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Design and rationale of the Reduction of Infarct Expansion and Ventricular Remodeling with Erythropoietin After Large Myocardial Infarction (REVEAL) trial

Chiara Melloni, MD, MHS^{a,b}, Sunil V. Rao, MD^{a,b,c}, Thomas J. Povsic, MD^{a,b}, Laura Melton, PhD^b, Raymond J. Kim, MD^{a,b}, Rakhi Kilaru, MS^b, Manesh Patel, MD^{a,b}, Mark Talan, MD, PhD^d, Luigi Ferrucci, MD, PhD^d, Dan L. Longo, MD^d, Edward G. Lakatta, MD^d, Samer S. Najjar, MD^{d,e}, and Robert A. Harrington, MD^{a,b}

^aDivision of Cardiology, Department of Medicine, Duke University School of Medicine, Durham, NC, USA

^bDuke Clinical Research Institute, Duke University Medical Center, Durham, NC, USA

^cDurham Veterans Affairs Medical Center, Durham, NC, USA

^dIntramural Research Program, National Institute on Aging, the National Institutes of Health, Baltimore, MD, USA

^eMedStar Health Research Institute, Washington, DC, USA

Abstract

Background—Acute myocardial infarction (MI) remains a leading cause of death despite advances in pharmacologic and percutaneous therapies. Animal models of ischemia/reperfusion have demonstrated that single-dose erythropoietin (EPO) may reduce infarct size, decrease apoptosis, and increase neovascularization, possibly through mobilization of endothelial progenitor cells (EPCs).

Study Design—REVEAL is a randomized, double-blind, placebo-controlled, multicenter trial evaluating the effects of epoetin alfa on infarct size and left ventricular (LV) remodeling in patients with large MIs. The trial comprises a dose-escalation safety phase and a single-dose efficacy phase using the highest acceptable epoetin alfa dose up to 60,000 units. Up to 250 STEMI patients undergoing primary or rescue percutaneous coronary intervention (PCI) will be randomized to intravenous epoetin alfa or placebo within 4 hours of successful reperfusion. The primary study endpoint is infarct size expressed as a percentage of LV mass, as measured by cardiac magnetic resonance imaging 2–6 days post study medication administration. Secondary endpoints will assess changes in EPC numbers and changes in indices of ventricular remodeling.

Conclusion—The REVEAL trial will evaluate the safety and efficacy of the highest tolerated single dose of epoetin alfa in patients who have undergone successful rescue or primary PCI for acute STEMI.

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Correspondence to: Chiara Melloni, MD, MHS, Department of Medicine–Cardiology, Duke University SOM, DUMC Box 3850, Durham, NC 27710, chiara.melloni@duke.edu.

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Despite advances in pharmacologic and percutaneous therapies for ST-segment elevation myocardial infarction (STEMI) over the past 2 decades, acute myocardial infarction (AMI) remains a leading cause of death and disability in the industrialized world.^{1,2} Patients surviving moderate to large AMIs may develop infarct expansion, left ventricular (LV) dilation, and heart failure.³ Major risk factors for this remodeling include size and location of the index infarction and the extent to which it involves the full thickness of myocardium; recurrence of ischemic insults; hemodynamic stresses on the myocardium; and apoptosis.^{4,5} Novel strategies to limit infarct size have been examined, including targeting specific pathways such as the complement system that may be involved in remodeling, but none have reduced infarct size or improved survival.^{6–8}

Endogenous repair mechanisms mediated through endothelial progenitor cells (EPCs) may attenuate remodeling. Animal models of ischemia/reperfusion indicate that erythropoietin (EPO) facilitates infarct size reduction and improvement in LV function, perhaps mediated via reduced apoptosis, increased neovascularization, mobilization of EPCs, and angiogenesis.^{9–16} Preliminary studies have shown promise;^{17,18} however, data from recent trials have raised safety concerns and suggest that potential benefits may be mitigated by prothrombotic effects, hematocrit elevation, and hypertension.^{17–22}

The Reduction of Infarct Expansion and Ventricular Remodeling with Erythropoietin After Large Myocardial Infarction (REVEAL) trial explores the safety and efficacy of intravenous (IV) recombinant human erythropoietin (epoetin alfa [Epogen; Procrit]) at the highest tolerated dose administered after primary or rescue percutaneous coronary intervention (PCI) for STEMI. REVEAL will also collect and analyze EPCs to explore mechanisms underlying potential clinical effects of EPO.

Methods

Study objectives

This phase II, randomized, double-blind, placebo-controlled study is designed to determine the safety and efficacy of IV epoetin alfa in reducing infarct size among STEMI patients. Because data regarding the safety of IV epoetin alfa are limited in this population, the study comprises two phases: (i) a safety evaluation of escalating doses of epoetin alfa in patients with first STEMI; and (ii) a single-dose efficacy phase comparing effects of the highest acceptable dose (up to 60,000 units) of epoetin alfa versus matching placebo on infarct size among STEMI patients undergoing primary/rescue PCI.

This study is supported by the Intramural Research Program of the National Institute on Aging, the National Institutes of Health, Bethesda, MD, USA. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper, and its final contents.

Endpoints

The primary endpoint is infarct size expressed as percentage of LV mass in the territory of the infarct-related artery (IRA), measured by delayed-enhancement cardiac magnetic resonance imaging (DE-CMR) at 2–6 days post study medication administration. Secondary endpoints are (i) change in numbers of circulating EPCs, measured up to 4 h after successful revascularization but before study drug administration and again at 24 and 48 h following study drug administration; and (ii) left ventricular ejection fraction (LVEF), LV volumes, and infarct size as determined by follow-up CMR 12 ± 2 weeks post PCI.

Study design

Figure 1 illustrates both phases of REVEAL. The final study population will include 210–250 subjects enrolled at 22 U.S. sites. Enrollment will continue until 110 patients have completed the primary efficacy assessment (CMR at 2–6 days post study drug administration).

Given uncertainty regarding an appropriate dose of epoetin alfa that will confer cardioprotective effects while limiting risk of thrombotic complications in STEMI patients, a dose-escalating design will be employed. During the dose-escalation phase, patients will be randomly allocated in a 2:1 ratio to either 15,000 units of IV epoetin alfa or placebo until 30 patients have received study medication. If the independent data and safety monitoring board (DSMB) concludes that a clinically unacceptable number of events did not occur at this dose, this dose-escalation/evaluation scheme will be repeated for doses of 30,000 and 60,000 units. The DSMB will subsequently assess the safety of the 60,000-unit cohort for enrollment in the efficacy phase (Appendix 1).

In the efficacy phase, participants will be randomly allocated in a 1:1 ratio to receive the highest dose of epoetin alfa deemed acceptable (15,000, 30,000, or 60,000 units) or placebo. Subjects from the dose-escalation phase randomized to the final efficacy dose or placebo will be included in the overall efficacy analysis.

Other treatments will be at the discretion of the treating physician, but concomitant medical therapy consistent with the current standard of STEMI care will be encouraged.¹

Eligibility criteria

Table 1 details REVEAL eligibility criteria. Subjects are eligible if they present with acute STEMI due to total occlusion (TIMI flow grade 0–1) of a major epicardial coronary artery or large branch vessel and undergo successful (<50% residual lesion or TIMI 2–3) primary or rescue angioplasty within 8 h of onset of ischemic symptoms. Subjects with a history of LV dysfunction (LVEF <50%), MI, CABG, or PCI in the IRA will be excluded due to potential confounding of infarct size measurement. Due to EPO's potential to increase blood viscosity with subsequent risk of thrombosis, patients with hematocrit above the institutional upper limit of normal at time of study drug administration will not be eligible.

Randomization

The study protocol conforms to International Conference on Harmonization/Good Clinical Practice standards. Participating sites will submit the protocol for approval by the local institutional review board. Subjects will be randomized using the allocation ratio for each dosing cohort and stratified by age (<70, ≥70 y) and infarct location (left anterior descending (LAD) vs. non-LAD), but not by site. The block randomization scheme will use varying block sizes for safety and efficacy phases.

Study drug administration

Epoetin alfa, formulated as a sterile, colorless liquid, will be supplied in single-dose vials identical to placebo. Study medication will be packaged in numbered kits and administered over approximately 10 minutes as a single IV infusion within 4 hours of successful revascularization. Centocor Ortho Biotech provided erythropoietin 20,000 units/mL in 1 mL multidose vials (NDC# 59676-0320-04) and matching placebo free of charge during the initial phase of the study. During the efficacy phase, Centocor Ortho Biotech decided to stop providing study drug and matching placebo. At this point, the NIA (the study sponsor) purchased study medication from Centocor Ortho Biotech through the NIH Clinical Center Pharmacy Department and contracted Florida Biologix (Alachua, FL) to manufacture

matching placebo 1 mL sterile saline vials. Neither Centocor Ortho Biotech nor Florida Biologix provided funding for, or had input into the design or the conduct of, the REVEAL trial.

CMR imaging

CMR scans will be performed at 2–6 days and 12 ± 2 weeks post study medication administration. Each examination will use cine-CMR for ventricular volumes and function, and DE-CMR for infarct size assessment and evaluation of microvascular obstruction.^{23,24} Images will be analyzed in blinded fashion in a central core laboratory (Duke Cardiovascular Magnetic Resonance Center, Durham, NC) (Appendix 2). Figure 2 outlines parameters to be measured on CMR techniques, provides an analysis schematic, and summarizes CMR endpoints.

EPC collection and analysis

Blood specimens will be collected using 8 mL cell preparation tubes (Becton-Dickinson) at baseline immediately before and at 24 (± 12 h) and 48 h (± 12 h) after study drug administration and processed by local site personnel.²⁵ The mononuclear cell fraction will be isolated using density gradient centrifugation according to manufacturer's instructions. Plasma will be removed and stored at -80°C and the cell layer transferred to a 50 mL conical tube, where cells will be washed with phosphate-buffered saline. Mononuclear cells will be suspended in a 10% DMSO supplied solution, cryopreserved at -80°C , and shipped on dry ice to the Duke EPC core lab. Samples will be thawed, cells washed and recovered, and EPCs enumerated based on expression of cell surface markers (CD34, CD133, and vascular endothelial growth factor receptor-2) as well as on levels of aldehyde dehydrogenase, a marker of multiple progenitor cell subtypes.²⁶ Plasma samples will form a biorepository for subsequent analysis. Because interpatient EPC levels vary considerably, primary EPC analysis will focus on changes in EPC counts over the course of the study.

Study assessments

Concomitant medications, clinical events, procedures, and blood samples will be collected at initial hospitalization, at 7, 14, and 30 days, and at 12 weeks (Figure 3).

Adverse events and additional safety assessments

For subjects receiving study medication, serious adverse events (SAEs) will be collected from randomization to 12 ± 2 weeks post randomization. For randomized subjects who do not receive study medication, SAEs will be recorded from randomization through 7 days after randomization. Nonserious adverse events (AEs) will be collected from randomization to 7 days after study medication administration.

Safety assessments will also include changes in hemoglobin levels during the first week and at 14 ± 4 days following study medication administration, and there will be careful evaluation for possible complications of IV epoetin alfa with regard to death or thrombotic events (Appendix 3).

Sample size calculation

The primary outcome of REVEAL is CMR-delineated infarct size (expressed as percentage of LV mass) in the territory of the IRA as measured at 2–6 days after study medication administration in subjects randomized to the highest acceptable dose of epoetin alfa or placebo.

The sample size for the first three cohorts in the dose-escalation phase is fixed, with 30 treated subjects at each dose level. Statistical power for the primary comparison is based on subjects in the highest acceptable dose group among these three cohorts, in a stratified analysis with subsequent enrollment into that group. The stratification will adjust for implicit differences that may exist between patients recruited in the dose-escalation and single-dose efficacy phases. Data regarding infarct size from a number of unpublished sources reveals consistently non-normal distribution; therefore, conventional methods of sample-size estimation were rejected in favor of an empirical approach based on available data carefully matched to the conditions of the present study.²⁷

We determined that a sample size of 55 subjects per arm plus the safety cohort subjects (20 active, 10 placebo) will provide adequate power to detect a difference of $\geq 20\%$ in infarct size between patients receiving placebo and those receiving the highest acceptable dose of epoetin alfa.

Data analysis

Data will be analyzed on a modified intent-to-treat basis: all subjects who receive study medication will be included, regardless of treatment assignment or protocol compliance. If problems occur with incorrect treatment assignments, an as-treated analysis may also be performed. Baseline differences for continuous variables will be evaluated using the *t* test or Wilcoxon rank-sum test; the Fisher exact mid-*p* test will be used for categorical variables. Treatment effects will be evaluated based on a two-sided significance level of 0.05, unless otherwise stated.

The primary efficacy comparison will be tested by means of a log-rank test. Secondary analyses investigating the hypotheses that (i) IV epoetin alfa alters the change in number of circulating EPCs from baseline and (ii) IV epoetin alfa will reduce infarct size and improve measures of LV function and remodeling at 12 ± 2 weeks after study medication administration will be performed. Infarct size at follow-up MRI (12 ± 2 weeks after study medication administration) will be evaluated using ANCOVA, with infarct size at 2–6 days as a covariate. Similarly, secondary efficacy parameters of systolic and diastolic LV volumes and LVEF (%), measured at 12 ± 2 weeks after study medication administration, will be evaluated using ANCOVA, with baseline measures as covariates.

Primary and secondary analyses will also be analyzed according to IRA (LAD vs. non-LAD) and patient age (<70 or ≥ 70 years).

There will be one formal interim test of efficacy after 20 subjects have been randomly assigned to each treatment group in the single-dose efficacy phase and have completed the first CMR. A Haybittle-Peto rule will be used for the interim test, which will be made using an alpha level of 0.001. Due to rounding, this will result in no penalty for the final comparison alpha at 0.05 and an overall alpha level of 0.05 will be maintained.

Discussion

The REVEAL study will evaluate the efficacy of a single IV dose of epoetin alfa, administered within 4 hours following successful reperfusion of an occluded coronary artery in STEMI patients, in limiting infarct size.

Hematopoietic and nonhematopoietic effects of erythropoietin

Erythropoietin is a 165 amino-acid glycoprotein whose production and secretion by renal peritubular cells and other tissues are regulated by tissue oxygen supply.²⁸ EPO is the main regulator of erythroid production, but the discovery of new sites of EPO production and

EPO receptors in different tissues and cells such as cardiomyocytes, cardiac fibroblasts, endothelial cells, and vascular smooth muscle cells suggests that EPO could also have pleiotropic effects.^{29,30}

In fact, EPO is now recognized as having antiapoptotic and antiinflammatory effects, as well as promoting neovascularization, mobilization of EPCs from bone marrow,³¹ and differentiation of EPCs into functionally active cells,^{31,32} thus amplifying angiogenesis.^{13,33} EPO enhances neovascularization by exerting mitogenic and chemotactic effects on endothelial cells and inducing them to migrate and proliferate to form new blood vessels.³⁴ In addition, EPO has been found to regulate production of neural progenitor cells from neural stem cells, both in cell cultures and in situ in the adult murine central nervous system.^{35,36}

As EPO's mechanisms of action have been elucidated and its antiapoptotic and angiogenic effects increasingly recognized, interest in its potential as a cytoprotective agent in both nervous and cardiovascular systems has grown.³⁷ In animal models of ischemic brain injuries, EPO has been shown to protect against ischemic and free radical-induced neuronal injury, prevent cellular inflammation, and improve neurologic recovery.^{38,39} In myocardial ischemia-reperfusion animal models, EPO administration decreased the number of apoptotic cells, reduced infarct size, and improved postischemic recovery.^{9–11,40} Increased neovascularization by EPCs also attenuates apoptosis, reduces collagen deposition and scar formation, and decreases the extent of LV remodeling.⁴¹

Although these beneficial effects were initially observed regardless of whether EPO was administered prior to^{10,42} or during ischemic injury,^{11,12,40} or at time of reperfusion,^{11,24} more recent studies indicate a therapeutic window beyond which the tissue-protective effects of EPO are attenuated or absent. Lipšic et al. demonstrated in a rodent model that reduction in apoptosis was more pronounced when EPO was administered at onset of ischemia (29% reduction) or after onset of reperfusion (38% reduction), as compared with pretreatment 2 hours before onset of ischemia (16% reduction).⁴³ Additionally, rodent studies by Moon et al. demonstrated that cytoprotective benefits were no longer observed if EPO was administered >8 hours after ischemic insult.⁴⁴ (This finding was however observed using a model of permanent coronary ligation—a different therapeutic window may apply to an ischemia/reperfusion model.) Because these observations suggest that timing of EPO delivery may be an important determinant of efficacy, we have mandated that epoetin alfa be administered within 4 hours of successful reperfusion in REVEAL.

Erythropoietin safety concerns

At the time REVEAL was conceived, clinical data on the safety and cytoprotective effects of IV EPO were limited. A study in patients with acute ischemic stroke demonstrated that administering EPO within 5 hours of symptom onset was safe and resulted in improved functional outcomes at 1 month; a trend toward a reduction in brain infarct size was also observed.¹⁸ In clinical studies of patients with heart failure, administration of EPO for correction of anemia often resulted in improved exercise capacity and LVEF,^{17,45} results confirmed in later studies.^{21,46} A pilot study testing a single high dose of darbepoetin alfa (~60,000 IU of epoetin alfa) demonstrated that darbepoetin can be safely administered in AMI patients.⁴⁷ A small single-center study demonstrated that short-term high-dose EPO (33×10^3 IU/3 consecutive days) in STEMI patients undergoing primary PCI was safe and had antiapoptotic, angiogenic, and anti-inflammatory effects with the potential to reduce infarct size.⁴⁸

However, more recent studies with conflicting efficacy results have raised safety concerns. In one study of STEMI patients treated with lytic therapy, a single dose of EPO was

demonstrated to be safe but did not favorably modify infarct size.⁴⁹ A pilot study evaluating the effect of 40,000 IU of EPO on myocardial damage in NSTEMI patients found no difference in infarct size between EPO-treated and non-EPO-treated patients and an increase in systolic blood pressure was noted.¹⁹ The more recent REVIVAL trial in STEMI patients undergoing primary PCI failed to demonstrate that EPO improves LVEF or reduces infarct size at 6 months as measured by CMR, and a trend toward more adverse clinical events was observed in treated patients.²² Other studies have shown that long-term use of EPO and other erythropoiesis-stimulating agents in patients with chronic kidney disease or cancer may increase risk of death, serious cardiovascular events, and tumor growth (in certain types of cancer) when given at doses beyond those necessary to correct anemia.³⁰⁻³⁴⁻⁵⁰ A recent study in patients with ischemic stroke suggests that EPO may not improve functional status; also, because the death rate was higher in the EPO group compared with placebo (particularly among patients receiving systemic thrombolysis), there may be important safety concerns as well.²⁰ In the recently reported HEBE III⁵¹ study in which 529 STEMI patients with successful PCI were randomized to either 60,000 units of IV epoetin alfa or placebo, EPO administration did not improve LVEF at 6 weeks but was associated with fewer major cardiovascular adverse events—a benefit driven primarily by reductions in heart failure. However, as Voors et al. note, these clinical outcomes warrant cautious interpretation, as the trial was not powered for clinical events.⁵¹

Responding to these data, the FDA in 2008 issued a warning and required a labeling change for EPO.⁵² REVEAL continued to enroll patients under the trial's robust safety plan and the DSMB watched carefully for potential safety signals. We note that in addition to the recently concluded HEBE III trial,⁵¹ there is another ongoing clinical trial evaluating the safety and efficacy of EPO in STEMI patients.³⁶

Conclusion

Experimental studies in cardiac acute infarction models have demonstrated a protective effect of EPO, but clinical studies report conflicting results. The REVEAL trial has a unique design consisting of a dose-escalation phase to assess safety, followed by an efficacy phase to determine whether tissue-protective effects established in animal models translate to the clinical arena. The use of CMR will allow efficacy determination in a reasonable patient cohort. The results of REVEAL will set the stage for a larger trial to evaluate the safety and efficacy of EPO in STEMI.

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Figure 1. Study flow

Epoetin alfa doses of 15,000, 30,000, and 60,000 units are planned for evaluation in the dose-escalation safety phase. Initiation of increasing doses is contingent on review of safety data by the DSMB. The single-dose efficacy phase will involve the highest dose deemed acceptable by the DSMB.

Figure 2. CMR evaluation

(A) Outline of CMR techniques, timing, and physiological parameters. (B) Example of a cine-CMR and DE-CMR short-axis image with planimetry for LVEF, LV volumes, and infarct size. Areas of microvascular obstruction will also be noted (green arrow). (C) Primary, secondary, and tertiary CMR endpoints are listed.

Assessment	Visit	Time	Assessments									
			Screening	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Screening	1	0	X									
Baseline	2	1		X								
Week 1	3	2			X							
Week 2	4	3				X						
Week 3	5	4					X					
Week 4	6	5						X				
Week 5	7	6							X			
Week 6	8	7								X		
Week 7	9	8									X	
Week 8	10	9										X
End of Study	11	10										

Figure 3. Study schedule

Table I**Eligibility criteria**

Inclusion criteria	
1	Age >21 y
2	Acute STEMI or new LBBB due to occlusion of major epicardial coronary artery or large branch vessel
3	Referral for primary/rescue angioplasty
4	Revascularization procedure within 8 h of onset of ischemic symptoms
5	TIMI 0–1 in culprit artery at beginning of coronary angiography
6	Successful IRA revascularization (<50% residual lesion or TIMI 2–3)
Exclusion criteria	
1	History of LVEF <50%, MI, or CABG
2	Prior PCI in IRA
3	Uncontrolled hypertension (SBP >180 mm Hg or DBP >110 mm Hg) at time of study drug administration
4	Cardiogenic shock defined as: <ol style="list-style-type: none"> Hypotension (SBP <100) despite use of vasopressors, or Evidence of tissue hypoperfusion (e.g., cold extremities and diminished urine output despite vasopressors and/or intra-aortic balloon pump)
5	Need for coronary surgical revascularization at time of index catheterization
6	Clinical indication for erythropoietin
7	Hypersensitivity to human albumin, mammalian cell-derived products, or erythropoietin Hematocrit >institutional upper limit of normal at time of study drug administration History of hypercoagulable disorder, thromboembolic event, or venous thrombosis History of stroke/transient ischemic attack History of seizures Contraindication to MRI (e.g., pacemaker, AICD, aneurysm clips, inability to lie flat for 45–60 min, inability to hold breath for 15 s) Contraindication to gadolinium contrast, such as eGFR <30 mL/min/1.73 m ² Pregnant/nursing AICD, automatic implantable cardioverter/defibrillator; CABG, coronary artery bypass graft; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; IRA, infarct-related artery; LBBB, left bundle branch block; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention; MRI, magnetic resonance imaging; SBP, systolic blood pressure; STEMI, ST-segment elevation myocardial infarction; TIMI, Thrombolysis in Myocardial Infarction