Recombinant Human Erythropoietin in the Treatment of Acute Ischemic Stroke

Hannelore Ehrenreich, MD, DVM; Karin Weissenborn, MD; Hilmar Prange, MD; Dietmar Schneider, MD; Christian Weimar, MD; Katja Wartenberg, MD; Peter D. Schellinger, MD; Matthias Bohn, PhD; Harald Becker, MD; Martin Wegrzyn, BS; Peter Jähnig, MBA; Manfred Herrmann, MD; Michael Knauth, MD; Mathias Bähr, MD; Wolfgang Heide, MD; Armin Wagner, MD; Stefan Schwab, MD; Heinz Reichmann, MD; Günther Schwendemann, MD; Reinhard Dengler, MD; Andreas Kastrup, MD; Claudia Bartels, PhD; for the EPO Stroke Trial Group

Background and Purpose—Numerous preclinical findings and a clinical pilot study suggest that recombinant human erythropoietin (EPO) provides neuroprotection that may be beneficial for the treatment of patients with ischemic stroke. Although EPO has been considered to be a safe and well-tolerated drug over 2 decades, recent studies have identified increased thromboembolic complications and/or mortality risks on EPO administration to patients with cancer or chronic kidney disease. Accordingly, the double-blind, placebo-controlled, randomized German Multicenter EPO Stroke Trial (Phase II/III; ClinicalTrials.gov Identifier: NCT00604630) was designed to evaluate efficacy and safety of EPO in stroke.

Methods—This clinical trial enrolled 522 patients with acute ischemic stroke in the middle cerebral artery territory (intent-to-treat population) with 460 patients treated as planned (per-protocol population). Within 6 hours of symptom onset, at 24 and 48 hours, EPO was infused intravenously (40 000 IU each). Systemic thrombolysis with recombinant tissue plasminogen activator was allowed and stratified for.

Results—Unexpectedly, a very high number of patients received recombinant tissue plasminogen activator (63.4%). On analysis of total intent-to-treat and per-protocol populations, neither primary outcome Barthel Index on Day 90 ($P=0.45$) nor any of the other outcome parameters showed favorable effects of EPO. There was an overall death rate of 16.4% (n=42 of 256) in the EPO and 9.0% (n=24 of 266) in the placebo group (OR, 1.98; 95% CI, 1.16 to 3.38; $P=0.01$) without any particular mechanism of death unexpected after stroke.

Conclusions—Based on analysis of total intent-to-treat and per-protocol populations only, this is a negative trial that also raises safety concerns, particularly in patients receiving systemic thrombolysis. **(*Stroke*. 2009;40:e647-e656.)**

Key Words: clinical trial ■ hematopoietic growth factor ■ neuroprotection ■ NIHSS ■ rtPA

Stroke remains a leading cause of death and disability throughout the industrialized world. The introduction of thrombolysis using recombinant tissue plasminogen activator (rtPA) in acute ischemic stroke has improved the clinical outcome of patients who present early after onset of symptoms. With multiple contraindications, however, thrombolysis applies to a restricted number of patients, leaving the vast majority without specific treatment. Therefore, there is an urgent need for new therapies, accessible for more patients, in particular those not qualifying for rtPA.

Apart from thrombolytic strategies, many agents targeting other aspects of stroke pathology were effective in animal models but unsuccessfully translated to humans.[1] In our monocentric proof-of-concept study, the "Göttingen EPO Stroke Study" (www.epo-study.de/index_eng.html), recombinant human erythropoietin (EPO) appeared safe and bene-

Received August 6, 2009; final revision received August 26, 2009; accepted September 4, 2009.

From the Division of Clinical Neuroscience (H.E., M.W., C.B.), Max Planck Institute of Experimental Medicine, Göttingen, Germany; Center for Neurological Medicine (K.W., R.D.), Hannover Medical School, Hannover, Germany; the Department of Neurology (H.P., M.B., A.K.), University Medical Center Göttingen, Georg-August-University, Göttingen, Germany; the Department of Neurology (D.S., A.W.), University Hospital of Leipzig, Leipzig, Germany; the Department of Neurology (C.W.), University of Duisburg-Essen, Essen, Germany; the Department of Neurology (K.W., H.R.), Stroke Center, University of Technology Dresden, Dresden, Germany; the Department of Neurology (P.D.S., S.S.), University Hospital of Erlangen, Erlangen, Germany; Central Pharmacy (M.B.), University Medical Center Göttingen, Georg-August-University, Göttingen, Germany; Applied Science and Technology (H.B.), Zwingenberg, Germany; Data Management and Biostatistical Services (P.J.), PAREXEL International GmbH, Berlin, Germany; the Department of Neuropsychology and Behavioral Neurobiology (M.H.), University of Bremen, Bremen, Germany; the Department of Neuroradiology (M.K.), University Medical Center Göttingen, Georg-August-University, Göttingen, Germany; the Department of Neurology (W.H.), General Hospital Celle, Celle, Germany; and the Department of Neurology (G.S.), University Hospital Bremen-Mitte, Bremen, Germany.

Correspondence to Hannelore Ehrenreich, MD, DVM, Division of Clinical Neuroscience, Max Planck Institute of Experimental Medicine, Hermann-Rein Str 3, 37075 Göttingen, Germany. E-mail ehrenreich@em.mpg.de

Stroke **is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.109.564872**

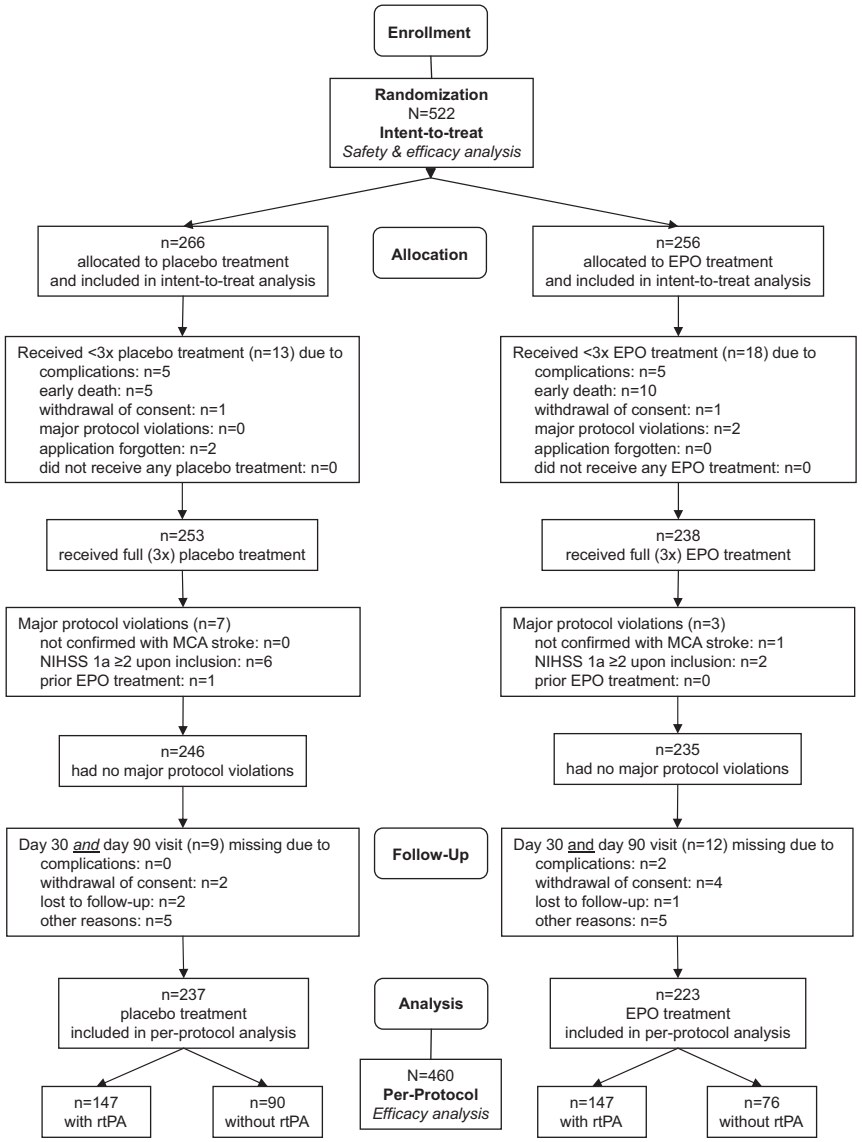

Figure 1. Overview of patient flow in the German Multicenter EPO Stroke Trial.

ficial on clinical outcome in patients with ischemic stroke of the middle cerebral artery (MCA) territory.[2] EPO theoretically represents an ideal compound for neuroprotection in brain disease. It binds specifically to neuronal EPO receptors where it acts in an antiapoptotic, antioxidant, anti-inflammatory, neurotrophic, neural stem cell–modulating and neuroplasticity-enhancing fashion. Independent of its hematopoietic effects, EPO proved neuroprotective/neuroregenerative in animal models of ischemia, hypoxia, or combinations thereof.[3,4] Despite a molecular weight of >30 000 Dalton, EPO, given at high doses, crosses the blood–brain barrier in an amount sufficient to exert neuroprotection.[5–7]

Since conclusion of the "Göttingen EPO Stroke Study" in 2001, the stroke landscape has changed due to approval and increasing use of rtPA in Germany.[8] We report here the results of the double-blind, placebo-controlled, randomized German Multicenter EPO Stroke Trial designed to further investigate efficacy and safety of EPO with/without systemic thrombolysis in patients with ischemic stroke in the MCA territory (ClinicalTrials.gov Identifier: NCT00604630).

Patients and Methods

Trial Design

In 2002, the randomized, double-blind, placebo-controlled German multicenter trial was approved by the Ethical Committee of the Georg-August-University of Göttingen as well as committees of participating study sites and performed according to ICH-GCP guidelines. From January 2003 through March 2008, 522 patients were enrolled by the responsible neurologists in the centers. Patients were randomly assigned to treatment after written informed consent, their own or a surrogate. A central managing pharmacy, acting independently from the principal investigator and the study centers, was responsible for randomization and study drug logistics, including shipment. Stratified block randomization for the 2 stratification factors, study centers and thrombolysis (rtPA), was employed using a fixed block size of 4 in each stratum. The randomization allocation sequence was generated by a random-number table. Vials containing study drug were numbered (randomization number) and additionally labeled with the strata (for rtPA or non-rtPA patients). Each study center was then supplied with vials for several patients. On inclusion, patients were assigned to study drug (allocation of the next available randomization number; 3 vials each with the same number) by the respective, blinded site investigator and remained on the same allocation throughout the study. Randomization number of the

Table 1. Patient Characteristics on Inclusion (ITT Population)

	ITT (N=522)		rtPA (n=331)		Non-rtPA (n=191)	
Variable	EPO (n=256)	Placebo (n=266)	EPO (n=166)	Placebo (n=165)	EPO (n=90)	Placebo (n=101)
Age, years, mean±SD (range)	68.6±12.4 (20–100)	68.2±12.8 (19–95)	66.8±12.5 (20–100)	66.2±13.4 (19–95)	71.9±11.5 (38–95)	71.5±11.2 (42–92)
Sex, male/female ratio (%)	141/115 (55/45)	141/125 (53/47)	92/74 (55/45)	93/72 (56/44)	49/41 (54/46)	48/53 (48/52)
Hemisphere, N (%)						
Left	118 (46.1)	125 (47.0)	77 (46.4)	80 (48.5)	41 (45.6)	45 (44.6)
Right	135 (52.7)	139 (52.3)	87 (52.4)	84 (50.9)	48 (53.3)	55 (54.5)
Both	1 (0.4)	2 (0.8)	0 (0.0)	1 (0.6)	1 (1.1)	1 (1.0)
Not applicable	2 (0.8)	0 (0.0)	2 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
Stroke subtype,* N (%)						
Cardiogenic embolism	130 (50.8)	121 (45.5)	85 (51.2)	73 (44.2)	45 (50.0)	48 (47.5)
Arterial embolism	61 (23.8)	75 (28.2)	41 (24.7)	47 (28.5)	20 (22.2)	28 (27.7)
Large artery occlusion	58 (22.7)	65 (24.4)	39 (23.5)	45 (27.3)	19 (21.1)	20 (19.8)
Paradox embolism	12 (4.7)	14 (5.3)	6 (3.6)	10 (6.1)	6 (6.7)	4 (4.0)
Lacunar infarction	11 (4.3)	9 (3.4)	5 (3.0)	3 (1.8)	6 (6.7)	6 (5.9)
Tandem arterial	0 (0.0)	3 (1.1)	0 (0.0)	3 (1.8)	0 (0.0)	0 (0.0)
Septic embolism	1 (0.4)	1 (0.4)	0 (0.0)	0 (0.0)	1 (1.1)	1 (1.0)
Unknown	24 (9.4)	30 (11.3)	16 (9.6)	20 (12.1)	8 (8.9)	10 (9.9)
Not applicable	2 (0.8)	0 (0.0)	2 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
Prior anticoagulation, N (%)						
No	156 (60.9)	165 (62.0)	109 (65.7)	114 (69.1)	47 (52.2)	51 (50.5)
Yes	96 (37.5)	99 (37.2)	55 (33.1)	50 (30.3)	41 (45.6)	49 (48.5)
Unknown	4 (1.6)	2 (0.8)	2 (1.2)	1 (0.6)	2 (2.2)	1 (1.0)
Hypertension, N (%)						
No	63 (24.6)	72 (27.1)	41 (24.7)	45 (27.3)	22 (24.4)	27 (26.7)
Yes	188 (73.4)	188 (70.7)	120 (72.3)	117 (70.9)	68 (75.6)	71 (70.3)
Borderline	5 (2.0)	6 (2.3)	5 (3.0)	3 (1.8)	0 (0.0)	3 (3.0)
Diabetes, N (%)						
No	181 (70.7)	182 (68.4)	113 (68.1)	113 (68.5)	68 (75.6)	69 (68.3)
Yes	57 (22.3)	67 (25.2)	38 (22.9)	39 (23.6)	19 (21.1)	28 (27.7)
Borderline	18 (7.0)	16 (6.0)	15 (9.0)	13 (7.9)	3 (3.3)	3 (3.0)
Unknown	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)
NIHSS†						
Mean±SD (range)	13.2±5.8 (4–32)	12.8±5.7 (4–30)	13.3±5.5 (4–31)	13.5±5.8 (4–30)	13.0±6.4 (4–32)	11.7±5.5 (4–27)
Median	13	12	13	13	11	11
MRI diffusion-weighted imaging, cm^3†						
Mean±SD (range)	39±55 (0–401)	36±59 (0–354)	37±54 (0–401)	30±49 (0–250)	43±56 (0–243)	46±72 (0.2–354)
Median	20	11	20	10	19	13
MRI FLAIR, cm^3†						
Mean±SD (range)	3.4±13.3 (0–130)	2.4±13.8 (0–182)	2.2±9.3 (0–84)	1.9±15.0 (0–182)	5.8±18.5 (0–130)	3.3±11.5 (0–103)
Median	0	0	0	0	0	0
Time to treatment, minutes†						
Mean±SD (range)	266±72 (42–442)	272±66 (78–485)	263±67 (45–410)	267±67 (110–480)	271±81 (42–442)	281±64 (78–485)
Mean arterial blood pressure, mm Hg†						
Mean±SD (range)	119±17 (83–178)	118±17 (78–179)	118±15 (85–171)	118±15 (79–179)	121±20 (83–178)	119±19 (78–170)
C-reactive protein, mg/L†						
Mean±SD (range)	12.2±33 (0.3–319)	10.7±21 (0.3–144)	10.8±26 (0.4–219)	8.5±15 (1.0–102)	14.9±42 (0.3–319)	14.4±28 (0.3–144)
Glucose, mg/dL†						
Mean±SD (range)	128±46 (67–531)	130±48 (64–367)	130±48 (73–531)	131±50 (64–367)	125±41 (67–295)	130±45 (78–360)

*Adds up to n >subgroup and >100% (some patients fulfill criteria for more than one subtype).

†Parameters based on n=489–519 (ITT), n=243–254 (ITT EPO), n=246–265 (ITT placebo), n=312–328 (ITT rtPA), n=158–165 (ITT rtPA EPO), n=154–163 (ITT rtPA placebo), n=176–189 (ITT non-rtPA), n=84–89 (ITT non-rtPA EPO), and n=92–100 (ITT non-rtPA placebo) due to missing data.

FLAIR indicates fluid-attenuated inversion recovery.

applied study drug was documented in the patient's case record form, and, after completion of study intervention (Day 3), this information was transmitted back to the central pharmacy for maintaining a demand-oriented stream of study drug delivery to the centers.

An independent scientific advisory and safety board was responsible for data reviews at 2 interim looks (at the stage of 60 and 263 patients) and at trial conclusion supported by an independent clinical research organization, PAREXEL International, Berlin. The prespecified safety stopping rules for the study in general and the interim looks in particular were suspected (unexpected) serious adverse reactions (in causal relation to EPO treatment). Prespecified efficacy stopping rules applied only for the second look after 263

Table 2. Main Outcome Parameters (Primary Outcome: Barthel Index on Day 90; Secondary Outcomes Exploratory)

	ITT (N=522)			rtPA (n=331)			Non-rtPA (n=191)		
Variable	EPO (n=256)	Placebo (n=266)	*P*	EPO (n=166)	Placebo (n=165)	*P*	EPO (n=90)	Placebo (n=101)	*P*
Barthel Index (Day 90)	56.5±42 (70)	59.2±41 (75)	0.45	59.5±41 (70)	63.5±41 (85)	0.38	50.9±42 (52.5)	52.2±41 (60)	0.83
Modified Rankin Scale (Day 90)	3.2±2.0 (3)	3.0±1.9 (3)	0.26	3.1±2.0 (3)	2.9±2.0 (3)	0.28	3.5±3.3 (4)	3.3±1.8 (4)	0.60
NIHSS (Day 90)	9.7±12 (5.5)	8.2±10 (4.5)	0.12	9.5±12 (5.0)	7.7±10 (4.0)	0.15	10.2±12 (6.0)	9.1±9 (7.0)	0.47
Δ NIHSS (Day 1–Day 90)	3.5±10 (6.0)	4.5±9 (6.0)	0.24	3.8±11 (6.0)	5.7±9 (6.0)	0.08	3.1±9 (5.0)	2.5±8 (4.0)	0.62
Barthel Index (Day 30)	49.5±43 (40)	51.4±42 (50)	0.61	50.8±43 (45)	54.9±43 (60)	0.39	47.0±43 (30)	45.6±40 (40)	0.82
Modified Rankin Scale (Day 30)	3.5±2.0 (4)	3.3±1.9 (4)	0.40	3.4±2.0 (4)	3.2±2.0 (4)	0.26	3.6±1.9 (4)	3.6±1.7 (4)	0.95
NIHSS (Day 30)	11.0±12 (7.0)	9.4±10 (6.0)	0.11	10.7±12 (7.0)	8.8±11 (5.0)	0.15	11.6±12 (8.0)	10.3±10 (8.0)	0.43
MRI diffusion-weighted imaging, cm^3 (Day 7)†	86±107 (43)	91±122 (32)	0.67	78±85 (46)	98±133 (34)	0.15	100±137 (43)	80±101 (31)	0.27
MRI FLAIR, cm^3 (Day 7)†	86±108 (47)	90±114 (37)	0.71	76±83 (47)	91±116 (38)	0.21	103±142 (48)	87±111 (37)	0.43
Δ MRI diffusion-weighted imaging (Day 7–Day 1)†	57±87 (19)	59±96 (19)	0.80	52±69 (24)	70±111 (24)	0.12	65±112 (12)	42±62 (15)	0.13

Data presented as mean±SD (median) unless otherwise indicated; significant differences are bold.

All comparisons based on independent *t* tests, 2-tailed.

†ITT: parameters based on n=429–445, n=205–214 (ITT EPO), n=224–231 (ITT placebo), n=267–280 (ITT rtPA), n=131–138 (ITT rtPA EPO), n=136–142 (ITT rtPA placebo), n=162–166 (ITT non-rtPA), n=74–77 (ITT non-rtPA EPO), and n=88–89 (ITT non-rtPA placebo) due to missing data; PP: parameters based on n=399–413, n=190–198 (PP EPO), n=209–215 (PP placebo), n=251–263 (PP rtPA), n=123–129 (PP rtPA EPO), n=128–134 (PP rtPA placebo), n=148–152 (PP non-rtPA), n=67–70 (PP non-rtPA EPO), and n=81–82 (PP non-rtPA placebo) due to missing data.

FLAIR indicates fluid-attenuated inversion recovery.

patients; early stopping of the trial due to proven efficacy was checked in a formal statistical interim analysis using the Lan and DeMets Type I error spending function approach with boundaries of the O'Brien and Fleming type to account for the sequential evaluation of the trial.[9,10]

Patients

Inclusion Criteria

Patients ≥18 years of age with ischemic stroke in the MCA territory were eligible. Inclusion required scoring ≥5 on the National Institutes of Health Stroke Scale (NIHSS; scores up to 42 indicate increasing severity[11]) and a time window of ≤6 hours from onset of symptoms to study drug infusion (time to treatment). Acute stroke was confirmed using diffusion-weighted (DWI) MRI. Fluid-attenuated inversion recovery (FLAIR) should essentially be free of fresh infarct signs (or at least show distinctly smaller lesion size compared with DWI) and rule out recent infarcts in the same territory.

Exclusion Criteria

Exclusion criteria were contraindications to MRI, fast resolving neurological symptoms, unclear time point of symptom onset, coma (NIHSS-1a ≥2), brain trauma/surgery within the last 4 weeks, subarachnoid/intracerebral hemorrhage, intracranial neoplasia, septic embolism, endocarditis, malignant hypertension, florid malignancy, myeloproliferative disorder, antibodies or allergy against EPO, pregnancy, or participation in other treatment trials.

Study Intervention

History, physical examination, routine laboratory and cranial CT were obtained for patients screened for eligibility before referral to MRI. Intravenous infusion of recombinant human EPO (Epoetin-alpha, provided by Johnson & Johnson, 40 000 IU in 50 mL isotonic electrolyte solution over 30 minutes) or placebo (solvent control; Johnson & Johnson) was started within 6 hours after symptom onset (Day 1; in case of thrombolysis, always thereafter) and repeated 24 hours and 48 hours later (cumulative dose of 120 000 IU per patient). The dose was chosen according to the previous EPO stroke study.[2]

Clinical Assessment

Patients were formally assessed at enrollment, 24 hours and 48 hours later, at Day 7, Day 30, and Day 90 by raters blinded to treatment allocation (double-blind study). In addition to NIHSS, assessments during follow-up included functional measures, Barthel Index (range from 100, indicating no deficit, to 0, indicating complete dependence or death[12]), and modified Rankin Scale (range from 0, indicating no residual symptoms, to 6, indicating death[13]) at Days 30 and 90 only. Barthel Index on Day 90 was the prespecified primary outcome measure. All scoring was performed by certified/trained examiners. For perpetuation of data quality/reliability, respective rater trainings were repeated yearly. Further outcome/safety measures were lesion size (MRI on inclusion and Day 7), routine laboratory, vital signs, and serious adverse events (SAE) monitoring. All SAE cases were immediately and carefully evaluated by the coordinating study center, documented in SAE forms, and reported to the respective regulatories. Evaluation was performed by studying chart records in addition to notes in the case record form as well as by comprehensive interview of the treating physicians. MRI analysis was based on DWI and FLAIR images using a manual semiautomatic standard volumetric procedure carried out by blinded, trained raters (DicomWorks software 1.3.5; P Puech, Lille & L Boussel, Lyon, France; http://dicom.online.fr). Inter- and intrarater reliability amounted to >0.95.

Table 2. Continued

	PP (n=460)			rtPA (n=294)			Non-rtPA (n=166)		
Variable	EPO (n=223)	Placebo (n=237)	*P*	EPO (n=147)	Placebo (n=147)	*P*	EPO (n=76)	Placebo (n=90)	*P*
Barthel Index (Day 90)	63.7±39 (80)	63.6±40 (85)	0.97	66.0±38 (85)	67.9±39 (90)	0.67	59.4±40 (70)	56.5±40 (62.5)	0.64
Modified Rankin Scale (Day 90)	2.9±1.9 (3)	2.8±1.9 (3)	0.80	2.8±1.9 (3)	2.6±1.9 (2)	0.50	3.1±1.8 (3)	3.2±1.8 (3)	0.72
NIHSS (Day 90)	7.3±9 (4.0)	7.1±8 (4.0)	0.72	7.6±10 (3.0)	6.4±8 (3.0)	0.24	6.8±7 (4.5)	8.2±8 (6.0)	0.25
Δ NIHSS (Day 1–Day 90)	5.3±8 (6.0)	5.5±7 (6.0)	0.79	5.4±9 (6.0)	6.9±7 (7.0)	0.10	**5.3±5 (5.5)**	**3.2±6 (4.0)**	**0.03**
Barthel Index (Day 30)	56.2±42 (60)	55.0±41 (60)	0.77	56.6±42 (60)	58.5±41 (70)	0.69	55.4±42 (62.5)	49.3±40 (42.5)	0.34
Modified Rankin Scale (Day 30)	3.1±1.9 (4)	3.1±1.8 (4)	0.98	3.1±1.9 (4)	3.0±1.9 (3)	0.47	3.2±1.8 (4)	3.4±1.7 (4)	0.32
NIHSS (Day 30)	8.7±10 (5.0)	8.2±9 (5.0)	0.52	8.9±10 (5.0)	7.6±9 (5.0)	0.23	8.3±9 (5.0)	9.1±8 (8.0)	0.57
MRI diffusion-weighted imaging, cm^3 (Day 7)†	79±96 (42)	92±125 (32)	0.23	75±81 (42)	100±136 (34)	0.07	87±119 (41)	80±104 (29)	0.70
MRI FLAIR, cm^3 (Day 7)†	78±93 (42)	91±115 (38)	0.20	73±80 (44)	94±118 (42)	0.09	87±113 (42)	86±111 (35)	0.97
Δ MRI diffusion-weighted imaging (Day 7–Day 1)†	51±80 (18)	61±99 (19)	0.28	**48±62 (24)**	**72±114 (25)**	**0.04**	58±106 (11)	43±65 (13)	0.33

Data presented as mean±SD (median) unless otherwise indicated; significant differences are bold.

All comparisons based on independent *t* tests, 2-tailed.

†ITT: parameters based on n=429–445, n=205–214 (ITT EPO), n=224–231 (ITT placebo), n=267–280 (ITT rtPA), n=131–138 (ITT rtPA EPO), n=136–142 (ITT rtPA placebo), n=162–166 (ITT non-rtPA), n=74–77 (ITT non-rtPA EPO), and n=88–89 (ITT non-rtPA placebo) due to missing data; PP: parameters based on n=399–413, n=190–198 (PP EPO), n=209–215 (PP placebo), n=251–263 (PP rtPA), n=123–129 (PP rtPA EPO), n=128–134 (PP rtPA placebo), n=148–152 (PP non-rtPA), n=67–70 (PP non-rtPA EPO), and n=81–82 (PP non-rtPA placebo) due to missing data.

FLAIR indicates fluid-attenuated inversion recovery.

Statistical Analysis

All statistical analyses were prespecified and prospective (no post hoc analyses except for the additional exploratory analyses denoted as such). They include safety evaluation of intent-to-treat (ITT) and end point evaluation of ITT as well as of per-protocol (PP) population. Because an unexpectedly high, ever-increasing number of patients received thrombolysis, the decision was made at first interim look to subdivide the study population for final analysis into an rtPA and a non-rtPA population. Barthel Index on Day 90 was determined as the primary outcome parameter with statistical sample size and power calculations based on the Göttingen EPO Stroke Study.[2] The calculated sample size provided 80% statistical power to detect a 10-point increase on primary outcome at an overall 2-sided alpha error rate of 0.05. As secondary (exploratory) end points, modified Rankin Scale, NIHSS, and imaging variables were used. The ITT population comprised all included and randomized patients (N=522) having received study medication at least once. Prespecified reasons for exclusion from the PP population were (1) incomplete (<3×) study medication; (2) major protocol violations (coma on inclusion, no MCA stroke, EPO pretreatment); and (3) lacking Days 30 and 90 follow-up (patients dying before Day 30 who received complete study medication are not excluded). Missing data for functional and neurological outcomes were imputed by using "last observation carried forward" (if no postbaseline value was available, worst possible outcome was imputed).

Data are presented as mean±SD (median in brackets) in text/tables and mean±SEM in figures with significance at $P<0.05$. Because there is only one primary end point decisive for the trial to be declared positive or negative, adjustment of the alpha value for multiplicity was not indicated. All other (secondary) outcome measures were exploratory (hypothesis-generating). Independent Student *t* test (2-tailed) and χ^2 test (2-tailed) were used for intergroup comparisons. Analysis of variance for repeated measures was applied to compare EPO versus placebo with respect to NIHSS score over time. Analysis of covariance for repeated measures with age as a covariate compared relative efficacy of EPO versus rtPA versus placebo. Statistical analyses were performed independently by PAREXEL International GmbH, Berlin, and scientists of the Max Planck Institute of Experimental Medicine, Göttingen.

Results

Patient Flow and Baseline Characteristics

All screened patients who met eligibility criteria, that is, a total of 522 patients were included and received study medication (ITT population), 491 (94.1%) received full study medication (Figure 1). Of these, 460 were treated per protocol (PP population). Ten patients (1.9%) were excluded from PP efficacy analysis due to major protocol violations and another 21 (4.0%) due to missing follow-up data. Reasons for nonreceiving medication per protocol (n=31) were application only once or twice due to stroke-related complications/early death, withdrawal of consent, protocol violations, and forgotten application. Except for the patients who died (Barthel Index=0), primary outcome, Barthel Index on Day 90, was unavailable for 29 other patients (5.6% total, 5.5% in the EPO versus 5.6% in the placebo group) and had to be

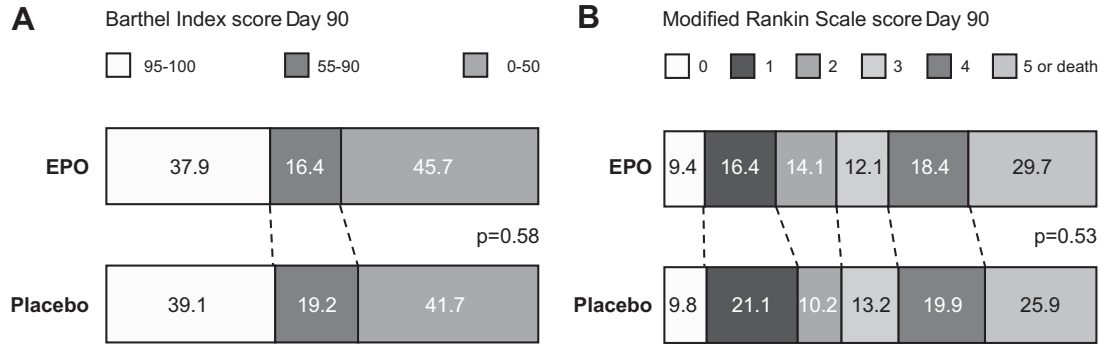

Figure 2. Results of main outcome parameters (ITT population). A, Primary outcome, Barthel Index on Day 90, expressed as distribution of Barthel Index scores in the ITT placebo (n=266) and EPO groups (n=256) revealed no treatment effect (χ^2 test, 2-tailed). Numbers denote the proportion of patients (%) in the respective score range. B, Likewise, distribution of modified Rankin Scale on Day 90 failed to show significant differences between ITT placebo (n=266) and EPO groups (n=256; χ^2 test, 2-tailed). Numbers denote the proportion of patients (%) in the respective score range.

imputed (n=21 imputed to "0"; n=8 last observation carried forward).

The ITT population had a mean age of 68.4 years, 282 males (54%), and a mean NIHSS score on inclusion of 13.0 (median, 12.5). Altogether, 331 ITT patients (63.4%) received rtPA. Subpopulations (rtPA and non-rtPA) were well balanced between EPO and placebo groups regarding typical baseline variables, representative of a stroke population (Table 1).

Clinical Outcomes

For efficacy purposes, the ITT population (N=522), the PP population (N=460) as well as the prespecified subpopulations, rtPA and non-rtPA, are presented with respective EPO and placebo groups listed next to each other (Table 2). Due to the scientific and thus also exploratory character of this trial, subgroup analysis of patients treated per protocol was also performed.

This is a negative trial: The primary outcome measure, Barthel Index on Day 90, failed to show a significant difference in all groups (ITT: EPO versus placebo: 56.9±42 versus 59.2±41; 95% CI, −4.41 to 9.86; $P=0.45$). This was also true for most secondary outcome parameters (modified Rankin Scale and NIHSS on Day 90, MRI parameters; Table 2; Figure 2A–B). In contrast, subgroup analysis revealed that the delta NIHSS (NIHSS Day 1 minus Day 90) showed a better outcome of patients receiving EPO in the PP non-rtPA group as compared with placebo (mean difference of 5.3±5.3 in EPO versus 3.2±6.4 in placebo; $P<0.03$). In the respective rtPA population, EPO provided no benefit. Regarding MRI

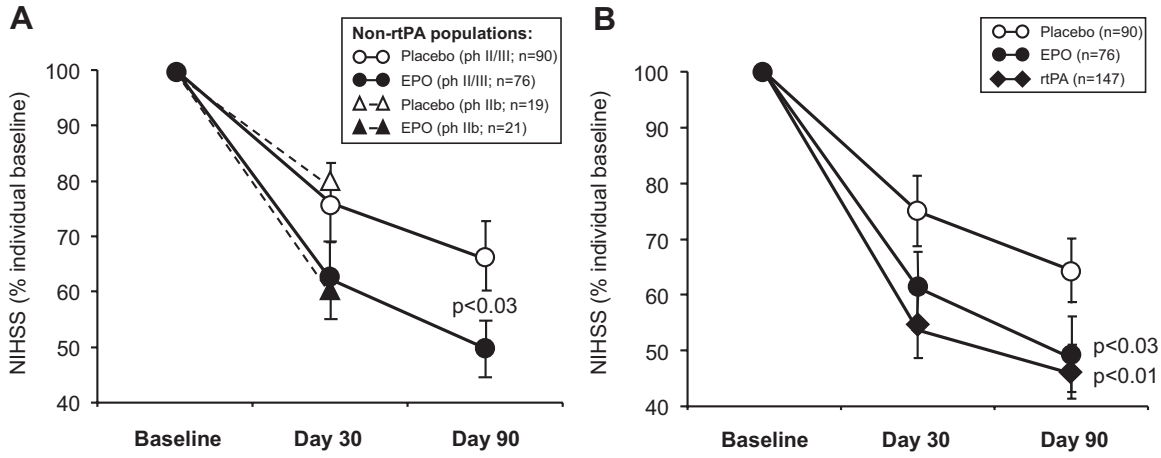

Figure 3. Exploratory subgroup analyses of EPO effects on recovery in acute ischemic stroke. A, Exploratory subgroup analysis (post hoc) of the PP non-rtPA population revealed that the German Multicenter EPO Stroke Trial (phase II/III) reproduced the findings of the previous Göttingen EPO Stroke Study (phase IIb; follow-up only until Day 30) with respect to course of NIHSS.[2] Note: NIHSS scores on study entry were comparable in both trials. Significance refers to the time×treatment interaction effect of the PP non-rtPA treatment groups of the multicenter trial determined with analysis of variance for repeated measures (NIHSS as the dependent variable, treatment assignment and time [Day 1 and Day 90] as independent variables). Mean±SEM presented. B, Relative efficacy of EPO monotreatment, rtPA monotreatment, and placebo monotreatment on neurological recovery in the PP population. EPO and rtPA show similar beneficial effects in this exploratory subgroup analysis (post hoc). Significance refers to the time×treatment interaction effect of EPO versus placebo and rtPA versus placebo determined with analysis of covariance for repeated measures (NIHSS as the dependent variable, treatment assignment and time [Day 1 and Day 90] as independent variables). Due to significant age differences between rtPA (66.7±13.6 years) and EPO (71.1±11.5 years; $P=0.02$) as well as between rtPA (66.7±13.6 years) and placebo groups (71.5±11.0 years; $P=0.005$), age was implemented as covariate to eliminate impact of age on recovery. Estimated marginal mean±SEM presented. For illustration of different recovery between treatment groups, the individual baseline NIHSS value is set to 100%, and subsequent values are expressed as % individual baseline (A, B).

data, only the PP rtPA population exhibited reduced evolution of lesion size in the EPO as compared with the placebo group from Day 1 to 7 (mean DWI difference of 48 ± 62 cm^3 in EPO versus 72 ± 114 cm^3 in patients receiving placebo; $P=0.04$; Table 2).

Additional Exploratory Analysis (Post hoc)

Because the PP non-rtPA population is the only adequate comparator for the first EPO stroke study population (rtPA not yet approved, all patients treated per protocol),[2] it was of scientific interest to align the respective NIHSS data of both trials in a post hoc analysis. As illustrated in Figure 3A, improvement from individual baseline to Day 30 (predefined end point of the first EPO stroke study) looks identical. To estimate the relative efficacy of EPO alone and of rtPA alone, the course of NIHSS was compared, after statistically adjusting for age, in placebo and EPO groups of the PP non-rtPA with the PP placebo group of the rtPA population (Figure 3B). EPO and rtPA curves are similar and distinct from placebo treatment.

Safety Analysis

For safety analysis, all ITT patients were included (Table 3; Supplemental Tables I to III; available at http://stroke.ahajournals.org). A total of 66 patients (12.6%) of the ITT population died, with significantly more deaths in the EPO compared with the placebo group (16.4% versus 9.0%; OR, 1.98; CI, 1.16 to 3.38; $P=0.01$). The highest death rate occurred within the first week (29 of 66 [43.9%]), mainly attributable to intracerebral hemorrhage[14] (13 of 66 [19.7%]), brain edema (10 of 66 [15.2%]), and thromboembolic events (10 of 66 [15.2%]). Subpopulation analysis revealed that the significantly increased death rate on EPO is found in the rtPA population as well (ITT: 16.3% versus 8.5%; OR, 2.10; CI, 1.10 to 4.16; $P=0.03$; PP: 12.2% versus 5.4%; OR, 2.42; CI, 1.02 to 5.77; $P=0.04$). In the non-rtPA population, a strong trend toward an increased death rate on EPO was also present in the ITT analysis (16.7% versus 9.9%; OR, 1.82; CI, 0.77 to 4.29; $P=0.17$) but did not translate to the PP population (7.9% versus 10.0%; OR, 0.77; CI, 0.26 to 2.28; $P=0.64$). Importantly, on inclusion (that is, before receiving any study medication), ITT non-rtPA EPO patients who died were more severely affected as compared with patients receiving placebo (NIHSS on Day 1: 20.4 ± 5.4 versus 13.3 ± 4.9; $P=0.003$). This misbalance is not only highly significant, but also highly predictive of a worse outcome. Consequently, there was a 2-fold higher very early death rate in the EPO group (after only one study drug application; Table 3; Figure 1).

Distribution of SAE in the ITT population was similar without reaching significance (31.2% versus 25.9% with at least one SAE in patients receiving EPO versus patients receiving placebo; OR, 1.30; CI, 0.89 to 1.90; $P=0.18$). Specifically, intracerebral hemorrhage subtypes (classification according to Safe Implementation of Thrombolysis in Stroke-MOnitoring STudy [SITS-MOST])[14] were comparably distributed among groups. Detailed additional safety analysis (see Supplemental Tables II and III) uncovered that, according to the rtPA summary of product characteristics, approximately 50% of the rtPA-treated patients in this trial had contraindications to rtPA, with (1) prior anticoagulation (25.7%), that is, application of cumarines (international normalized ratio >1.3), heparins or thrombocyte aggregation inhibitors; (2) time window >3 hours (16.1%); (3) age >80 years (13.6%); and (4) infarct more than one third of the MCA region (9.0%) being the most prominent contraindications. Among the rtPA-treated patients of the ITT population with fatal outcome, 12 (44.4%) in the EPO but only 4 (28.6%) in the placebo group had one rtPA contraindication ($P=0.05$; Supplemental Tables II and III).

As expected, there was a slight difference over time regarding hemoglobin, hematocrit, erythrocytes, reticulocytes, iron, and ferritin between EPO and placebo ITT patients, reproducing findings of the first EPO stroke study.[2] Looking at individual patients' charts, however, unblinding due to these values would have been highly unlikely. Patients receiving EPO essentially kept their baseline hemoglobin, hematocrit, and erythrocyte levels, whereas patients receiving placebo showed a temporary drop thereof. Other hematologic parameters, including platelets, did not differ between groups over time. Parameters of blood chemistry and coagulation were comparable on EPO and placebo. In both groups, there was a typical stroke-related, persistent elevation of glucose, C-reactive protein, and erythrocyte sedimentation rate (Supplemental Table I).

Discussion

The German Multicenter EPO Stroke Trial, a Phase II/III trial designed to reproduce the promising results of the Göttingen EPO Stroke Study,[2] that is, improved clinical recovery on EPO in patients with ischemic stroke, turned out to be a negative trial. Primary outcome Barthel Index as well as all other outcome measures failed to show any benefit on analysis of total ITT or PP populations. In addition, there was a higher death rate in patients receiving EPO as compared with patients receiving placebo, particularly in those who were pretreated with thrombolysis. In the EPO arm, there was an increased risk of serious complications: death, intracerebral hemorrhage, brain edema, and thromboembolic events without any particular mechanism of death being unexpected in a comparable stroke population. The increased death rate in the rtPA population is still unexplained and may result from a combination of (as yet unknown) factors and/or potential rtPA–EPO interactions. In contrast, in the ITT non-rtPA population, the tendency toward a higher death rate in the EPO group might be explained by higher stroke severity of the dead patients on inclusion (before any study medication was applied). Notably, compared with previous prominent stroke trials including patients with comparable stroke severity, the ITT death rate under EPO of 16.4% lies in the expected range, whereas that under placebo (9.0%) is unexpectedly low.[15–17]

When the trial was started in 2003, it was nonpredictable that $>60\%$ of patients would receive thrombolysis. This high proportion may arise from (1) the close time window for rtPA and EPO in our protocol; (2) the restriction to MCA strokes (one domain of thrombolysis); and (3) the experienced recruiting centers, feeling urged by the medical community to conduct thrombolysis to avoid possible criticism of underuse

Table 3. Safety Analysis I: Treatment-Emergent Serious Adverse Events, Including Deaths

	ITT (N=522)		rtPA (n=331)		Non-rtPA (n=191)		PP (n=460)		rtPA (n=294)		Non-rtPA (n=166)	
Variable	EPO (n=256)	Placebo (n=266)	EPO (n=166)	Placebo (n=165)	EPO (n=90)	Placebo (n=101)	EPO (n=223)	Placebo (n=237)	EPO (n=147)	Placebo (n=147)	EPO (n=76)	Placebo (n=90)
Deaths, N (%)*	**42 (16.4)**	**24 (9.0)**	**27 (16.3)**	**14 (8.5)**	15 (16.7)	10 (9.9)	24 (10.8)	17 (7.2)	**18 (12.2)**	**8 (5.4)**	6 (7.9)	9 (10.0)
OR [CI]	1.98	[1.16–3.38]	2.10	[1.10–4.16]	1.82	[0.77–4.29]	1.56	[0.82–2.99]	2.42	[1.02–5.77]	0.77	[0.26–2.28]
Time point of death, N (%)*												
Day 1–7	20 (7.8)	9 (3.4)	14 (8.4)	8 (4.8)	6 (6.7)	1 (1.0)	6 (2.7)	2 (0.8)	6 (4.1)	2 (1.4)	0 (0.0)	0 (0.0)
Day 8–14	6 (2.3)	5 (1.9)	5 (3.0)	2 (1.2)	1 (1.1)	3 (3.0)	6 (2.7)	5 (2.1)	5 (3.4)	2 (1.4)	1 (1.3)	3 (3.3)
Day 15–30	8 (3.1)	5 (1.9)	3 (1.8)	2 (1.2)	5 (5.6)	3 (3.0)	6 (2.7)	5 (2.1)	3 (2.0)	2 (1.4)	3 (3.9)	3 (3.3)
>Day 30	8 (3.1)	5 (1.9)	5 (3.0)	2 (1.2)	3 (3.3)	3 (3.0)	6 (2.7)	5 (2.1)	4 (2.7)	2 (1.4)	2 (2.6)	3 (3.3)
Not applicable	214 (83.6)	242 (91.0)	139 (83.7)	151 (91.5)	75 (83.3)	91 (90.1)	199 (89.2)	220 (92.8)	129 (87.8)	139 (94.6)	70 (92.1)	81 (90.0)
Cause of death, N (%)*												
Intracerebral hemorrhage†	11 (4.3)	2 (0.8)	8 (4.8)	1 (0.6)	3 (3.3)	1 (1.0)	2 (0.9)	1 (0.4)	2 (1.4)	1 (0.7)	0 (0.0)	0 (0.0)
Asymptomatic	2 (0.8)	0 (0.0)	2 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Symptomatic	9 (3.5)	2 (0.8)	6 (3.6)	1 (0.6)	3 (3.3)	1 (1.0)	2 (0.9)	1 (0.4)	2 (1.4)	1 (0.7)	0 (0.0)	0 (0.0)
Brain edema	6 (2.3)	4 (1.5)	5 (3.0)	4 (2.4)	1 (1.1)	0 (0.0)	4 (1.8)	0 (0.0)	4 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)
Thromboembolic	6 (2.3)	4 (1.5)	5 (3.0)	3 (1.8)	1 (1.1)	1 (1.0)	5 (2.2)	4 (1.7)	4 (2.7)	3 (2.0)	1 (1.3)	1 (1.1)
Others	17 (6.6)	13 (4.9)	8 (4.8)	5 (3.0)	9 (10.0)	8 (7.9)	12 (5.4)	11 (4.6)	7 (4.8)	3 (2.0)	5 (6.6)	8 (8.9)
Unknown	2 (0.8)	1 (0.4)	1 (0.6)	1 (0.6)	1 (1.1)	0 (0.0)	1 (0.4)	1 (0.4)	1 (0.7)	1 (0.7)	0 (0.0)	0 (0.0)
Not applicable	214 (83.6)	242 (91.0)	139 (83.7)	151 (91.5)	75 (83.3)	91 (90.1)	199 (89.2)	220 (92.8)	129 (87.8)	139 (94.6)	70 (92.1)	81 (90.0)
Treatment-emergent SAE (including deaths),* N (%)												
Patients without SAE	176 (68.8)	197 (74.1)	111 (66.9)	119 (72.1)	65 (72.2)	78 (77.2)	165 (74.0)	182 (76.8)	103 (70.1)	110 (74.8)	62 (81.6)	72 (80.0)
Patients with 1 SAE	59 (23.0)	54 (20.3)	38 (22.9)	38 (23.0)	21 (23.3)	16 (15.8)	40 (17.9)	44 (18.6)	29 (19.7)	32 (21.8)	11 (14.5)	12 (13.3)
Patients with ≥2 SAE	21 (8.2)	15 (5.6)	17 (10.2)	8 (4.8)	4 (4.4)	7 (6.9)	18 (8.1)	11 (4.6)	15 (10.2)	5 (3.4)	3 (3.9)	6 (6.7)
OR [CI] for ≥1 SAE	1.30	[0.89–1.90]	1.28	[0.80–2.05]	1.30	[0.68–2.51]	1.16	[0.76–1.78]	1.27	[0.76–2.12]	0.90	[0.42–1.96]
Type of treatment-emergent SAE, N (%)§												
Intracerebral hemorrhage†	18 (7.0)	12 (4.5)	14 (8.4)	8 (4.8)	4 (4.4)	4 (4.0)	8 (3.6)	9 (3.8)	7 (4.8)	8 (5.4)	1 (1.3)	1 (1.1)
Asymptomatic	7 (2.7)	7 (2.6)	7 (4.2)	6 (3.6)	0 (0.0)	1 (1.0)	4 (1.8)	7 (3.0)	4 (2.7)	6 (4.1)	0 (0.0)	1 (1.1)
Symptomatic	11 (4.3)	5 (1.9)	7 (4.2)	2 (1.2)	4 (4.4)	3 (3.0)	4 (1.8)	2 (0.8)	3 (2.0)	2 (1.4)	1 (1.3)	0 (0.0)
Brain edema	20 (7.8)	14 (5.3)	15 (9.0)	10 (6.1)	5 (5.6)	4 (4.0)	15 (6.7)	8 (3.4)	12 (8.2)	5 (3.4)	3 (3.9)	3 (3.3)
Thromboembolic	29 (11.3)	18 (6.8)	25 (15.1)	13 (7.9)	4 (4.4)	5 (5.0)	27 (12.1)	16 (6.8)	24 (16.3)	11 (7.5)	3 (3.9)	5 (5.6)
Others	41 (16.0)	41 (15.4)	26 (15.7)	23 (13.9)	15 (16.7)	18 (17.8)	32 (14.3)	33 (13.9)	23 (15.6)	17 (11.6)	10 (13.2)	16 (17.8)
Unknown	2 (0.8)	1 (0.4)	1 (0.6)	1 (0.6)	1 (1.1)	0 (0.0)	1 (0.4)	1 (0.4)	1 (0.7)	1 (0.7)	0 (0.0)	0 (0.0)
Not applicable	176 (68.8)	197 (74.1)	111 (66.9)	119 (72.1)	65 (72.2)	78 (77.2)	165 (74.0)	182 (76.8)	103 (70.1)	110 (74.8)	62 (81.6)	72 (80.0)

Significant differences ($P<0.05$) are bold.

*χ^2 test, 2-tailed.

†Classification according to SITS-MOST protocol (asymptomatic intracerebral hemorrhages include HI1, HI2, PH1, asymptomatic PH2, PHr1, PHr2; symptomatic intracerebral hemorrhages are defined by the presence of PH2 with an increase of NIHSS by ≥4 points, or PH2 leading to death).

§Adds up to n>subgroup and >100% (some patients fulfill criteria for more than one treatment-emergent SAE).

"Thromboembolic" refers to thromboembolic events such as second stroke, myocardial infarction, splenic, and so on infarctions, 3-level thrombosis, large artery occlusion, lung embolism; "Others" refers to other SAE and adverse events such as aspiration pneumonia, epileptic seizures, invasive procedures, sepsis, lung edema, heart failure/decompensation (eg, atrial fibrillation), multiorgan failure, skin rash, fractures, severe anemia, pseudomembranous colitis.

For safety analyses II and III, see Supplemental Material.

SAE indicates serious adverse event.

of rtPA (eg, refs. 18 and 19). Due to the increasing application of rtPA, the total number of non-rtPA patients (n <200), the only actual comparator of the previous trial, is lower than that calculated to be necessary for reproducing the effect of EPO (n >500). Therefore, unplanned and unexpected, the study is "underpowered".

During the 5-year course of the trial, there was a constantly rising rate of rtPA treatments, reflecting approval of the medication for treatment of ischemic stroke in Germany and an increasingly more aggressive approach to treatment of patients with stroke worldwide in the absence of any other treatment options.[18–23] Whereas in the previous study[2] as well as in both interim looks of the present trial, no safety concerns of EPO treatment, even in the rtPA population, were discerned, this changed on final analysis. This raises the question as to whether the higher number of deaths in the EPO arm may be related to some potential interaction with rtPA, which was always given before EPO.

Although many therapeutic stroke trials have been undertaken in the past, no neuroprotective/neuroregenerative phar-

macological treatment has been identified to date.[1] With the NIHSS score difference from Day 1 to 90, a sensitive measure of neurological symptom development over time after stroke, the PP non-rtPA subpopulation may have had a small advantage of EPO treatment. This effect was observed only in this particular parameter (delta NIHSS) and, therefore, a result by chance cannot be excluded. However, despite the small number of non-rtPA patients in the present trial, EPO has alluded twice to a beneficial signal on outcome in patients with ischemic stroke who did not receive thrombolysis, overshadowed by an increased death rate in the second trial. Further support for a potentially beneficial signal in ischemia comes from a very recent Phase II trial reporting on reduction of delayed ischemic deficits after aneurysmal subarachnoid hemorrhage.[24] In this trial, using comparable doses of EPO, no safety concerns were identified.

The mechanism of action of EPO is different from the clot-dissolving strategy pursued by thrombolysis. It would, therefore, have been most attractive to see that the neuroprotective approach using EPO, aimed at salvaging potentially viable brain tissue from spreading of death signals, and thrombolysis, targeting reopening of the feeding artery, had provided additive beneficial outcome. However, the unexpected observation that a combination of EPO and rtPA is not advantageous, and may even be detrimental, poses at present a contraindication for acute EPO treatment in patients receiving rtPA.

In the PP rtPA population, there was a reduction in evolution of lesion from Day 1 to 7 in patients receiving EPO despite values comparable with patients receiving placebo on inclusion. This clearly did not translate into clinical readouts and was not observed in the PP non-rtPA population with their potential clinical benefit. Because this analysis was based only on patients who had both MRIs performed as planned and excluded patients who were unable to go to the scanner on Day 1 and/or Day 7, we conducted an additional analysis in which all missing MRI data were imputed by assigning to the respective patient the largest lesion size observed in all patients on the respective MRI day. This certainly "imperfect" statistical approach to remedying a potential lesion size ascertainment bias expectedly eliminated the difference in lesion size evolution between groups (data not shown). Nevertheless, 2 other explanations should be considered for the dissociation between imaging and clinical outcome: (1) evolution of lesion does not matter in the range observed, that is, the penumbra concept has to be revisited. Interestingly, weak correlations between lesion size and functional outcome are known (eg, ref. 25). Follow-up MRIs on Day 30 or 90 would have been desirable to substantiate these findings but were financially and logistically impossible to obtain; and (2) the apparent benefit gained by reduction in lesion size disappears on combining rtPA and EPO due to yet unclear interactions enhancing the functional damage. Further bench work is required to understand probable interactions of the 2 strategies. Such research may ultimately help elucidate mechanisms of increased death rates reported or suspected in association with EPO in conditions ranging from adult cancer over renal failure to preterm neonates.[26–31]

Although the causes of the observed increased death rate on EPO are far from clear, rtPA-treated patients should be excluded from acute poststroke EPO application. Further speculations on the increased mortality in the EPO arm comprise (1) an imbalance of stroke severity on inclusion in the dead patients; (2) an outcome by chance, considering the obvious undermortality in the placebo group; and (3) a negative influence of the escalating violations of thrombolysis contraindications, which amounted to 50% of the rtPA treatments. These assumptions are supported by the lack of safety concerns in the pilot study and in 2 interim looks of the present trial. In summary, strict adherence to comprehensive safety protocols is mandatory for any potential follow-up trial on EPO in acute ischemic stroke. Similar to the era of clinical introduction of rtPA with its well-recognized safety risks, careful risk–benefit considerations will have to precede any such trials.

Appendix

The EPO Stroke Trial Group

The following centers participated in the German Multicenter EPO Stroke Trial: Hannover,* Göttingen,† Bremen,‡ Celle,§ Erlangen,‖ Leipzig,¶ Dresden,** Essen,†† Braunschweig,‡‡ Berlin,§§ Aachen.‖‖

Enrolling investigators (in alphabetical order): B. Ahl,* U. Becker,** M. Bigalke,‡ M. Ebke,‡ M. Feldmann,‡ G. Gahn,** A. Goldbecker,* K. Gröschel,† Z. Gumienny,§ J. Hanssen,§ C. Hobohm,¶ U. Johansson,†† M. Köhrmann,‖ P. Marx,§§ D. Michalski,¶ G. Moldrich,† J. Noth,‖‖ T. Nowe,‖ J. Schaumberg,‡ S. Schnaudigel,† S. Schwarting,† B. Sommer,‡ J. Stewen,§ J. Thomsen,‡ A. Tountopoulou,* A. Tryc,* K. Wessel,‡‡ H. Worthmann,* and G. Wortmann.‡ Neuroradiologists: H. Becker,* M. Bester,† A. Dörfler,‖ F. Donnerstag,* M. Forsting,†† S. Jacob,† R. von Kummer,** H. Lanfermann,* T. Mitrovics,‡ H. J. Roth,¶ M. Schlamann,†† M. Stiefel,† U. Thiemann,‡ and B. F. Tomandl.‡ Study pharmacists: K. Linke and J. Müller. Coordinating study center (Division of Clinical Neuroscience, Max Planck Institute of Experimental Medicine, Göttingen): C. Aust, K. Hannke, C. Norra, S. Sperling, and N. Stender. Members of the scientific advisory and safety board: H. C. Diener, Essen (Neurology), O. Gefeller, Erlangen (Biostatistics), D. Neubert, Berlin (Pharmacology), and P. Rieckmann, Vancouver, Canada (Neurology).

Acknowledgments

The German Multicenter EPO Stroke Trial, an investigator initiated trial, has been supported by the Max Planck Society and by a research grant from Johnson & Johnson (J&J)–Ortho Biotech. J&J was not involved in designing and conducting the trial, in analyzing and interpreting the data, or in preparation of the manuscript and in the decision to publish. We express our gratitude to the members of the independent scientific advisory and safety board: H. C. Diener, Essen; O. Gefeller, Erlangen; D. Neubert, Berlin; and P. Rieckmann, Vancouver, Canada. We thank all patients and relatives for study participation. Furthermore, we are indebted to all contributing study nurses (I. Gerhardt and D. Urban, Leipzig; D. Stangenberg, Celle; K. Fleischer, Dresden; A. Schickert-Schleicher and A. Schmidt, Erlangen; and M. Dietzold, Essen) for their excellent and friendly assistance.

Disclosures

H.E. holds a patent on the use of EPO for treatment of cerebral ischemia.

References

1. O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1026 experimental treatments in acute stroke. *Ann Neurol*. 2006;59:467–477.
2. Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, Rustenbeck H-H, Breiter N, Jacob S, Knerlich F, Bohn M, Poser W, Rüther E, Kochen M, Gefeller O, Gleiter C, Wessel TC, De Ryck M, Itri L, Prange H, Cerami A, Brines M, Sirén A-L. Erythropoietin therapy for acute stroke is both safe and beneficial. *Molecular Medicine*. 2002;8: 495–505.
3. Brines M, Cerami A. Emerging biological roles for erythropoietin in the nervous system. *Nat Rev Neurosci*. 2005;6:484–494.
4. Juul S. Recombinant erythropoietin as a neuroprotective treatment: in vitro and in vivo models. *Clin Perinatol*. 2004;31:129–142.
5. Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C, Itri LM, Cerami A. Erythropoietin crosses the blood–brain barrier to protect against experimental brain injury. *Proc Natl Acad Sci U S A*. 2000;97:10526–10531.
6. Ehrenreich H, Degner D, Meller J, Brines M, Béhé M, Hasselblatt M, Woldt H, Falkai P, Knerlich F, Jacob S, Von Ahsen N, Maier W, Brück W, Rüther E, Cerami A, Becker W, Sirén A-L. Erythropoietin. A candidate compound for neuroprotection in schizophrenia. *Molecular Psychiatry*. 2004;9:42–54.
7. Banks WA, Jumbe NL, Farrell CL, Niehoff ML, Heatherington AC. Passage of erythropoietic agents across the blood–brain barrier: a comparison of human and murine erythropoietin and the analog darbepoetin alfa. *Eur J Pharmacol*. 2004;505:93–101.
8. Weimar C, Kraywinkel K, Maschke M, Diener HC. Intravenous thrombolysis in German stroke units before and after regulatory approval of recombinant tissue plasminogen activator. *Cerebrovasc Dis*. 2006;22: 429–431.
9. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics*. 1979;35:549–556.
10. Lan KK, Zucker DM. Sequential monitoring of clinical trials: the role of information and brownian motion. *Stat Med*. 1993;12:753–765.
11. Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, Spilker J, Holleran R, Eberle R, Hertzberg V, et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke*. 1989;20: 864–870.
12. Mahoney FI, Barthel DW. Functional evaluation: the Barthel Index. *Md State Med J*. 1965;14:61–65.
13. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke*. 1988;19:604–607.
14. Anonymous. SITS-MOST (EMEA) study protocol. Available at: www.Acutestroke.Org/index.Php?Module=contentexpress&func=display&ceid=29&meid=6. 2002. Accessed August 25th, 2009.
15. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. 1995;333:1581–1587.
16. Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, Diener HC, Ashwood T, Wasiewski WW, Emeribe U. Nxy-059 for the treatment of acute ischemic stroke. *N Engl J Med*. 2007;357:562–571.
17. Diener HC, Schneider D, Lampl Y, Bornstein NM, Kozak A, Rosenberg G. Dp-b99, a membrane-activated metal ion chelator, as neuroprotective therapy in ischemic stroke. *Stroke*. 2008;39:1774–1778.
18. Kaste M. Thrombolysis: what more does it take? *Stroke*. 2005;36: 200–202.
19. Barber PA, Zhang J, Demchuk AM, Hill MD, Buchan AM. Why are stroke patients excluded from tPA therapy? An analysis of patient eligibility. *Neurology*. 2001;56:1015–1020.
20. Bravata DM. Intravenous thrombolysis in acute ischaemic stroke: optimising its use in routine clinical practice. *CNS Drugs*. 2005;19:295–302.
21. Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, Larrue V, Lees KR, Medeghri Z, Machnig T, Schneider D, von Kummer R, Wahlgren N, Toni D. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med*. 2008;359:1317–1329.
22. Gomez-Choco M, Obach V, Urra X, Amaro S, Cervera A, Vargas M, Chamorro A. The response to IV rt-PA in very old stroke patients. *Eur J Neurol*. 2008;15:253–256.
23. Schmülling S, Rudolf J, Strotmann-Tack T, Grond M, Schneweis S, Sobesky J, Thiel A, Heiss WD. Acetylsalicylic acid pretreatment, concomitant heparin therapy and the risk of early intracranial hemorrhage following systemic thrombolysis for acute ischemic stroke. *Cerebrovasc Dis*. 2003;16:183–190.
24. Tseng MY, Hutchinson PJ, Richards HK, Czosnyka M, Pickard JD, Erber WN, Brown S, Kirkpatrick PJ. Acute systemic erythropoietin therapy to reduce delayed ischemic deficits following aneurysmal subarachnoid hemorrhage: a phase II randomized, double-blind, placebo-controlled trial. *J Neurosurg*. 2009;111:171–180.
25. Johnston KC, Wagner DP, Wang XQ, Newman GC, Thijs V, Sen S, Warach S. Validation of an acute ischemic stroke model: does diffusion-weighted imaging lesion volume offer a clinically significant improvement in prediction of outcome? *Stroke*. 2007;38:1820–1825.
26. Fauchère JC, Dame C, Vonthein R, Koller B, Arri S, Wolf M, Bucher HU. An approach to using recombinant erythropoietin for neuroprotection in very preterm infants. *Pediatrics*. 2008;122:375–382.
27. Singh AK, Szczech L, Tang KL, Barnhart H, Sapp S, Wolfson M, Reddan D. Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med*. 2006;355:2085–2098.
28. Bennett CL, Silver SM, Djulbegovic B, Samaras AT, Blau CA, Gleason KJ, Barnato SE, Elverman KM, Courtney DM, McKoy JM, Edwards BJ, Tigue CC, Raisch DW, Yarnold PR, Dorr DA, Kuzel TM, Tallman MS, Trifilio SM, West DP, Lai SY, Henke M. Venous thromboembolism and mortality associated with recombinant erythropoietin and darbepoetin administration for the treatment of cancer-associated anemia. *JAMA*. 2008;299:914–924.
29. Leyland-Jones B, Semiglazov V, Pawlicki M, Pienkowski T, Tjulandin S, Manikhas G, Makhson A, Roth A, Dodwell D, Baselga J, Biakhov M, Valuckas K, Voznyi E, Liu X, Vercammen E. Maintaining normal hemoglobin levels with epoetin alfa in mainly nonanemic patients with metastatic breast cancer receiving first-line chemotherapy: a survival study. *J Clin Oncol*. 2005;23:5960–5972.
30. Wright JR, Ung YC, Julian JA, Pritchard KI, Whelan TJ, Smith C, Szechtman B, Roa W, Mulroy L, Rudinskas L, Gagnon B, Okawara GS, Levine MN. Randomized, double-blind, placebo-controlled trial of erythropoietin in non-small-cell lung cancer with disease-related anemia. *J Clin Oncol*. 2007;25:1027–1032.
31. Dronca RS, Steensma DP. VTE and mortality associated with erythropoiesis-stimulating agents in cancer-associated anemia. *Nat Clin Pract Oncol*. 2008;5:504–505.