Stroke in Patients With Type 2 Diabetes Mellitus, Chronic Kidney Disease, and Anemia Treated With Darbepoetin Alfa

The Trial to Reduce Cardiovascular Events With Aranesp Therapy (TREAT) Experience

Hicham Skali, MD, MSc; Hans-Henrik Parving, MD, DMSc; Patrick S. Parfrey, MD; Emmanuel A. Burdmann, MD, PhD; Eldrin F. Lewis, MD; Peter Ivanovich, MD; Sai Ram Keithi-Reddy, MD; Janet B. McGill, MD; John J.V. McMurray, MD; Ajay K. Singh, MD; Scott D. Solomon, MD; Hajime Uno, PhD; Marc A. Pfeffer, MD, PhD, on behalf of the TREAT Investigators

Background—More strokes were observed in the Trial to Reduce Cardiovascular Events With Aranesp Therapy (TREAT) among patients assigned to darbepoetin alfa. We sought to identify baseline characteristics and postrandomization factors that might explain this association.

Methods and Results—A multivariate logistic regression model was used to identify baseline predictors of stroke in 4038 patients with diabetes mellitus, chronic kidney disease, and anemia randomized to receive darbepoetin alfa or placebo. To determine whether postrandomization blood pressure, hemoglobin level, platelet count, or treatment dose were responsible for the increased risk related to darbepoetin alfa, we performed a nested case-control analysis (1:10 matching) identifying nonstroke controls with propensity matching. The risk of stroke was doubled with darbepoetin alfa. Overall, 154 patients had a stroke, 101/2012 (5.0%) in the darbepoetin alfa arm and 53/2026 (2.6%) in the placebo arm (hazard ratio 1.9; 95% confidence interval, 1.4–2.7). Independent predictors of stroke included assignment to darbepoetin alfa (odds ratio 2.1; 95% confidence interval, 1.5–2.9), history of stroke (odds ratio 2.0; 95% confidence interval, 1.4–2.9), more proteinuria, and known cardiovascular disease. In patients assigned to darbepoetin alfa, postrandomization systolic and diastolic blood pressure, hemoglobin level, platelet count, and darbepoetin alfa dose did not differ between those with and without stroke. Additional sensitivity analyses using maximal values, latest values, or changes over varying periods of exposure yielded similar results.

Conclusions—The 2-fold increase in stroke with darbepoetin alfa in TREAT could not be attributed to any baseline characteristic or to postrandomization blood pressure, hemoglobin, platelet count, or dose of treatment. These readily identifiable factors could not be used to mitigate the risk of darbepoetin alfa–related stroke.

Clinical Trial Registration—http://www.clinicaltrials.gov. Unique identifier: NCT00093015.

(*Circulation*. 2011;124:2903-2908.)

Key Words: anemia ■ diabetes mellitus ■ kidney diseases ■ erythropoietin ■ risk ■ stroke

The presence of anemia has been consistently associated with a higher risk of cardiovascular (CV) events in adjusted analyses from both epidemiological observations and clinical trials encompassing broad and diverse populations.[1–7] Among patients with type 2 diabetes mellitus and nondialysis chronic kidney disease (CKD), those with anemia had a ≈60% to 90% greater adjusted relative risk of death, myocardial infarction, or stroke in a pooled analysis of 4 major epidemiological cohorts.[8] However, in the placebo-controlled Trial to Reduce Cardiovascular Events With Aranesp Therapy (TREAT),[9] correction of anemia in patients with type 2 diabetes mellitus and nondialysis CKD with the

Received March 13, 2011; accepted September 28,2011.

From the Cardiovascular Division (H.S., E.F.L., S.D.S., J.J.V.M., M.A.P.) and Renal Division (A.K.S.), Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, and Department of Biostatistics (H.U.), Harvard School of Public Health, Boston, MA; Department of Medical Endocrinology, Rigshospitalet, University of Copenhagen, Coopenhagen, and Faculty of Health Science, Aarhus University, Aarhus, Denmark (H.H.P.); Division of Nephrology, Health Sciences Center, St. Johns, Newfoundland, Canada (P.S.P.); Division of Nephrology, University of Sao Paulo Medical School, Brazil (E.A.B.); Division of Nephrology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL (P.I.); Global Clinical Development, Amgen, Thousand Oaks, CA (S.R.K.R.); Division of Endocrinology, Metabolism, and Lipid Research, Washington University, St. Louis, MO (J.B.M.); British Heart Foundation Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK (J.J.V.M.).

Guest Editor for this article was David J. Moliterno, MD.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.111.030411/-/DC1.

Correspondence to Marc A. Pfeffer, MD, PhD, Brigham and Women's Hospital, Cardiovascular Division, 75 Francis St, Boston, MA 02115. E-mail mpfeffer@rics.bwh.harvard.edu

erythropoiesis-stimulating agent (ESA) darbepoetin alfa had no effect, overall, on the composite end point of death or nonfatal CV events. Moreover, we observed a statistically significant and clinically important increase in the rate of stroke in patients assigned to darbepoetin alfa.

Editorial see p 2805
Clinical Perspective on p 2908

To aid clinical decision making in prescribing ESA therapy, we examined baseline characteristics and postrandomization factors, including blood pressure, platelet count, achieved hemoglobin level, and treatment dose, which might explain the heightened risk of stroke associated with darbepoetin alfa.

Methods

The Trial to Reduce Cardiovascular Events With Aranesp Therapy was a randomized, double-blind, placebo-controlled trial of patients with type 2 diabetes mellitus, nondialysis CKD (estimated glomerular filtration rate [eGFR] of 20–60 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$) and anemia (hemoglobin level ≤11 g/dL) designed to determine whether treatment with darbepoetin alfa would reduce the risk of (1) a cardiovascular composite of death, myocardial infarction, heart failure, stroke, or hospitalization for myocardial ischemia, (2) a renal composite of death or end-stage renal disease (initiation of dialysis), or both. Subjects were randomly assigned to receive either darbepoetin alfa to attempt to achieve a hemoglobin level of 13 g/dL or placebo with rescue darbepoetin alfa if their hemoglobin level was <9 g/dL. Hemoglobin levels and vital signs were assessed every 2 weeks during the titration period and monthly thereafter. The rationale and design,[10] baseline characteristics,[11] and the primary results[9] of the trial have been published. The Trial to Reduce Cardiovascular Events With Aranesp Therapy is registered at http://www.clinicaltrials.gov, unique identifier: NCT00093015. The randomization groups were similar without any clinically meaningful baseline differences except for history of heart failure, which was more prevalent in the placebo arm.

All potential end points, including stroke, were prospectively defined and adjudicated by a central committee that was blinded to treatment assignment and hemoglobin levels. Stroke was defined as a focal neurological deficit of sudden onset lasting >24 hours or resulting in death and which was not due to another readily identifiable cause (ie, brain tumor or trauma). Neuroimaging was not mandated by protocol and performed as deemed necessary for clinical reasons. Where available, imaging reports were used to categorize stroke as hemorrhagic or nonhemorrhagic (cases without imaging results were considered as nonhemorrhagic strokes). For patients who experienced >1 stroke, only the first stroke was used in this analysis.

Statistical Analysis

Distributions of baseline characteristics are presented as median (interquartile range) for the overall population. In this posthoc analysis, univariate comparisons for baseline characteristics between patients who experienced a stroke and those who did not were performed with 2-sample Wilcoxon rank-sum test or the Fisher exact test. History of cardiovascular diseases was derived from the clinical database as previously used and a history of stroke or transient ischemic attack was part of the definition of history of cardiovascular diseases.[9] The urinary protein-to-creatinine ratio was log transformed. The cumulative incidence function of stroke was presented by treatment arm and history of stroke, and the treatment effect was estimated using a proportional subdistribution hazards approach.[12] Potential baseline predictors of stroke were selected on the basis of clinically known risk factors for stroke as well as factors identified in the univariate analysis. Logistic regression was used for outcomes analysis. A stepwise forward selection procedure ($P<0.10$) was performed to help build a multivariable model. Variables considered clinically relevant that were not statistically significant were retained in the model. In addition, interaction among treatment allocation and statistically significant baseline variables was prospectively evaluated.

To assess whether the increased risk of stroke in patients treated with darbepoetin alfa could be explained by postrandomization factors, we conducted a nested-case control analysis within each treatment arm. Using the baseline variables selected in the logistic regression model, a propensity score for stroke was developed within each treatment arm separately to select subjects, free of stroke by the end of the trial, who would serve as controls. Within each treatment arm, every patient experiencing a stroke (case) was matched to 10 controls that had an equivalent duration of follow-up (1:10 nearest neighbor propensity-score matching).

Postrandomization factors studied included hemoglobin level, blood pressure, platelet count, and dose of darbepoetin alfa. For each factor, we compared the latest value available in the 3 months preceding the stroke (or equivalent parallel period for controls) between cases and controls in each treatment arm separately using generalized Hodges-Lehmann median difference test for clustered data.[13] In additional sensitivity analyses, we compared the average of the latest available values (up until the stroke or the equivalent period for controls), the highest value in the latest 3 months, and highest during entire follow-up. We also varied the case-control ratio (1:1, 1:5, and 1:15). A P value of <0.05 was considered statistically significant. All analyses were performed with Stata 11.1 (StataCorp, College Station, TX), and R 2.10.2 (R Project for Statistical Computing, Vienna, Austria).

Results

Over a median follow-up period of 29.1 months, 154/4038 (3.8%) of patients randomized had a stroke, 53/2026 (2.6%, 1.1 per 100 patient-years) in the placebo arm and 101/2012 (5.0%, 2.1 per 100 patient-years) in the darbepoetin alfa arm; hazard ratio 1.92, 95% confidence interval, 1.38 to 2.68. Of the total of 154 strokes, 133 (86.4%) were categorized as nonhemorrhagic (ischemic cause: 112 [72.7%]; unknown: 21 [13.6%]) and 21 (13.6%) as hemorrhagic. Each type of stroke was numerically more frequent in the darbepoetin alfa group compared with the placebo group: nonhemorrhagic, 87 (4.3%) versus 46 (2.3%), and hemorrhagic, 14 (0.7%) versus 7 (0.3%), respectively.

Baseline Predictors of Stroke

In addition to treatment assignment to darbepoetin alfa, univariate predictors of stroke included history of cardiovascular diseases, specifically history of stroke, or transient ischemic attack. Although a history of stroke or transient ischemic attack was part of the definition of history of cardiovascular diseases, both remained statistically significantly associated with incident stroke during follow-up. Patients who had a stroke during the trial also had higher urinary protein–to-creatinine ratio and glycohemoglobin and lower body mass index (BMI), eGFR, serum albumin, hemoglobin, and transferrin saturation. Imbalances in baseline medication use were also found, with stroke patients more likely to be on a vitamin K antagonists or insulin and less likely to be on an oral hypoglycemic agent (Table 1). In a multivariate logistic regression model of predictors of stroke (Table 2), the 2 predictors with the highest z score were assignment to darbepoetin alfa therapy (odds ratio 2.08; 95% confidence interval, 1.47–2.94), and a history of stroke or transient ischemic attack (odds ratio 2.00; 95% confidence interval, 1.36–2.94). Other independent baseline predictors

Table 1. Baseline Characteristics

Variable	Stroke N=154 (3.8%)	No Stroke N=3884 (96.2%)	*P*
Darbepoetin alfa	101 (65.6)	1911 (49.2)	<0.0001
Age, y	69 (62, 76)	68 (60, 75)	0.08
Male gender	71 (46.1)	1655 (42.6)	0.41
Race			0.02
White	104 (67.5)	2466 (63.5)	
Black	37 (24.0)	778 (20.0)	
Other	13 (8.4)	640 (16.5)	
Known duration of diabetes mellitus, months	195 (121, 279)	184 (99, 259)	0.14
BMI, kg/m^2	29.0 (25.3, 34.1)	30.3 (26.3, 35.2)	0.03
Current smoking	8 (5.2)	196 (5.0)	0.85
History of CV disease	123 (79.9)	2519 (64.9)	<0.0001
Coronary artery disease	79 (51.3)	1712 (44.1)	0.08
Heart failure	58 (37.7)	1289 (33.2)	0.26
Myocardial infarction	35 (22.7)	706 (18.2)	0.17
Stroke	36 (23.4)	411 (10.6)	<0.0001
Transient ischemic attack	18 (11.7)	247 (6.4)	0.02
Peripheral arterial disease	35 (22.7)	744 (19.2)	0.30
Atrial fibrillation	21 (13.7)	404 (10.4)	0.23
SBP, mm Hg	138 (127, 150)	136 (122, 148)	0.17
DBP, mm Hg	74 (64, 80)	70 (64, 80)	0.23
Heart rate, bpm	72 (66, 80)	72 (64, 80)	0.08
eGFR, ml · min^{-1} · 1.73 m^{-2}	31 (24, 41)	34 (26, 43)	0.05
Ratio of total protein (mg/dL) to creatinine (mg/dL) in urine	0.7 (0.2, 2.6)	0.4 (0.1, 1.8)	0.001
Serum albumin, g/dL	3.9 (3.6, 4.2)	4.0 (3.7, 4.3)	0.01
Glycated hemoglobin, %	7.2 (6.5, 8.2)	7.0 (6.2, 8.0)	0.05
Hemoglobin, g/dL	10.2 (9.5, 10.9)	10.4 (9.8, 10.9)	0.01
Platelets, 10^9/L	241 (202, 296)	243 (198, 293)	0.51
Ferritin, ng/mL	152 (76, 257)	132 (66, 257)	0.13
Transferrin saturation, %	24 (20, 31)	23 (18, 28)	0.01
Total cholesterol, mg/dL	171 (148, 213)	169 (142, 201)	0.18
LDL, mg/dL	88 (68, 123)	85 (63, 111)	0.08
Insulin	89 (57.8)	1900 (48.9)	0.03
Oral iron therapy	64 (41.6)	1644 (42.3)	0.85
Oral hypoglycemic agent	70 (45.5)	2223 (57.2)	0.01
Statin or lipid-lowering agent	88 (57.1)	2507 (64.5)	0.07
Aspirin or antiplatelet agent	77 (50.0)	1873 (48.2)	0.68
Vitamin K antagonist	18 (11.7)	259 (6.7)	0.02

Values presented as median (IQR) or N (%). BMI indicates body mass index; CV, cardiovascular; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; and LDL, low-density lipoprotein.

Comparisons testing with nonparametric Wilcoxon test for continuous variables and Fisher exact test for categorical variables.

included urinary protein–to-creatinine ratio, insulin therapy, history of CV disease, and lower serum hemoglobin level or BMI.

We tested whether any of the independent baseline predictors of stroke modified the risk of this event related to treatment with darbepoetin alfa. None of the interaction tests were statistically significant (all were $P>0.1$). In particular, although the darbepoetin alfa–related risk of stroke was higher in patients with prior stroke (HR 2.99; 95% confidence interval, 1.40–6.36) than in those without prior stroke (HR 1.61; 95% confidence interval, 1.14–2.41), the test for interaction was not significant ($P=0.25$, the Figure).

Table 2. Multivariate Baseline Predictors of Stroke

Variable	OR	z Score	*P*	95 % CI
Darbepoetin alfa	2.08	4.16	<0.001	1.47 to 2.94
History of stroke/TIA	2.00	3.53	<0.001	1.36 to 2.94
Log protein:creatinine ratio	1.21	2.98	0.003	1.07 to 1.37
Insulin use	1.48	2.23	0.026	1.05 to 2.09
History of CV disease	1.60	2.08	0.038	1.03 to 2.49
Hb, 1 g/dL down	1.18	2.02	0.043	1.01 to 1.39
BMI, 1 kg/m^2 down	1.03	2.02	0.044	1.00 to 1.05
Age, 10 y up	1.20	1.95	0.051	1.00 to 1.44
Heart rate, bpm up	1.01	1.76	0.079	1.00 to 1.03
Statin or other lipid-lowering agent	0.74	−1.73	0.084	0.53 to 1.04
Vitamin K antagonist	1.66	1.66	0.097	0.91 to 3.02
Black race (vs white)	1.30	1.28	0.201	0.87 to 1.95
Other race (vs white)	0.49	−2.32	0.020	(0.27 to 0.89)
eGFR, 1 mL · min^{-1} · 1.73 m^{-2} down	1.01	0.90	0.370	(0.99 to 1.02)
History of atrial fibrillation	0.90	−0.36	0.722	(0.51 to 1.58)
SBP, 10 mm Hg down	1.01	0.17	0.869	(0.92 to 1.10)

C-statistic=0.72 (95% CI 0.68–0.76). This was not statistically different from a more parsimonious model that included only treatment assignment, history of stroke or transient ischemic attack, log protein:creatinine ratio, insulin therapy, history of CV disease, serum hemoglobin level, BMI, age, and race (C-statistic=0.70; 95% CI, 0.66–0.74).

OR indicates odds ratio; CI, confidence interval; TIA, transient ischemic attack; CV, cardiovascular; Hb, hemoglobin; BMI, body mass index; bpm, beats per minute; eGFR, estimated glomerular filtration rate; and SBP, systolic blood pressure.

Variables not retained in multivariate model that were significant in univariate analyses: serum albumin, glycated hemoglobin, and transferrin saturation.

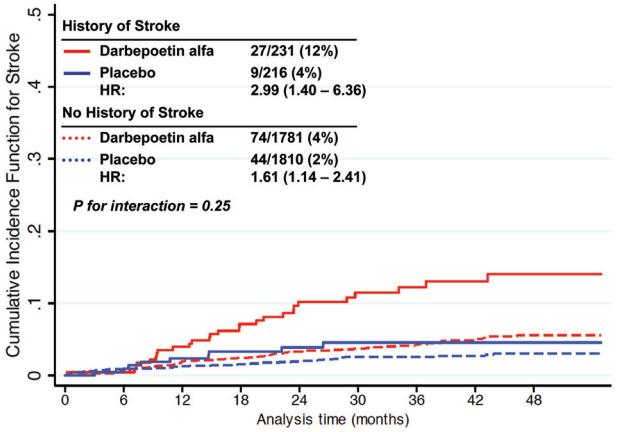

Figure. Cumulative incidence function of stroke by treatment arm and history of stroke. HR indicates hazard ratio.

Table 3. Latest Values Before Stroke (1:10 Matching)

Most Recent Value in Latest 90 days	Darbepoetin Alfa					Placebo*				
	Stroke Cases (N=101)		No Stroke Controls (N=1010)			Stroke Cases (N=52)		No Stroke Controls (N=520)		
	N	Median (IQR)	N	Median (IQR)	*P*	N	Median (IQR)	N	Median (IQR)	*P*
Systolic BP (mm Hg)	86	135 (120, 150)	923	134 (121, 145)	0.98	44	136 (122, 148)	468	137 (124, 150)	0.52
Diastolic BP, mm Hg	86	72 (64, 80)	923	72 (66, 80)	0.62	44	70 (63, 80)	468	72 (64, 80)	0.76
Hemoglobin, mg/dL	80	12.3 (11.1, 13.1)	864	12.5 (11.7, 13.2)	0.10	42	10.4 (9.4, 10.8)	438	10.4 (9.7, 11.3)	0.20
Platelet count, 1000/dL	38	230 (172, 301)	470	225 (185, 281)	0.87	26	225 (182, 291)	253	236 (194, 281)	0.50
Darbepoetin alfa dose, μg/mo	80	150 (100, 300)	866	150 (80, 300)	0.79	42	0 (0, 0)	436	0 (0, 0)	0.40

All controls were pooled from patients who were free of stroke by the end of the trial. Data presented as median (IQR). Varying sample size contingent on available data for particular variable. All pairwise comparisons tested with generalized Hodges-Lehmann median difference test for clustered data.

*Rescue darbepoetin alfa in placebo arm, expressed as n/N (%), was 6/43 (14) in stroke cases and 82/434 (19) in no stroke controls (P=0.54).

IQR indicates interquartile range; BP, blood pressure.

Postrandomization Factors

In a treatment-stratified, nested, case-control analysis, there was no difference between patients who experienced a stroke and those who did not in terms of latest blood pressure value, hemoglobin level, platelet count, or dose of darbepoetin alfa (Table 3). Among patients assigned to darbepoetin alfa experiencing a stroke (n=86), the last-measured blood pressure in the 90 days preceding the event was 135/72 mm Hg compared with 134/72 mm Hg over the same time interval in patients who did not have a stroke (n=923; P>0.50). In patients in the darbepoetin alfa–treated group experiencing a stroke, the last-measured hemoglobin concentration in the 90 days preceding stroke was 12.3 mg/dL compared with 12.5 mg/dL in darbepoetin alfa–treated patients without a stroke (P=0.09).

In the placebo-treated group, there were also no differences in any of the evaluated factors between those with and without a stroke (Table 3). Moreover, the proportion of patients receiving rescue treatment with darbepoetin alfa was not different between cases with strokes and their nonstroke controls (14% versus 19%, P=0.54; Table 3). Postrandomization iron parameters also did not significantly differ between subjects with and without stroke. Within each treatment arm, the latest ferritin and transferrin saturation values preceding a stroke event were similar among cases and controls. The number of subjects being treated with intravenous iron preceding the event was similar among cases and controls (darbepoetin alfa: cases: 15/101 [14.9%] versus controls: 16/101 [15.8%] and placebo: cases: 10/52 [19.2%] versus controls: 7/52 [13.5%]).

Additional sensitivity analyses examining the latest, highest, and average value of blood pressure, hemoglobin, platelet count, and dose of darbepoetin alfa in the 90 days preceding the event (or during the entire follow-up) each produced similar results, as did varying the case-to-control ratio. In each analysis, the postrandomization blood pressure, hemoglobin level, platelet count, and dose of darbepoetin alfa were similar between the cases with a stroke and their nonstroke controls (see online-only Data Supplement).

Discussion

We found that in this cohort of patients with type 2 diabetes mellitus, nondialysis CKD, and anemia, allocation to darbepoetin alfa was associated with a statistically significant doubling of the risk of stroke. The risk of stroke related to darbepoetin alfa did not appear to be associated with any baseline characteristic. Furthermore, this risk was not mediated by postrandomization factors previously implicated as mechanisms of ESA-related adverse outcomes,[14,15] including increase in hemoglobin level, blood pressure, or platelet number, and was not related to dose of darbepoetin alfa.

Although a number of baseline characteristics predicted risk of stroke in the overall TREAT population, none of these modified the risk of stroke related to darbepoetin alfa. In particular, history of stroke did not modify the risk related to darbepoetin (ie, the risk of stroke with this ESA was increased both in patients with and without a prior history of stroke). Interestingly, we observed an inverse relationship between BMI and risk of stroke where subjects with lower BMI at baseline were at higher risk of stroke. This association was recently described in a cohort of subjects with diabetes mellitus[16] and may carry important prognostic information, given that lower BMI at the time of stroke was also associated with a higher risk of death.[17]

Of the postrandomization variables examined, increase in hemoglobin has most often been proposed as a mediator of ESA-related CV risk, in part because it raises blood viscosity and vascular resistance and may increase blood pressure.[15,18] The initial rapid rise in hemoglobin concentration, the so-called ramp phase, has been of particular concern.[19] However, we did not find any association between stroke and average achieved hemoglobin, change in hemoglobin, or peak hemoglobin during varying follow-up periods in either darbepoetin alfa–treated or placebo-treated patients. In fact, the achieved hemoglobin level was lower in the stroke cases than in the nonstroke controls although this difference was not statistically significant. Furthermore, there was a marked temporal disassociation between the early rise in hemoglobin and the late augmentation in stroke rates,[9] which does not support concerns about the ramp phase of treatment.[19]

Although earlier studies in patients with severe anemia suggested that blood pressure may increase in up to one third of patients treated with recombinant human erythropoietin, there was no difference in systolic blood pressure between the treatment groups in TREAT, and a 1.7-mm Hg difference in

diastolic pressure was observed (higher in the darbepoetin alfa arm).[9] More importantly, in the present analysis, there was no association between postrandomization blood pressure and the incidence of stroke despite extensive analyses. Specifically, the latest systolic or diastolic pressure preceding stroke in cases (or during the equivalent period in nonstroke controls) was not different between cases and controls within each treatment arm. The same was true for mean and highest pressures measured preceding stroke or at any time during the entire follow-up period. In addition, as previously reported, there was no statistically significant difference between treatment groups in reporting of hypertension as an adverse event by investigators: 24.4% in patients randomized to darbepoetin alfa compared with 22.0% in those assigned to placebo ($P=0.07$).[9]

Increases in platelet count, reactivity, or both may also occur after ESA administration, and could promote a prothrombotic milieu.[20] In patients undergoing dialysis in the Normal-Hematocrit study, the risk of thrombosis affecting the vascular access site was increased in the higher-hemoglobin target group compared with the lower-hemoglobin target group: 243 patients (39%) versus 176 patients (29%), $P=0.001$.[21] In TREAT, thromboembolic events were also reported more frequently in patients treated with darbepoetin alfa compared with placebo (venous: 2.0% versus 1.1%, $P=0.02$; arterial: 8.9% versus 7.1%, $P=0.04$).[9] In the current analyses, however, there was no association between various assessments of platelet count and incident stroke in either treatment arm.

Dose-related nonerythropoietic toxicity has also been invoked as a possible mechanism for ESA-related risk.[22] In the German Multicenter Erythropoietin Stroke Trial, there was an increase in mortality among acute-stroke patients randomized to just 3 doses of epoetin alfa given over 48 hours.[23] In TREAT, the median monthly dose of darbepoetin alfa administered to patients who had a stroke was not different than the dose administered to the matched nonstroke controls. Furthermore, in the placebo arm, there was no relation between the risk of stroke and use of darbepoetin alfa as rescue therapy. We have also reported that poor initial hemoglobin response to an ESA (despite escalating dosing) was associated with increased risk of death or cardiovascular events in TREAT.[24] However, the rate of stroke was not higher in patients with a poor initial hemoglobin response to darbepoetin alfa therapy.

Some limitations of this analysis need to be acknowledged. The increase in stroke with darbepoetin alfa could be a chance finding. However, the similar observation in the study by Parfrey and colleagues[25] and the relatively large number of strokes in TREAT argue against this. Furthermore, given the clinical importance of stroke, it is safer to adopt a conservative approach and assume this increased risk to be actual.[26] Subtle differences in blood pressure between treatment groups might have been detected using more intensive methods such as ambulatory monitoring. Greater modification of antihypertensive medication in the darbepoetin alfa group by investigators might also have concealed an increase in blood pressure in the treatment group compared with placebo. Although platelet count did not differ between treatments, darbepoetin alfa might have changed platelet reactivity, which was not measured. Although we did not have complete or comprehensive imaging data on stroke type or information on stroke severity and outcome, this end point was prospectively defined and centrally adjudicated without knowledge of randomization arm or hemoglobin levels.

In summary, darbepoetin alfa doubled the risk of stroke in patients with type 2 diabetes mellitus, nondialysis CKD, and anemia. No baseline characteristic was associated with darbepoetin alfa–related risk of stroke. Similarly, despite extensive analyses of postrandomization blood pressure, hemoglobin, platelet count, and dose of treatment, no factor previously identified as a potential mechanism for ESA-related cardiovascular risk explained the increase in risk of stroke observed with darbepoetin alfa. Measurement of these readily available follow-up parameters cannot be used to mitigate the risk of darbepoetin alfa–related stroke.

Sources of Funding

The TREAT trial was supported by Amgen. This analysis was conducted independently by the academic coauthors using the TREAT database at the Brigham and Women's Hospital, Boston, MA.

Disclosures

Drs Parving, Parfrey, Burdmann, Lewis, Ivanovich, McGill, McMurray, Singh, Solomon, and Pfeffer have received research support and/or consulting fees or honoraria from Amgen. Dr Keithi-Reddy is an employee of Amgen. The other authors report no conflicts.

References

1. Sarnak MJ, Tighiouart H, Manjunath G, MacLeod B, Griffith J, Salem D, Levey AS. Anemia as a risk factor for cardiovascular disease in the Atherosclerosis Risk in Communities (ARIC) study. *J Am Coll Cardiol*. 2002;40:27–33.
2. Ezekowitz JA, McAlister FA, Armstrong PW. Anemia is common in heart failure and is associated with poor outcomes: insights from a cohort of 12 065 patients with new-onset heart failure. *Circulation*. 2003;107:223–225.
3. Li S, Collins AJ. Association of hematocrit value with cardiovascular morbidity and mortality in incident hemodialysis patients. *Kidney Int*. 2004;65:626–633.
4. Go AS, Yang J, Ackerson LM, Lepper K, Robbins S, Massie BM, Shlipak MG. Hemoglobin level, chronic kidney disease, and the risks of death and hospitalization in adults with chronic heart failure: The Anemia in Chronic Heart Failure: Outcomes and Resource Utilization (ANCHOR) study. *Circulation*. 2006;113:2713–2723.
5. O'Meara E, Clayton T, McEntegart MB, McMurray JJV, Lang CC, Roger SD, Young JB, Solomon SD, Granger CB, Ostergren J, Olofsson B, Michelson EL, Pocock S, Yusuf S, Swedberg K, Pfeffer MA, CHARM Committees and Investigators. Clinical correlates and consequences of anemia in a broad spectrum of patients with heart failure: results of the Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM) program. *Circulation*. 2006;113:986–994.
6. Giraldez RR, Sabatine MS, Morrow DA, Mohanavelu S, McCabe CH, Antman EM, Braunwald E. Baseline hemoglobin concentration and creatinine clearance composite laboratory index improves risk stratification in ST-elevation myocardial infarction. *Am Heart J*. 2009;157:517–524.
7. Groenveld HF, Januzzi JL, Damman K, van Wijngaarden J, Hillege HL, van Veldhuisen DJ, van der Meer P. Anemia and mortality in heart failure patients: a systematic review and meta-analysis. *J Am Coll Cardiol*. 2008;52:818–827.
8. Vlagopoulos PT, Tighiouart H, Weiner DE, Griffith J, Pettitt D, Salem DN, Levey AS, Sarnak MJ. Anemia as a risk factor for cardiovascular disease and all-cause mortality in diabetes: the impact of chronic kidney disease. *J Am Soc Nephrol*. 2005;16:3403–3410.
9. 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 JJV, 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.
10. Mix TC, Brenner RM, Cooper ME, de Zeeuw D, Ivanovich P, Levey AS, McGill JB, McMurray JJ, Parfrey PS, Parving HH, Pereira BJ, Remuzzi G, Singh AK, Solomon SD, Stehman-Breen C, Toto RD, Pfeffer MA. Rationale–Trial to Reduce Cardiovascular Events With Aranesp Therapy (TREAT): evolving the management of cardiovascular risk in patients with chronic kidney disease. *Am Heart J*. 2005;149:408–413.
11. Pfeffer MA, Burdmann EA, Chen CY, Cooper ME, de Zeeuw D, Eckardt KU, Ivanovich P, Kewalramani R, Levey AS, Lewis EF, McGill J, McMurray JJV, Parfrey P, Parving H-H, Remuzzi G, Singh AK, Solomon SD, Toto R, Uno H, TREAT Investigators. Baseline characteristics in the Trial to Reduce Cardiovascular Events With Aranesp Therapy (TREAT). *Am J Kidney Dis*. 2009;54:59–69.
12. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94:496–509.
13. Newson R. Confidence intervals for rank statistics: percentile slopes, differences, and ratios. *Stata J*. 2006;6:497–520.
14. Vaziri ND. Cardiovascular effects of erythropoietin and anemia correction. *Curr Opin Nephrol Hypertens*. 2001;10:633–637.
15. Raine AE. Hypertension, blood viscosity, and cardiovascular morbidity in renal failure: implications of erythropoietin therapy. *Lancet*. 1988;1:97–100.
16. Dallongeville J, Bhatt DL, Steg PG, Ravaud P, Wilson PW, Eagle KA, Goto S, Mas JL, Montalescot G. Relation between body mass index, waist circumference, and cardiovascular outcomes in 19 579 diabetic patients with established vascular disease: The REACH Registry. *Eur J Cardiovasc Prev Rehabil*. Published ahead of print, February 25, 2011. DOI:10.1177/1741826710394305. http://cpr.sagepub.com/content/early/2011/02/24/1741826710394305.full.pdf+html. Accessed November 1, 2011.
17. Vemmos K, Ntaios G, Spengos K, Savvari P, Vemmou A, Pappa T, Manios E, Georgiopoulos G, Alevizaki M. Association between obesity and mortality after acute first-ever stroke: the obesity-stroke paradox. *Stroke*. 2011;42:30–36.
18. Raine AE, Roger SD. Effects of erythropoietin on blood pressure. *Am J Kidney Dis*. 1991;18:76–83.
19. Unger EF, Thompson AM, Blank MJ, Temple R. Erythropoiesis-stimulating agents: time for a reevaluation. *N Engl J Med*. 2010;362:189–192.
20. Vaziri ND, Zhou XJ. Potential mechanisms of adverse outcomes in trials of anemia correction with erythropoietin in chronic kidney disease. *Nephrol Dial Transplant*. 2009;24:1082–1088.
21. Besarab A, Bolton WK, Browne JK, Egrie JC, Nissenson AR, Okamoto DM, Schwab SJ, Goodkin DA. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. *N Engl J Med*. 1998;339:584–590.
22. Ghezzi P, Bernaudinb M, Bianchic R, Blomgren K. Erythropoietin: not just about erythropoiesis. *Lancet*. 2010;375:2142.
23. Ehrenreich H, Weissenborn K, Prange H, Schneider D, Weimar C, Wartenberg K, Schellinger PD, Bohn M, Becker H, Wegrzyn M, Jähnig P, Herrmann M, Knauth M, Bähr M, Heide W, Wagner A, Schwab S, Reichmann H, Schwendemann G, Dengler R, Kastrup A, Bartels C, EPO Stroke Trial Group. Recombinant human erythropoietin in the treatment of acute ischemic stroke. *Stroke*. 2009;40:e647–e656.
24. 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. Erythropoietic response and outcomes in kidney disease and type 2 diabetes. *N Engl J Med*. 2010;363:1146–1155.
25. Parfrey PS, Foley RN, Wittreich BH, Sullivan DJ, Zagari MJ, Frei D. Double-blind comparison of full and partial anemia correction in incident hemodialysis patients without symptomatic heart disease. *J Am Soc Nephrol*. 2005;16:2180–2189.
26. Pfeffer MA, Skali H. Can there be any surrogate for safety? *Dialogues Cardiovasc Med*. 2010;15:130–139.

CLINICAL PERSPECTIVE

Although anemia has been associated with higher mortality and morbidity in subjects with diabetes mellitus and nondialysis chronic kidney disease, treatments with erythropoiesis-stimulating agents have not led to improvements in prognosis. In the Trial to Reduce Cardiovascular Events With Aranesp Therapy (TREAT), a concerning increase in the risk of darbepoetin alfa–related stroke was observed. We examined the role of baseline predictors and postrandomization factors that might explain this heightened risk of stroke with darbepoetin alfa. We found that in this cohort of patients with type 2 diabetes mellitus, nondialysis chronic kidney disease, and anemia, the risk of stroke related to darbepoetin alfa did not appear to be associated with any baseline characteristic. Despite extensive sensitivity analyses, this risk did not seem to be mediated by postrandomization factors previously implicated as mechanisms of erythropoiesis-stimulating agent–related adverse outcomes, including increase in hemoglobin level, blood pressure, or platelet number, and was not related to dose of darbepoetin alfa. Therefore, clinicians cannot rely on monitoring these readily available follow-up parameters to mitigate the risk of darbepoetin alfa–related stroke.

Stroke in Patients With Type 2 Diabetes Mellitus, Chronic Kidney Disease, and Anemia Treated With Darbepoetin Alfa: The Trial to Reduce Cardiovascular Events With Aranesp Therapy (TREAT) Experience

Hicham Skali, Hans-Henrik Parving, Patrick S. Parfrey, Emmanuel A. Burdmann, Eldrin F. Lewis, Peter Ivanovich, Sai Ram Keithi-Reddy, Janet B. McGill, John J.V. McMurray, Ajay K. Singh, Scott D. Solomon, Hajime Uno and Marc A. Pfeffer

Circulation. 2011;124:2903-2908; originally published online November 21, 2011;
doi: 10.1161/CIRCULATIONAHA.111.030411
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539

SUPPLEMENTAL MATERIAL

Stroke in Patients with Type 2 Diabetes, Chronic Kidney Disease and Anemia Treated with Darbepoetin Alfa. The TREAT Experience

Supplemental tables
Sensitivity analyses

Abbreviations used in tables:
SBP: systolic blood pressure (mmHg)
DBP: diastolic blood pressure (mmHg)
Hb: hemoglobin (g/dL)
PLT: platelet count (10^9/L)

Notes:
All controls were pooled from patients who were stroke free by the end of the trial.
All pairwise comparisons tested with generalized Hodges-Lehmann median difference test for clustered data.
Sample size in each cell varied contingent on available data for particular variable.

Table 1A: Change from baseline value of post-randomization factors for cases and controls (1:1 matching) in each treatment arm.

	Darbepoetin alfa			*Placebo*		
Change from baseline value to:	**No Stroke Controls N=101**	**Stroke Cases N = 101**	**P value**	**No Stroke Controls N=52**	**Stroke Cases N = 52**	**P value**
Highest SBP	N = 101	N = 101	0.84	N = 52	N = 52	0.12
	21 (5, 34)	18 (5, 40)		14 (0, 31)	21 (5, 32)	
Highest DBP	N = 101	N = 101	0.59	N = 52	N = 52	0.15
	10 (4, 19)	11 (5, 20)		6 (0, 15)	10 (0, 18)	
Latest SBP	N = 101	N = 101	0.65	N = 52	N = 52	0.17
	-1 (-14, 11)	0 (-16, 13)		0 (-8, 18)	-2 (-16, 10)	
Latest DBP	N = 101	N = 101	0.66	N = 52	N = 52	0.64
	0 (-10, 8)	0 (-10, 7)		0 (-4, 5)	0 (-8, 6)	
Highest Hb	N = 101	N = 100	0.37	N = 52	N = 52	0.40
	3.8 (2.6, 4.5)	4.0 (2.8, 4.8)		0.9 (0.3, 1.6)	1.2 (0.4, 2.0)	
Latest Hb	N = 101	N = 100	0.78	N = 52	N = 52	0.36
	1.9 (0.9, 2.8)	1.9 (0.6, 3.0)		0.0 (-0.5, 0.7)	0.0 (-1.1, 0.7)	
Highest Dose	N = 101	N = 100	0.78	N = 52	N = 52	0.56
	100 (40, 400)	160 (35, 310)		0 (0, 15)	0 (0, 30)	
Latest Dose	N = 101	N = 100	0.61	N = 52	N = 52	0.46
	20 (-30, 180)	50 (-20, 180)		0 (0, 0)	0 (0, 0)	
Highest PLT	N = 101	N = 98	0.88	N = 52	N = 52	0.89
	5 (0, 33)	6 (0, 36)		0 (0, 37)	0 (0, 18)	
Latest PLT	N = 101	N = 98	0.96	N = 52	N = 52	0.99
	-3 (-33, 21)	0 (-24, 13)		0 (-31, 2)	0 (-32, 2)	

Table 1B: Average value over entire follow-up of post-randomization factors for cases and controls (1:1 matching) in each treatment arm.

	Darbepoetin alfa			*Placebo*		
Average value over follow-up	**No Stroke Controls N=101**	**Stroke Cases N = 101**	**P value**	**No Stroke Controls N=52**	**Stroke Cases N = 52**	**P value**
Average SBP	N = 101	N = 101	0.50	N = 52	N = 52	0.62
	133 (124, 140)	132 (126, 146)		137 (127, 146)	138 (133, 145)	
Average DBP	N = 101	N = 101	0.55	N = 52	N = 52	0.94
	73 (67, 78)	74 (67, 79)		73 (68, 79)	75 (67, 78)	
Average Hb	N = 101	N = 100	0.01	N = 52	N = 52	0.38
	12.3 (11.6, 12.6)	12.1 (11.3, 12.5)		10.4 (9.7, 11.0)	10.2 (9.6, 10.7)	
Average weekly dose	N = 101	N = 100	0.92	N = 52	N = 52	0.28
	43 (10, 139)	54 (8, 125)		0 (0, 2)	0 (0, 4)	
Average PLT	N = 101	N = 99	0.96	N = 52	N = 52	0.43
	240 (198, 277)	245 (197, 295)		228 (189, 263)	245 (200, 278)	

Table 1C: Change from baseline value of post-randomization factors for cases and controls (1:1 matching) in each treatment arm.

	Darbepoetin alfa			*Placebo*		
Change from baseline to value in last 90 days	**No Stroke Controls N=101**	**Stroke Cases N = 101**	**P value**	**No Stroke Controls N=52**	**Stroke Cases N = 52**	**P value**
Highest SBP90	N = 92	N = 86	0.71	N = 46	N = 44	0.81
	7 (-2, 20)	7 (0, 23)		0 (-2, 20)	0 (-5, 23)	
Highest DBP90	N = 92	N = 86	0.37	N = 46	N = 44	0.49
	6 (0, 11)	6 (0, 14)		0 (0, 5)	0 (0, 11)	
Latest SBP90	N = 92	N = 86	0.80	N = 46	N = 44	0.35
	-1 (-14, 12)	0 (-16, 13)		0 (-8, 17)	-1 (-15, 8)	
Latest DBP90	N = 92	N = 86	0.55	N = 46	N = 44	0.83
	0 (-10, 8)	0 (-10, 8)		0 (-8, 5)	-1 (-8, 4)	
Highest Hb90	N = 87	N = 80	0.51	N = 42	N = 42	0.85
	2.7 (2.0, 3.6)	2.9 (1.9, 3.9)		0.6 (0.0, 1.1)	0.6 (0.0, 1.2)	
Latest Hb90	N = 87	N = 80	0.81	N = 42	N = 42	0.67
	2.0 (0.9, 2.8)	2.2 (0.7, 3.2)		0.1 (-0.4, 0.7)	0.0 (-0.4, 0.8)	
Highest PLT90	N = 51	N = 38	0.85	N = 28	N = 26	0.83
	-14 (-35, 12)	-3 (-38, 24)		0 (-22, 0)	0 (-27, 0)	
Latest PLT90	N = 51	N = 38	0.87	N = 28	N = 26	0.83
	-14 (-36, 12)	-3 (-42, 24)		0 (-22, 0)	0 (-27, 0)	

Table 1D: Average value over most recent 90 days of post-randomization factors for cases and controls (1:1 matching) in each treatment arm.

	Darbepoetin alfa			*Placebo*		
Average value over most recent 90 days	**No Stroke Controls N=101**	**Stroke Cases N = 101**	**P value**	**No Stroke Controls N=52**	**Stroke Cases N = 52**	**P value**
Average SBP90	N = 92	N = 86	0.60	N = 46	N = 44	0.62
	132 (124, 151)	136 (127, 146)		135 (127, 154)	137 (124, 150)	
Average DBP90	N = 92	N = 86	0.48	N = 46	N = 44	0.94
	73 (66, 79)	75 (67, 81)		72 (68, 80)	74 (64, 79)	
Average Hb90	N = 87	N = 80	0.05	N = 42	N = 42	0.90
	12.6 (11.8, 13.2)	12.2 (11.3, 12.9)		10.4 (9.6, 11.0)	10.3 (9.8, 10.9)	
Average PLT90	N = 51	N = 39	0.50	N = 28	N = 26	0.73
	239 (182, 293)	230 (172, 301)		210 (179, 264)	225 (182, 291)	

Table 2A: Change from baseline value of post-randomization factors for cases and controls (1:5 matching) in each treatment arm.

	Darbepoetin alfa			***Placebo***		
Change from baseline to:	No Stroke Controls N=505	Stroke Cases N = 101	P value	No Stroke Controls N=260	Stroke Cases N = 52	P value
Highest SBP	N = 505	N = 101	0.70	N = 260	N = 52	0.08
	20 (10, 36)	18 (5, 40)		14 (0, 30)	21 (5, 31.5)	
Highest DBP	N = 505	N = 101	0.65	N = 260	N = 52	0.58
	12 (5, 20)	11 (5, 20)		9 (0, 15)	10 (0, 18)	
Latest SBP	N = 505	N = 101	0.80	N = 260	N = 52	0.40
	-2 (-14, 10)	0 (-16, 13)		0 (-10, 10)	-1.5 (-15.5, 10)	
Latest DBP	N = 505	N = 101	0.85	N = 260	N = 52	0.25
	0 (-8, 8)	0 (-10, 7)		0 (-4.5, 5)	0 (-8, 6)	
Highest Hb	N = 504	N = 100	0.97	N = 259	N = 52	0.75
	3.8 (3.0, 4.7)	4.0 (2.8, 4.8)		1.2 (0.3, 2.2)	1.2 (0.4, 2.0)	
Latest Hb	N = 504	N = 100	0.12	N = 259	N = 52	0.11
	2.1 (1.2, 3.0)	1.9 (0.6, 3.0)		0.1 (-0.6, 1.1)	0.0 (-1.1, 0.7)	
Highest Dose	N = 505	N = 100	0.50	N = 259	N = 52	0.57
	140 (40, 440)	160 (35, 310)		0 (0, 20)	0 (0, 30)	
Latest Dose	N = 505	N = 100	0.67	N = 259	N = 52	0.97
	30 (-40, 200)	50 (-20, 180)		0 (0, 0)	0 (0, 0)	
Highest PLT	N = 504	N = 98	0.83	N = 258	N = 52	0.48
	6 (0, 37)	6 (0, 36)		0 (0, 31)	0 (0, 18)	
Latest PLT	N = 504	N = 98	0.87	N = 258	N = 52	0.34
	0 (-34, 14)	0 (-24, 13)		0 (-22, 8)	0 (-32, 2)	

Table 2B: Average value over entire follow-up of post-randomization factors for cases and controls (1:5 matching) in each treatment arm.

	Darbepoetin alfa			***Placebo***		
Average value over follow-up	No Stroke Controls N=505	Stroke Cases N = 101	P value	No Stroke Controls N=260	Stroke Cases N = 52	P value
Average SBP	N = 505	N = 101	0.88	N = 260	N = 52	0.43
	134 (127, 143)	132 (126, 146)		137 (129, 146)	138 (133, 145)	
Average DBP	N = 505	N = 101	0.94	N = 260	N = 52	0.31
	73 (68, 78)	74 (67, 79)		72 (66, 78)	75 (67, 78)	
Average Hb	N = 505	N = 100	<0.001	N = 259	N = 52	0.04
	12.3 (11.8, 12.6)	12.1 (11.3, 12.5)		10.4 (9.9, 11.1)	10.2 (9.6, 10.7)	
Average weekly dose	N = 505	N = 100	0.68	N = 259	N = 52	0.18
	56 (9, 142)	54 (8, 125)		0 (0, 2)	0 (0, 4)	
Average PLT	N = 505	N = 99	0.24	N = 258	N = 52	0.67
	230 (188, 272)	245 (197, 295)		238 (198, 279)	245 (200, 278)	

Table 2C: Change from baseline value of post-randomization factors for cases and controls (1:5 matching) in each treatment arm.

	Darbepoetin alfa			***Placebo***		
Change from baseline to value in last 90 days	No Stroke Controls N=505	Stroke Cases N = 101	P value	No Stroke Controls N=260	Stroke Cases N = 52	P value
Highest SBP90	N = 463	N = 86	0.43	N = 234	N = 44	0.61
	6 (-3, 20)	6.5 (0, 23)		1 (0, 20)	0 (-4.5, 23)	
Highest DBP90	N = 463	N = 86	0.33	N = 234	N = 44	0.61
	5 (0, 12)	6 (0, 14)		2 (0, 10)	0 (0, 11)	
Latest SBP90	N = 463	N = 86	0.66	N = 234	N = 44	0.47
	-2 (-14, 10)	0 (-16, 13)		0 (-10, 10)	-0.5 (-14.5, 7.5)	
Latest DBP90	N = 463	N = 86	0.74	N = 234	N = 44	0.18
	0 (-8, 8)	0 (-10, 8)		0 (-4, 5)	-0.5 (-7.5, 4)	
Highest Hb90	N = 435	N = 80	0.83	N = 222	N = 42	0.61
	2.9 (2.0, 3.8)	2.9 (1.9, 3.9)		0.6 (0.0, 1.5)	0.6 (0.0, 1.2)	
Latest Hb90	N = 435	N = 80	0.45	N = 222	N = 42	0.23
	2.2 (1.3, 3.0)	2.2 (0.7, 3.2)		0.1 (-0.4, 1.2)	0.0 (-0.4, 0.8)	
Highest PLT90	N = 223	N = 38	0.78	N = 124	N = 26	0.30
	-4 (-36, 12)	-3 (-38, 24)		0 (-10, 2)	0 (-27, 0)	
Latest PLT90	N = 223	N = 38	0.72	N = 124	N = 26	0.31
	-4 (-39, 12)	-3 (-42, 24)		0 (-10, 2)	0 (-27, 0)	

Table 2D: Average value over most recent 90 days of post-randomization factors for cases and controls (1:5 matching) in each treatment arm.

	Darbepoetin alfa			***Placebo***		
Average value over most recent 90 days	No Stroke Controls N=505	Stroke Cases N = 101	P value	No Stroke Controls N=260	Stroke Cases N = 52	P value
Average SBP90	N = 463	N = 86	0.38	N = 234	N = 44	0.78
	133 (123, 146)	136 (127, 146)		137 (127, 148)	137 (124, 150)	
Average DBP90	N = 463	N = 86	0.43	N = 234	N = 44	0.97
	73 (67, 80)	75 (67, 81)		72 (66, 80)	74 (64, 79)	
Average Hb90	N = 436	N = 80	0.01	N = 222	N = 42	0.49
	12.6 (11.9, 13.1)	12.2 (11.3, 12.9)		10.4 (9.6, 11.2)	10.3 (9.8, 10.9)	
Average PLT90	N = 223	N = 39	0.85	N = 124	N = 26	0.69
	231 (183, 279)	230 (172, 301)		228 (192, 282)	225 (182, 291)	

Table 3A: Change from baseline value of post-randomization factors for cases and controls (1:10 matching) in each treatment arm.

	Darbepoetin alfa			***Placebo***		
Change from baseline to:	No Stroke Controls N=1010	Stroke Cases N = 101	P value	No Stroke Controls N=520	Stroke Cases N = 52	P value
Highest SBP	N = 1010	N = 101	0.97	N = 520	N = 52	0.07
	20 (8,33)	18 (5,40)		14 (0,30)	21 (5,31.5)	
Highest DBP	N = 1010	N = 101	0.90	N = 520	N = 52	0.86
	11 (5,20)	11 (5,20)		10 (0,16)	10 (0,18)	
Latest SBP	N = 1010	N = 101	0.74	N = 520	N = 52	0.51
	-2 (-14,10)	0 (-16,13)		0 (-12,10)	-1.5 (-15.5,10)	
Latest DBP	N = 1010	N = 101	0.95	N = 520	N = 52	0.32
	0 (-8,8)	0 (-10,7)		0 (-6,5)	0 (-8,6)	
Highest Hb	N = 1006	N = 100	0.32	N = 518	N = 52	0.94
	3.7 (2.8,4.5)	4.0 (2.8,4.8)		1.2 (0.3,2.1)	1.2 (0.4,1.9)	
Latest Hb	N = 1006	N = 100	0.38	N = 518	N = 52	0.35
	2.0 (1.1,2.9)	1.8 (0.6,3)		0 (-0.6,0.8)	0 (-1.1,0.7)	
Highest Dose	N = 1009	N = 100	0.82	N = 518	N = 52	0.73
	130 (40,400)	160 (35,310)		0 (0,30)	0 (0,30)	
Latest Dose	N = 1009	N = 100	0.67	N = 518	N = 52	0.96
	30 (-40,200)	50 (-20,180)		0 (0,0)	0 (0,0)	
Highest PLT	N = 1008	N = 98	1.00	N = 517	N = 52	0.37
	5 (0,36)	6 (0,36)		0 (0,29)	0 (0,17.5)	
Latest PLT	N = 1008	N = 98	0.61	N = 517	N = 52	0.32
	-0.9 (-35,11)	0 (-24,12.6)		0 (-20.4,10)	0 (-32,1.5)	

Table 3B: Average value over entire follow-up of post-randomization factors for cases and controls (1:10 matching) in each treatment arm.

	Darbepoetin alfa			***Placebo***		
Average value over follow-up	No Stroke Controls N=1010	Stroke Cases N = 101	P value	No Stroke Controls N=520	Stroke Cases N = 52	P value
Average SBP	N = 1010	N = 101	0.79	N = 520	N = 52	0.51
	134.5 (127.2,143.5)	131.8 (126.4,145.8)		136.6 (128.5,146.2)	138 (133.1,144.9)	
Average DBP	N = 1010	N = 101	0.46	N = 520	N = 52	0.29
	72.8 (67.1,77.9)	73.7 (67.4,78.8)		71.9 (66.5,78.3)	74.8 (66.7,78.3)	
Average Hb	N = 1009	N = 100	<0.001	N = 518	N = 52	0.03
	12.3 (11.7,12.6)	12.1 (11.3,12.5)		10.4 (9.8,11.1)	10.2 (9.6,10.7)	
Average weekly dose	N = 1009	N = 100	0.68	N = 518	N = 52	0.18
	51.1 (7.2,136.3)	53.8 (7.8,124.7)		0 (0,1.7)	0 (0,4)	
Average PLT	N = 1009	N = 99	0.23	N = 518	N = 52	0.68
	228 (187,271.1)	244.7 (196.6,295)		238.4 (198.2,279.5)	245.2 (200.2,277.8)	

Table 3C: Change from baseline value of post-randomization factors for cases and controls (1:10 matching) in each treatment arm.

	Darbepoetin alfa			***Placebo***		
Change from baseline to value in last 90 days	No Stroke Controls N=1010	Stroke Cases N = 101	P value	No Stroke Controls N=520	Stroke Cases N = 52	P value
Highest SBP90	N = 923	N = 86	0.34	N = 468	N = 44	0.57
	7 (-4,20)	6.5 (0,23)		2 (0,20)	0 (-4.5,23)	
Highest DBP90	N = 923	N = 86	0.26	N = 468	N = 44	0.57
	5 (0,10)	6 (0,14)		2 (0,10)	0 (0,11)	
Latest SBP90	N = 923	N = 86	0.61	N = 468	N = 44	0.55
	-2 (-14,10)	0 (-16,13)		0 (-12,10)	-0.5 (-14.5,7.5)	
Latest DBP90	N = 923	N = 86	0.63	N = 468	N = 44	0.27
	0 (-8,8)	0 (-10,8)		0 (-5.5,5)	-0.5 (-7.5,4)	
Highest Hb90	N = 864	N = 80	0.89	N = 438	N = 42	0.78
	2.8 (2,3.8)	2.9 (1.9,3.9)		0.4 (0,1.3)	0.6 (0,1.2)	
Latest Hb90	N = 864	N = 80	0.66	N = 438	N = 42	0.75
	2.2 (1.2,3)	2.2 (0.7,3.2)		0 (-0.5,0.8)	0 (-0.4,0.8)	
Highest PLT90	N = 470	N = 38	0.99	N = 253	N = 26	0.19
	-2.0 (-34,14)	-3.3 (-38,24)		0 (-6,7)	0 (-27,0)	
Latest PLT90	N = 470	N = 38	0.92	N = 253	N = 26	0.20
	-3.2 (-35,14)	-3.3 (-41.9,24)		0 (-7.9,7)	0 (-27,0)	

Table 3D: Average value over most recent 90 days of post-randomization factors for cases and controls (1:10 matching) in each treatment arm.

	Darbepoetin alfa			***Placebo***		
Average value over most recent 90 days	No Stroke Controls N=1010	Stroke Cases N = 101	P value	No Stroke Controls N=520	Stroke Cases N = 52	P value
Average SBP90	N = 923	N = 86	0.38	N = 468	N = 44	0.78
	133.7 (124,145)	135.6 (126.5,145.7)		136.7 (126.7,147.4)	137 (124,149.8)	
Average DBP90	N = 923	N = 86	0.43	N = 468	N = 44	0.97
	72.8 (66.2,78.7)	74.6 (66.5,80.5)		71.9 (65.3,80)	73.7 (64,79)	
Average Hb90	N = 866	N = 80	0.03	N = 438	N = 42	0.45
	12.5 (11.8,13.1)	12.2 (11.2,12.9)		10.4 (9.7,11.2)	10.3 (9.8,10.9)	
Average PLT90	N = 470	N = 39	0.86	N = 253	N = 26	0.50
	225.1 (185.2,282)	230 (172,301.4)		236 (194,281)	225 (181.9,291)	

Table 4A: Change from baseline value of post-randomization factors for cases and controls (1:15 matching) in each treatment arm.

	Darbepoetin alfa			Placebo		
Change from baseline to:	**No Stroke Controls N = 1395**	**Stroke Cases N = 101**	**P value**	**No Stroke Controls N = 780**	**Stroke Cases N = 52**	**P value**
Highest SBP	N = 1395	N = 101	0.81	N = 780	N = 52	0.14
	20 (7, 32)	18 (5, 40)		0 (15, 30)	5 (21, 31.5)	
Highest DBP	N = 1395	N = 101	0.98	N = 780	N = 52	0.86
	10 (5, 20)	11 (5, 20)		0 (9.5, 16)	0 (10, 18)	
Latest SBP	N = 1395	N = 101	0.85	N = 780	N = 52	0.30
	0 (-14, 10)	0 (-16, 13)		-10 (0, 10)	-15.5 (-1.5, 10)	
Latest DBP	N = 1395	N = 101	0.75	N = 780	N = 52	0.45
	0 (-8, 8)	0 (-10, 7)		-6 (0, 5)	-8 (0, 6)	
Highest Hb	N = 1390	N = 100	0.10	N = 778	N = 52	0.90
	3.6 (2.7, 4.4)	4.0 (2.8, 4.8)		0.3 (1.2, 2.1)	0.4 (1.2, 2.0)	
Latest Hb	N = 1390	N = 100	0.50	N = 778	N = 52	0.29
	2.0 (1.0, 2.9)	1.9 (0.6, 3.0)		-0.5 (0.0, 0.8)	-1.1 (0.0, 0.7)	
Highest Dose	N = 1394	N = 100	0.75	N = 778	N = 52	0.67
	120 (40, 400)	160 (35, 310)		0 (0, 30)	0 (0, 30)	
Latest Dose	N = 1394	N = 100	0.47	N = 778	N = 52	0.95
	30 (-40, 200)	50 (-20, 180)		0 (0, 0)	0 (0, 0)	
Highest PLT	N = 1392	N = 98	0.68	N = 777	N = 52	0.60
	1 (0, 35)	6 (0, 36)		0 (0, 26)	0 (0, 18)	
Latest PLT	N = 1392	N = 98	0.51	N = 777	N = 52	0.35
	-2 (-37, 9)	0 (-24, 13)		-21 (0, 7)	-32 (0, 2)	

Table 4B: Average value over entire follow-up of post-randomization factors for cases and controls (1:15 matching) in each treatment arm.

	Darbepoetin alfa			Placebo		
Average value over follow-up	**No Stroke Controls N = 1395**	**Stroke Cases N = 101**	**P value**	**No Stroke Controls N = 780**	**Stroke Cases N = 52**	**P value**
Average SBP	N = 1395	N = 101	0.94	N = 780	N = 52	0.25
	134.4 (126.9, 143.2)	131.8 (126.4, 145.8)		128.2 (135.9, 145.1)	133.1 (138.0, 144.9)	
Average DBP	N = 1395	N = 101	0.33	N = 780	N = 52	0.34
	72.5 (67.0, 77.8)	73.7 (67.4, 78.8)		66.5 (72.1, 78.2)	66.7 (74.8, 78.3)	
Average Hb	N = 1394	N = 100	<0.001	N = 778	N = 52	0.02
	12.3 (11.8, 12.7)	12.1 (11.3, 12.5)		9.8 (10.5, 11.1)	9.6 (10.2, 10.7)	
Average weekly dose	N = 1394	N = 100	0.98	N = 778	N = 52	0.19
	49.0 (4.9, 143.2)	53.8 (7.8, 124.7)		0 (0, 1.6)	0 (0, 4.0)	
Average PLT	N = 1393	N = 99	0.35	N = 777	N = 52	0.55
	231 (188, 275)	245 (197, 295)		199 (239, 280)	200 (245, 278)	

Table 4C: Change from baseline value of post-randomization factors for cases and controls (1:15 matching) in each treatment arm.

	Darbepoetin alfa			Placebo		
Change from baseline to value in last 90 days	**No Stroke Controls N = 1395**	**Stroke Cases N = 101**	**P value**	**No Stroke Controls N = 780**	**Stroke Cases N = 52**	**P value**
Highest SBP90	N = 1282	N = 86	0.80	N = 699	N = 44	0.44
	7 (-3, 20)	6.5 (0, 23)		132 (143, 160)	131 (140, 159)	
Highest DBP90	N = 1282	N = 86	0.37	N = 699	N = 44	0.77
	5 (0, 12)	6 (0, 14)		70 (78, 84)	70 (78, 84)	
Latest SBP90	N = 1282	N = 86	0.80	N = 699	N = 44	0.35
	0 (-14, 10)	0 (-16, 13)		125 (137, 150)	122 (135.5, 148)	
Latest DBP90	N = 1282	N = 86	0.79	N = 699	N = 44	0.42
	0 (-8, 8)	0 (-10, 8)		64 (70, 80)	62.5 (70, 80)	
Highest Hb90	N = 1221	N = 80	0.68	N = 661	N = 42	0.89
	2.8 (1.9, 3.7)	2.9 (1.9, 3.9)		0.0 (0.5, 1.3)	0.0 (0.6, 1.2)	
Latest Hb90	N = 1221	N = 80	0.96	N = 661	N = 42	0.57
	2.0 (1.1, 2.9)	2.2 (0.7, 3.2)		-0.4 (0.0, 0.9)	-0.4 (0.0, 0.8)	
Highest PLT90	N = 687	N = 38	0.71	N = 372	N = 26	0.35
	-4 (-39, 10)	-3 (-38, 24)		-10 (0, 0)	-27 (0, 0)	
Latest PLT90	N = 687	N = 38	0.67	N = 372	N = 26	0.40
	-5 (-40, 10)	-3 (-42, 24)		-11 (0, 0)	-27 (0, 0)	

Table 4D: Average value over most recent 90 days of post-randomization factors for cases and controls (1:15 matching) in each treatment arm.

	Darbepoetin alfa			Placebo		
Average value over most recent 90 days	**No Stroke Controls N = 1395**	**Stroke Cases N = 101**	**P value**	**No Stroke Controls N = 780**	**Stroke Cases N = 52**	**P value**
Average SBP90	N = 1282	N = 86	0.38	N = 699	N = 44	0.95
	134.0 (124.0, 145.0)	135.6 (126.5, 145.7)		126.7 (136.7, 147.0)	124.0 (137.0, 149.8)	
Average DBP90	N = 1282	N = 86	0.19	N = 699	N = 44	0.83
	72.7 (66.0, 78.7)	74.6 (66.5, 80.5)		65.3 (72.0, 80.0)	64.0 (73.7, 79.0)	
Average Hb90	N = 1225	N = 80	0.02	N = 661	N = 42	0.24
	12.5 (11.8, 13.1)	12.2 (11.3, 12.9)		9.7 (10.5, 11.2)	9.8 (10.3, 10.9)	
Average PLT90	N = 687	N = 39	0.85	N = 372	N = 26	0.52
	226 (185, 273)	230 (172, 301)		192 (238, 282)	182 (225, 291)	