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Anemia, erythropoietin and iron in heart failure

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Hyporesponsiveness to Darbepoetin Alfa in patients with heart failure and anemia in the RED-HF study: clinical and prognostic associations

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ABSTRACT

Background

A poor response to erythropoiesis-stimulating agents such as darbepoetin alfa (DA) has been associated with adverse outcomes in patients with diabetes, 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 Reduction of Events by Darbepoetin alfa in Heart Failure (RED-HF) trial, in which 1008 patients with systolic heart failure and anemia (hemoglobin level 9.0–12.0 g/dL) were randomized to DA. We examined the relationship between the hematopoietic response to DA 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 “non-responders”. The median initial hemoglobin change in non-responders (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 ACE inhibitors and/or ARB’s were independently associated with non-response. Although a low endogenous erythropoietin level helped to differentiate responders from non-responders, its predictive value in a multivariable model was poor (c-statistic=0.69). Non-responders had a higher rate of all-cause death or first heart failure hospitalization (hazard ratio [HR] 1.25; 95% confidence interval (CI) 1.02–1.54) and a higher risk of all-cause mortality (HR 1.30; 95% CI 1.04–1.63) than responders.

Conclusion

A poor response to DA 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.

INTRODUCTION

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 Reduction of Events by Darbepoetin Alfa in Heart Failure trial (RED-HF).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 As the syndromes of HF and chronic kidney disease 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 hypo-responsiveness of the bone marrow.⁸⁻¹⁰ We assessed the hematopoietic response to DA in anemic HF patients in RED-HF.

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 (NYHA) functional class II, III, or IV, and left ventricular ejection fraction (LVEF) \leq 40%, with a Hb 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 [TSAT], $<$ 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 two doses of the study drug during the first 4 weeks (N=52 and N=53, respectively), those who reached the primary composite

endpoint in the first 4 weeks (N=35 and N=29, respectively) or those where the change in Hb 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 Hb level between baseline and week 5 (after the two 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 “non-responders” whereas subjects in the upper three quartiles were considered as “responders”.

Statistical analysis

Values are presented as means and standard deviation (\pm SD), median and interquartile range (IQR) or number and percentage when appropriate. Differences between baseline variables were assessed using analysis of variance, t-tests and chi-square 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 non-responsiveness. Additionally, the incremental value of endogenous erythropoietin on predicting response was assessed by calculating the net reclassification improvement (NRI), integrated discrimination improvement (IDI) and the Harrell’s 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 two-sided and a p-value of < 0.05 was considered statistically significant.

RESULTS

Patients

Non-responders (N=252) had an initial median reduction in Hb of 0.2 g/dL (interquartile range [IQR] -0.6 – 0.0) and Hb levels in this group remained lower throughout the trial despite higher average monthly doses of DA. No differences existed in levels of iron, ferritin, TSAT or co-treatment with oral or intravenous iron or red cell transfusions between responder and non-responders. For results, see **Supplemental Tables 1 and 2**, and **Supplemental Figure 1**.

Predictors of a poor hematopoietic response

The results of the analysis of predictors of hematopoietic response are summarized in **Table 1** and **Supplemental Table 3**. Female sex, higher baseline Hb or BUN, low serum sodium and the absence of treatment with an ACEi or ARB were most strongly associated with a poor hematopoietic response, although the overall predictive value of the model was poor with a Harrell's c-statistic of 0.68. CRP and iron status were not associated with Hb-response. Adding endogenous erythropoietin level to the model resulted in significantly improved reclassification ($P < 0.01$) but not discrimination.

Table 1 – Predictors of a poor response

Baseline variables	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		
Haemoglobin, g/dL	1.368 (1.077, 1.737)	0.01
Reticulocytes (%)	0.830 (0.696, 0.991)	0.04
TIBC (ug/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 B12 (pg/mL)	1.000 (1.000, 1.000)	0.03
Cholesterol (mg/dL)	0.998 (0.994, 1.001)	0.18
Medications		
ACEi and/or ARB	0.572 (0.366, 0.895)	0.01

* Stepwise multivariable linear regression (with $p < 0.2$ as stay criteria and $p < 0.1$ as entry criteria). CI = confidence interval, BMI = body-mass index, MCV = mean corpuscular volume, TSAT = transferrin saturation, TIBC = total iron binding capacity, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, eGFR = estimated glomerular filtration rate, BUN = blood urea nitrogen, ALT = alanine transaminase, AST = aspartate transaminase, HbA1C = glycated hemoglobin, WBC = white blood cells, ACEi = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker.

Outcomes

The primary endpoint occurred in 137 (54.4%) non-responders and in 361 (47.8%) responders (**Figure 1**). 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 [HR] 1.25; 95% confidence interval [CI] 1.02 – 1.54) and all-cause mortality (HR: 1.30; 95% CI: 1.04 – 1.63), (**Supplemental Table 4**). Regarding adverse events, no differences were observed between non-responders and respond-

ers 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 vs. 18.5; $P=0.03$) was observed in the non-responders (**Supplemental Table 5**). We found no significant interactions between tertiles of baseline endogenous erythropoietin levels and treatment with DA on outcome.

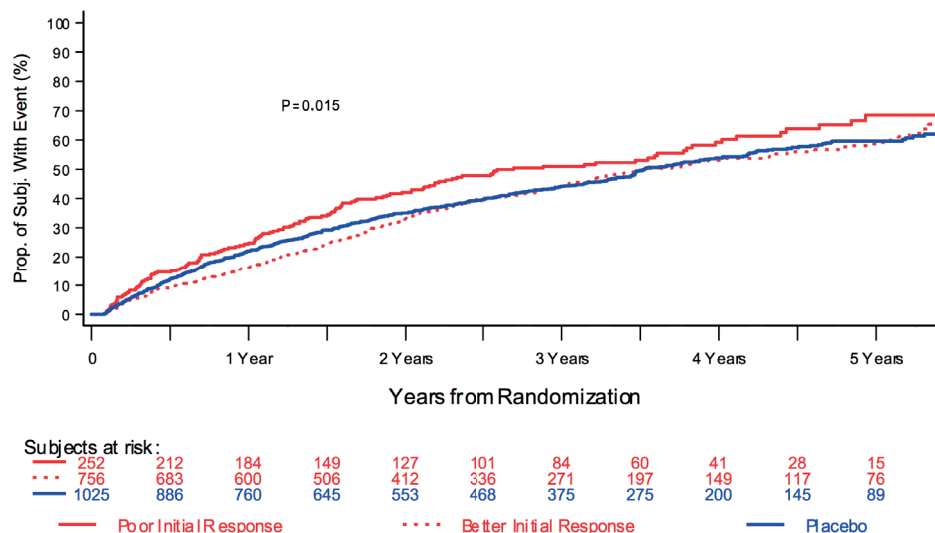


Figure 1 – Rates of the primary composite endpoint for those with a poor compared to a better initial hematopoietic response. Kaplan-Meier plot on the primary composite endpoint of all cause death or first hospitalization for worsening heart failure of patients with a poor initial response compared to those with a better initial response and placebo. The P-value for difference between poor compared to a better initial hematopoietic response is noted.

DISCUSSION

The main finding of the present study was that approximately one quarter of HF patients do not exhibit an initial increase in Hb in response to treatment with an ESA, a finding similar to that in anemic patients with chronic kidney disease and type 2 diabetes enrolled in TREAT.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 non-responders, despite use of higher doses of DA.

This is the first study in patients with HF evaluating hypo-responsiveness 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. Non-responders might have a lower physiological set-point for their Hb level, but our findings seem to suggest the opposite; non-responders have high levels of endogenous erythropoietin at baseline which remained relatively high after 6 months of treatment, while 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 B12 deficiency, secondary hyperparathyroidism, hemolysis or in response to certain drugs such as ACEi and ARBs.¹² In our study, only a few parameters were very 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 non-response.

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 were unfortunately not available. Another etiology of anemia in patients with HF is chronic blood loss associated with the use of antiplatelets and oral anti-coagulants. Although the use of antiplatelets did not differ significantly between response groups, the rate of oral anti-coagulants in the non-responders was almost 50% higher compared to responders (31.3 vs. 23.0%, $P=0.01$). Taken together, these results highlight the need for the assessment of the underlying etiology of anemia, specifically hemodilution and chronic blood loss, but also iron deficiency as this is relatively easily corrected with intravenous iron.

As 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, as the dose of DA was adjusted to the hematological response of the patient, hyporesponsiveness to DA was accompanied by higher doses used. Therefore, effects of these two 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 HF⁴, as well as the TREAT Trial⁶, the findings of the current sub analysis on hyporesponsiveness may help to identify which patients do not respond to ESA, which could subsequently lead to discontinu-

ation 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 etiology. Potential future therapies for anemia in HF include hepcidin antagonists, hypoxia inducible factor stabilizers and intravenous iron.

CONCLUSION

In HF patients with anemia, a poor initial Hb 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.

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DISCLOSURES

PvdM received consultancy fees and an unrestricted grant from Vifor Pharma NGB declares no disclosures MAP received consultancy fees and travel fees from Amgen Inc. KO is an employee from Amgen Inc. ISA received travel fees and RED-HF board membership fees from Amgen Inc. BDW received consulting and speaker fees from Boehringer Ingelheim and Bayer and a travel grant from Novartis. JMcM received travel fees and RED-HF board membership fees from Amgen Inc. KS received consultancy fees, travel fees and a grant from Amgen Inc. JBY received board membership fees from Amgen Inc. SDS received consultancy fees, travel fees and a grant from Amgen Inc. DJvV received consultancy fees and board membership fees from Vifor Pharma and Amgen Inc.

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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					
<i>Serum erythropoietin</i>					
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
<i>Hematological</i>					
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

Supplemental Table 1 – Baseline characteristics (continued)

Variable	Non-responders		Responders		P-value
	Q1 (N = 252)	Q2 (N = 253)	Q3 (N = 251)	Q4 (N = 252)	
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
<i>Kidney parameters</i>					
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
<i>Liver parameters</i>					
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
<i>Electrolytes</i>					
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
<i>Vitamins</i>					
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
<i>Other</i>					
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
Antiplatelets, 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

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			
<i>Serum erythropoietin</i>			
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
<i>Hematological</i>			
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

Supplemental Table 2. Baseline characteristics (continued)

Variable	Non-responders (N = 252)	Responders (N = 756)	P-value
<i>Kidney parameters</i>			
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
<i>Liver parameters</i>			
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
<i>Electrolytes</i>			
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
<i>Vitamins</i>			
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
<i>Other</i>			
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
Antiplatelets, n (%)	161 (64)	509 (67)	0.32
Oral anticoagulants, n (%)	79 (31.3)	177 (23)	0.01

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	P-value
Endogenous erythropoietin			
<i>Discrimination</i>			
C-statistic	0.68	0.69	0.40
<i>Reclassification</i>			
IDI	Reference	0.018 (0.007, 0.029)	<0.01
NRI	Reference	0.337 (0.175, 0.499)	<0.01

* 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)

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

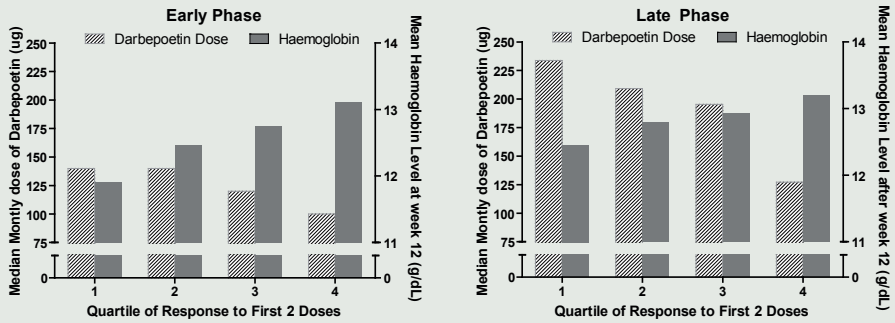
Events rates are shown for patients who received at least two doses during the first 4 weeks, did not have the primary composite endpoint within 4 weeks, and the change in hemoglobin level at week 5 was known.

* 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 cerebrovascular 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.

Part II

Iron

