 – 26.2)	17.1 (15.3 – 19.1)	1.28 (1.04 – 1.58)	1.26 (1.02 – 1.57)
All-cause mortality	13.9 (12.5 – 15.3)	17.9 – 14.8 – 21.5)	14.1 (12.5 – 15.7)	1.30 (1.05 – 1.61)	1.30 (1.04 – 1.63)
Hosp. worsening HF	12.0 (10.6 – 13.5)	14.2 (11.1 – 17.7)	11.0 (9.5 – 12.6)	1.27 (0.97 – 1.66)	1.17 (0.88 – 1.55)
CV death	11.4 (10.2 – 12.7)	14.8 (12.0 – 18.1)	11.4 (10.1 – 13.0)	1.32 (1.04 – 1.67)	1.33 (1.04 – 1.71)
CV death/MI/stroke	13.1 (11.7 – 14.5)	16.6 (13.5 – 20.1)	13.2 (11.6 – 14.8)	1.28 (1.02 – 1.60)	1.28 (1.01 – 1.63)
All cause death/MI/stroke	15.5 (14.0 – 17.0)	19.6 (16.3 – 23.4)	15.4 (13.8 – 17.2)	1.29 (1.05 – 1.59)	1.30 (1.05 – 1.62)
Myocardial infarction	2.6 (2.0 – 3.3)	3.0 (1.8 – 4.7)	2.0 (1.4 – 2.7)	1.52 (0.87 – 2.65)	1.65 (0.92 – 2.95)
Stroke	1.0 (0.7 – 1.5)	1.3 (0.6 – 2.6)	1.4 (0.9 – 2.0)	0.98 (0.45 – 2.17)	0.86 (0.38 – 1.95)

Legend. Events rates are shown for patients who received at least two doses during the first 4 weeks, did not have the primary composite endpoint within 4 weeks, and the change in hemoglobin level at week 5 was known.

* Hazard ratios are for patients with a poor response as compared with those with a better response.

† Adjusted for baseline age, sex, NYHA functional class, hospitalization for heart failure within 6 months before randomization (yes vs. no), diabetes (yes vs. no), log serum creatinine level, ejection fraction, cause of heart failure (ischemic vs. non-ischemic), body-mass index, left bundle-branch block (yes vs. no), history of atrial fibrillation or flutter (yes vs. no), systolic blood pressure, region and type of device.

CI = confidence interval, CV = cardiovascular, hosp. wors. HF = hospitalization for worsening heart failure, MI = myocardial infarction

Supplemental Table 5. Adverse events

	Placebo (N = 1025)	Non-responders (N = 252)	Responders (N = 756)	Poor vs. Better
	Exposure adjusted subject incidence rate (/100subject-year)			P- Value*
Any Event of Interest	41.1	42.4	35.6	0.08
Antibody-mediated pure red cell aplasia	0.0	0.0	0.0	-
Cardiac failure	23.5	24.1	18.5	0.03
Cerebrovascular disorders	1.9	3.3	2.1	0.17
Hemorrhagic cerebrovascular conditions	1.2	2.1	1.3	0.24
Ischemic cerebrovascular conditions	1.3	2.5	1.8	0.35
Cerebrovascular disorders, not specified as hemorrhagic or ischemic	0.0	0.0	0.0	-
Convulsions	0.2	0.2	0.2	0.93
Dialysis vascular access thrombosis	0.0	0.2	0.0	0.33
Embolic and thrombotic events	4.9	7.2	6.1	0.40
Arterial†	3.1	3.9	3.3	0.56
Venous‡	0.8	1.2	1.3	0.85
Vessel type unspecified and mixed arterial and venous§	1.1	2.5	1.8	0.37
Hypersensitivity	4.2	3.6	4.6	0.36
Hypertension	3.0	2.4	3.7	0.13
Ischemic heart disease	7.4	7.6	6.4	0.38
Malignancies	2.8	3.1	2.7	0.62

Legend. Events rates are shown for patients who received at least two doses during the first 4 weeks, did not have the primary composite endpoint within 4 weeks, and the change in hemoglobin level at week 5 was known.

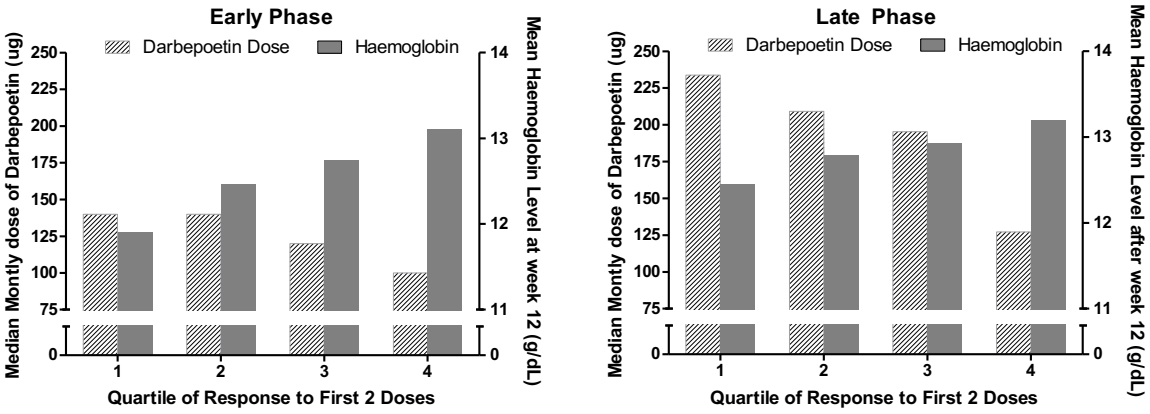
* P values were calculated with the use of Pearson's chi-square test, except for hemodialysis-related vascular access thrombosis and convulsions, for which Fisher's exact test was used.

† Included in the arterial-events category are the following preferred terms: acute myocardial infarction, amaurosis fugax, carotid-artery occlusion, cerebrovascular insufficiency, coronary arterial stent insertion, coronary-artery occlusion, ischemic stroke, lacunar infarction, myocardial infarction, percutaneous coronary intervention, peripheral arterial occlusive disease, peripheral-artery thrombosis, peripheral embolism, retinal-artery occlusion, and transient ischemic attack.

‡ Included in the venous-events category are the following preferred terms: deep-vein thrombosis, jugular-vein thrombosis, pulmonary embolism, retinal-vein occlusion, thrombophlebitis, superficial thrombophlebitis, and limb venous thrombosis.

§ Included in the unspecified events are the following preferred terms: bone infarction, cerebral infarction, cerebral ischemia, cerebrovascular accident, coronary-artery thrombosis, embolism, hemorrhagic stroke, hemiparesis, hemiplegia, heparin-induced thrombocytopenia, intestinal infarction, intracardiac thrombus, shunt occlusion, splenic infarction, thrombosis, thrombosis in device, mesenteric-vessel thrombosis, and thrombosis prophylaxis.

Supplemental Figure 1. Hemoglobin levels and dose of Darbepoetin Alfa used



The median dose of Darbepoetin Alfa used and the mean hemoglobin levels reached at week 12 (early phase) and after week 12 (late phase) divided by quartiles of initial hemoglobin response at week 5.