

Human Polymerized Hemoglobin for the Treatment of Hemorrhagic Shock when Blood Is Unavailable: The USA Multicenter Trial

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- BACKGROUND:** Human polymerized hemoglobin (PolyHeme, Northfield Laboratories) is a universally compatible oxygen carrier developed to treat life-threatening anemia. This multicenter phase III trial was the first US study to assess survival of patients resuscitated with a hemoglobin-based oxygen carrier starting at the scene of injury.
- STUDY DESIGN:** Injured patients with a systolic blood pressure ≤ 90 mmHg were randomized to receive field resuscitation with PolyHeme or crystalloid. Study patients continued to receive up to 6 U of PolyHeme during the first 12 hours postinjury before receiving blood. Control patients received blood on arrival in the trauma center. This trial was conducted as a dual superiority/noninferiority primary end point.
- RESULTS:** Seven hundred fourteen patients were enrolled at 29 urban Level I trauma centers (79% men; mean age 37.1 years). Injury mechanism was blunt trauma in 48%, and median transport time was 26 minutes. There was no significant difference between day 30 mortality in the as-randomized (13.4% PolyHeme versus 9.6% control) or per-protocol (11.1% PolyHeme versus 9.3% control) cohorts. Allogeneic blood use was lower in the PolyHeme group (68% versus 50% in the first 12 hours). The incidence of multiple organ failure was similar (7.4% PolyHeme versus 5.5% control). Adverse events (93% versus 88%; $p = 0.04$) and serious adverse events (40% versus 35%; $p = 0.12$), as anticipated, were frequent in the PolyHeme and control groups, respectively. Although myocardial infarction was reported by the investigators more frequently in the PolyHeme group (3% PolyHeme versus 1% control), a blinded committee of experts reviewed records of all enrolled patients and found no discernable difference between groups.
- CONCLUSIONS:** Patients resuscitated with PolyHeme, without stored blood for up to 6 U in 12 hours postinjury, had outcomes comparable with those for the standard of care. Although there were more adverse events in the PolyHeme group, the benefit-to-risk ratio of PolyHeme is favorable when blood is needed but not available. (*J Am Coll Surg* 2009;208:1–13. © 2008 by the American College of Surgeons)

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A critical unmet need exists for a universally compatible, oxygen (O_2)-carrying fluid when blood is unavailable.^{1,2} Natural disasters and threat of terrorism heighten the urgency. There are 47,000,000 Americans who live more than 1 hour from a trauma center,³ and most ambulances do not carry blood. Trauma patients with prehospital shock

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Abbreviations and Acronyms

AE	=	adverse event
AIS	=	Abbreviated Injury Score
Hb	=	hemoglobin
HBOC	=	hemoglobin-based oxygen carrier
IDMC	=	independent data safety monitoring committee
MOF	=	multiple organ failure
SAE	=	serious adverse event
SBP	=	systolic blood pressure

not receiving blood until arrival at the hospital have mortality rates of 17% to 54%,⁴⁻⁷ and mortality increases with time and distance to definitive care. Rural settings account for only 20% of the population but 60% of trauma deaths.⁸ In addition, stored red blood cells (RBCs) are sometimes incompatible for in-hospital use because of inadequate inventory or immunologic states such as autoimmune hemolytic anemia, and certain religious groups will not accept RBC transfusion.⁹ Finally, allogeneic RBC transfusions may provoke adverse immunoinflammatory responses in high-risk patients.^{10,11}

PolyHeme (hemoglobin glutamer-256 [human]; polymerized hemoglobin, pyridoxylated; Northfield Laboratories), is a hemoglobin-based O₂ carrier (HBOC) derived from human blood,¹²⁻²⁰ which has been demonstrated to have life-sustaining capability^{1,9,21-26} and attenuate the postinjury immunoinflammatory response invoked in the pathogenesis of multiple organ failure (MOF).^{1,27-30} PolyHeme was designed to avoid the vasoconstriction issues seen with earlier tetrameric hemoglobin preparations.^{5,31-33} These vasoactive tetramer properties were presumed to be from transendothelial extravasation of the small molecular weight tetramer, leading to abluminal binding of nitric oxide (NO) and causing unopposed vasoconstriction.³⁴⁻³⁶ So Northfield developed a virtually tetramer-free, polymerized hemoglobin to yield substantially larger molecules that would not readily extravasate.¹²⁻²⁰

Earlier clinical experience in hospitalized patients^{1,9,21-26} showed that PolyHeme can provide oxygen-carrying capacity at otherwise life-threatening hemoglobin (Hb) levels. Patients have survived with RBC Hb below 1g/dL while receiving up to 20 U (10 L, 1,000 g) of PolyHeme.⁹ These observations document the ability of PolyHeme to maintain critical oxygen transport during ongoing blood loss at life-threatening Hb levels and, collectively, provided the basis for initiation of this trial. The objective of this study was to assess survival of patients in hemorrhagic shock with treatment initiated at the scene comparing PolyHeme with standard of care (crystalloid in the field followed by stored RBCs at hospital arrival). The protocol was based on two potential survival benefits: early replacement of oxygen-

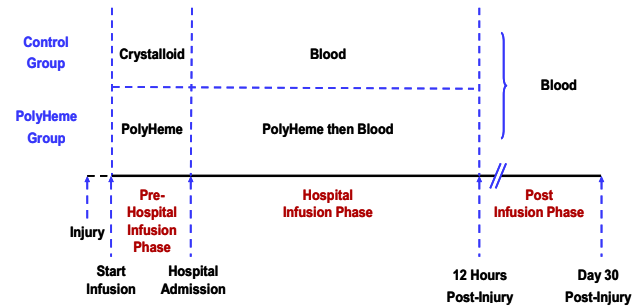


Figure 1. Study design. Patients were randomized 50:50 into each treatment group. PolyHeme patients received PolyHeme in the field, then PolyHeme (up to 6 U) for up to 12 hours, and blood in the hospital, as needed. Control patients received crystalloid in the field, then RBC transfusion in the hospital, as needed.

carrying capacity in a setting where blood is unavailable, and use of PolyHeme in lieu of allogeneic RBCs during the first 12 hours postinjury to reduce the immunoinflammatory response and subsequent organ dysfunction.

METHODS

Study design

In this controlled, open-label trial, patients were randomized to PolyHeme or control groups in the prehospital setting. Inclusion criteria were presumed acute blood loss from trauma, class III hemorrhagic shock (ie, systolic blood pressure [SBP] \leq 90 mmHg), and age 18 years or older. Exclusion criteria included imminent death, cardiopulmonary resuscitation, severe head injury (ie, Glasgow Coma Scale [GCS] \leq 5), pregnancy, or religious objection to blood products. Patient enrollment occurred under FDA regulation 21CFR§50.24 providing for exception from informed consent.³⁷ Study sites were Level I trauma centers. The study design is outlined in Figure 1. Patients received up to 6 U (50 g hemoglobin/unit) of PolyHeme beginning at the scene of injury and during the first 12 hours postinjury. If needed, stored RBCs were given thereafter. Control patients received crystalloid in the field and stored RBCs as needed in the hospital. Transfusion triggers were based on recent National Institutes of Health Glue Grant protocols for resuscitation of patients in hemorrhagic shock.³⁸

Study end points

The primary efficacy end point was day 30 mortality. Secondary efficacy end points were day 30 mortality for injury-type subgroups (blunt versus penetrating), day 1 mortality, allogeneic blood use through day 1, and the incidence of MOF through day 30. MOF scores were calculated using the Denver MOF score,¹⁰ which, in brief, evaluates four organ systems (lung, kidney, liver, and heart), each graded 0 to 3 with an MOF threshold of 4 or more

after 48 hours postinjury. Primary and secondary safety end points included day 1 mortality, day 30 mortality, adverse events (AEs), and serious adverse events (SAEs).

Statistical analysis

The primary efficacy analysis was a dual superiority/noninferiority assessment of day 30 mortality.³⁹⁻⁴⁴ The study design assumed a mortality rate of 17% for the control group based on published series.⁴⁻⁷ The superiority hypothesis assumed that the PolyHeme group would have a 7% lower mortality rate compared with controls. The superiority outcomes were based on the potential benefit of providing an oxygen carrier during prehospital critical anemia, and avoiding allogeneic blood transfusion-related MOF in the first 12 hours. The noninferiority hypothesis assumed that the PolyHeme group would have no more than a 7% higher mortality rate compared with controls. Different noninferiority margins were considered, but 7% was chosen based on available medical literature, feasibility of the study, earlier work in acute blood loss patients compared with historic controls,⁹ and a study of injured and bleeding patients who were administered blood en route to the medical center.⁴⁵ The implication of noninferiority outcomes is that PolyHeme would not be used interchangeably with available RBCs. Rather, in contrast with traditional noninferiority trials, noninferiority outcomes in this trial would allow the benefit to be extrapolated to settings in which RBCs are needed but not available.

Using a one-sided 0.05 α level, the study was powered for both hypotheses at 720 patients. An independent data monitoring committee (IDMC) composed of three experts in trauma, critical care, and biostatistics convened after enrollment of 60, 120, 250, and 500 patients to perform blinded safety analyses. A blinded adaptive power analysis was performed after 250 patients to ensure that no increase in the trial size was necessary. In the final analysis, the confidence interval on the difference between mortalities was used in a nonparametric analysis of covariance (ANCOVA)³⁹⁻⁴⁴ adjusting for the following covariates: age, gender, mechanism of injury, Injury Severity Score (ISS), Glasgow Coma Scale (GCS), field SBP, and amount of prerandomization crystalloid. The α -level was adjusted to correct for the IDMC interim analyses.

RESULTS

Enrollment occurred from January 2004 to July 2006 at 29 Level I trauma centers. Demographics and baseline characteristics were comparable between groups (Table 1), with the exception of coagulation status. Within the 12-hour postinjury interval, 53% of patients in the PolyHeme group received 1 to 2 U, 15% received 3 to 5 U, and 32%

received 6 U. In the control group, 7% of patients received 1 to 2 U of RBCs, 10% received 3 to 5 U, and 34% received at least 6 U in the initial 12-hour postinjury interval. At 6 and 12 hours postinjury, PolyHeme patients had a mean Hb of 10.4 g/dL and 10.3 g/dL, respectively; control patients had a mean Hb of 11.2 g/dL and 10.8 g/dL at 6 and 12 hours postinjury, respectively.

Cohort analyses

Three prespecified cohorts were analyzed (Fig. 2). Six of the 720 patients received no study treatment. The primary efficacy analysis was based on the remaining 714 patients analyzed according to the treatment to which they were randomized ("as-randomized"). Safety was analyzed in the 714 patients based on the treatment actually received ("as-treated"). The per-protocol cohort included 590 randomized patients who did not violate predefined major eligibility or treatment regimen criteria. There were 124 (17%) patients with major protocol violations (71 PolyHeme, 53 control).

Primary efficacy end point

Day 30 mortality rates and confidence intervals for the three cohorts and the major protocol violations group are shown in Table 2 and Figure 3. There was no significant difference in mortality between the PolyHeme and control groups. The upper limit of the confidence interval exceeded the 7% noninferiority threshold in the as-randomized (7.65%) and as-treated cohorts (7.06%). In the per-protocol cohort, the upper limit of the confidence interval was below the 7% threshold (6.21%).

After the day 30 assessment, four patient deaths in the as-randomized cohort were reported. One patient died on study day 32 and was randomized to control, but received PolyHeme. The second patient died on study day 34 and was randomized to and received control. The third patient died on study day 41 and was randomized to and received control. The fourth patient died on study day 194 and was randomized to and received PolyHeme. Two additional patients died (one in the PolyHeme group and one control) who were randomized and did not receive any treatment with study fluids (saline, PolyHeme, or RBCs).

Secondary efficacy end points

Day 1 mortality was not significantly different between PolyHeme and control patients in any cohort (as-randomized, 35 of 350 [10.0%] versus 27 of 364 [7.4%]; as-treated, 34 of 349 [9.7%] versus 28 of 365 [7.7%]; per-protocol, 20 of 279 [7.2%] versus 22 of 311 [7.1%], respectively). This is an important end point, because day 1 represented a clinically relevant prolonged interval of de-

Table 1. Baseline Characteristics and Demographics

Characteristic	As randomized (n = 714)		As treated (n = 714)		Per protocol (n = 590)		Protocol violations (n = 124)	
	PolyHeme (n = 350)	Control (n = 364)	PolyHeme (n = 349)	Control (n = 365)	PolyHeme (n = 279)	Control (n = 311)	PolyHeme (n = 71)	Control (n = 53)
Age, y*	36.3 (0.8)	37.9 (0.9)	35.9 (0.8)	38.3 (0.9)	36.7 (0.9)	38.2 (0.9)	35.0 (1.7)	35.9 (2.3)
Age category, APACHE, y†								
≤ 44	242 (69)	251 (69)	247 (71)	246 (67)	195 (70)	211 (68)	47 (66)	40 (76)
45–54	65 (19)	57 (16)	62 (18)	60 (16)	48 (17)	51 (16)	17 (24)	6 (11)
55–64	27 (8)	27 (7)	25 (7)	29 (8)	21 (8)	24 (8)	6 (9)	3 (6)
65–74	11 (3)	16 (4)	10 (3)	17 (5)	10 (4)	14 (5)	1 (1)	2 (4)
≥ 75	5 (1)	13 (4)	5 (1)	13 (4)	5 (2)	11 (4)	0 (0)	2 (4)
Male†	272 (78)	289 (79)	268 (77)	293 (80)	218 (78)	252 (81)	54 (76)	37 (70)
Ethnicity†								
Caucasian	160 (46)	170 (47)	156 (45)	174 (48)	127 (46)	151 (49)	33 (47)	19 (36)
African American	124 (35)	120 (33)	124 (36)	120 (33)	97 (35)	102 (33)	27 (38)	18 (34)
Hispanic	53 (15)	61 (17)	57 (16)	57 (16)	43 (15)	48 (15)	10 (14)	13 (25)
Asian	10 (3)	7 (2)	9 (3)	8 (2)	9 (3)	4 (1)	1 (1)	3 (6)
Other	3 (< 1)	6 (2)	3 (< 1)	6 (2)	3 (1)	6 (2)	0	0
Height, cm*	174.5 (0.6)	173.4 (0.6)	174.3 (0.6)	173.6 (0.6)	174.8 (0.7)	173.9 (0.6)	173.4 (1.7)	170.1 (2.3)
Weight, kg*	82.4 (1.1)	83.2 (1.2)	82.6 (1.1)	83.0 (1.2)	82.9 (1.3)	84.0 (1.2)	80.0 (2.5)	78.1 (3.6)
BMI, kg/m ² *	27.0 (0.4)	27.7 (0.4)	27.1 (0.3)	27.6 (0.4)	27.1 (0.4)	27.9 (0.4)	26.5 (0.9)	26.6 (1.2)
ISS*	19.9 (0.8)	19.4 (0.7)	20.1 (0.7)	19.2 (0.7)	19.1 (0.8)	19.1 (0.8)	22.9 (1.9)	21.2 (1.9)
ISS category†								
Mild/moderate (< 9)	56 (16)	60 (16)	51 (15)	65 (18)	47 (17)	53 (17)	9 (13)	7 (13)
Serious (9–15)	107 (31)	101 (28)	107 (31)	101 (28)	87 (31)	90 (29)	20 (28)	11 (21)
Severe (16–24)	61 (17)	74 (20)	60 (17)	75 (21)	51 (18)	67 (22)	10 (14)	7 (14)
Critical/maximal (25–75)	126 (36)	123 (34)	129 (37)	120 (33)	94 (34)	99 (32)	32 (45)	24 (49)
Maximal (36–75)	48 (14)	41 (11)	46 (13)	43 (12)	36 (13)	36 (12)	12 (17)	5 (9)
Unsurvivable (75)	3 (< 1)	1 (< 1)	3 (< 1)	1 (< 1)	1 (< 1)	1 (< 1)	2 (3)	0
Mechanism of injury†								
Blunt	166 (47)	174 (48)	165 (47)	175 (48)	138 (49)	154 (50)	28 (39)	20 (40)
Penetrating	184 (53)	186 (52)	183 (53)	187 (52)	141 (51)	156 (50)	43 (61)	30 (60)
Transport mode†								
Air	121 (35)	122 (34)	117 (34)	126 (35)	99 (35)	112 (36)	22 (31)	10 (19)
Ground	229 (65)	242 (66)	232 (66)	239 (65)	180 (65)	199 (64)	49 (69)	43 (81)
Median transport time, min*	26	26	26	26	27	26	24	26
SBP randomization, mmHg*	77.9 (0.7)	77.8 (0.6)	77.2 (0.6)	78.4 (0.7)	77.2 (0.7)	77.3 (0.6)	82.5 (3.3)	82.5 (2.7)
SBP < 60 mmHg†	17 (5)	19 (5)	16 (5)	20 (6)	12 (4)	19 (6)	4 (6)	1 (2)
Zero/no SBP obtained†	30 (9)	19 (5)	27 (8)	22 (6)	0	0	27 (39)	22 (41)
GCS randomization*	13.7 (0.1)	13.6 (0.1)	13.7 (0.1)	13.7 (0.1)	13.8 (0.1)	13.7 (0.1)	13.4 (0.3)	13.3 (0.4)
GCS-randomization category†								
≤ 5	4 (1)	2 (< 1)	4 (1)	2 (< 1)	0	0	4 (6)	2 (4)
6–8	17 (5)	18 (5)	18 (5)	17 (5)	15 (5)	17 (5)	2 (3)	1 (2)
9–12	35 (10)	49 (13)	34 (10)	50 (14)	28 (10)	40 (13)	7 (10)	9 (17)
13–15	294 (84)	295 (81)	293 (84)	296 (81)	236 (85)	254 (82)	58 (82)	41 (77)
Hb at randomization, g/dL*‡	13.2 (0.2)	13.0 (0.2)	13.2 (0.2)	13.0 (0.2)	13.3 (0.2)	13.0 (0.2)	12.8 (0.6)	12.7 (0.7)
PT at randomization, sec*	29.4 (2.6) [§]	20.9 (1.7)	29.8 (2.6) [§]	20.0 (1.6)	28.3 (2.8) [§]	20.2 (1.7)	37.4 (8.2)	28.5 (7.5)
aPTT at randomization, s*	67.4 (7.3) [§]	46.6 (4.4)	68.6 (7.2) [§]	44.3 (4.2)	63.8 (7.7) [§]	43.1 (4.1)	92.6 (22.0)	80.1 (24.0)
Hb at ED admission, g/dL*‡	12.3 (0.1) [§]	11.5 (0.2)	12.3 (0.1) [§]	11.5 (0.2)	12.5 (0.2) [§]	11.4 (0.2)	11.6 (0.4)	11.8 (0.4)
PT at ED admission, s*	20.7 (1.2)	21.0 (1.3)	20.7 (1.2)	20.9 (1.3)	20.1 (1.3)	21.0 (1.4)	23.4 (3.1)	20.3 (2.7)
aPTT at ED admission, s*	49.4 (3.8)	49.3 (4.1)	49.3 (3.7)	49.4 (4.1)	46.0 (3.9)	49.3 (4.5)	63.2 (10.1)	49.0 (9.2)

*Expressed as mean (SE).
 †Expressed as n (%); percentages are based on the number of patients in each treatment group divided by number of patients with nonmissing values.
 ‡To convert to g/L, multiply by 0.1.
 §p < 0.05 compared with control.
 aPTT, activated partial thromboplastin time; BMI, body mass index; ED, emergency department; GCS, Glasgow Coma Scale; Hb, hemoglobin; ISS, Injury Severity Score; SBP, systolic blood pressure.

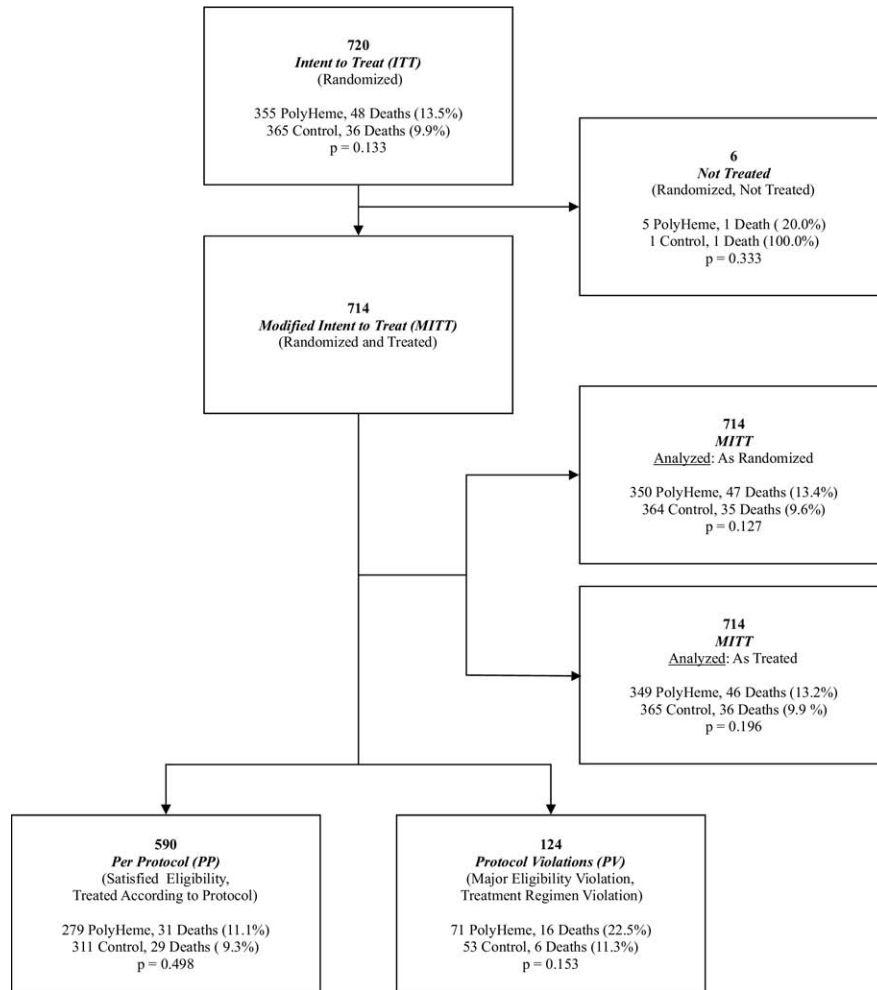


Figure 2. Study patient enrollment. Patients were assessed for eligibility by paramedics in the field at the scene of injury; 722 patients were enrolled into the study. Two patients had their data withheld by the local institutional review board. Seven hundred twenty patients comprised the modified intent-to-treat population (MITT). The MITT population was analyzed two ways: “as randomized,” in which patients were analyzed in the group to which they were randomized (regardless of which treatment the patients received) and “as treated,” in which patients were analyzed according to the treatment actually received. Of the 720 patients, 590 satisfied all the eligibility criteria and were treated according to the protocol; 124 did not meet eligibility criteria or had a treatment regimen different than that described in the protocol.

layed access to RBCs. The administration of PolyHeme, up to 6 U, in the first 12 hours simulated such a delay in access to stored RBCs.

Deaths from blunt trauma were statistically higher in the PolyHeme group, but there was no treatment-by-covariate interaction for mechanism of injury in any cohort (Table 2). Mortality differed considerably between PolyHeme and control patients with blunt injury who had protocol violations (Table 2). By comparison, the mortality between PolyHeme and control patients sustaining penetrating wounds was virtually identical, where the distribution of major protocol violations was equivalent.

Exposure to allogeneic stored blood was a secondary efficacy end point. In the initial 12 hours postinjury, 238 (68%) PolyHeme patients did not require RBCs compared with 183 (50%) control patients ($p < 0.001$). By day 1, 201 (57%) PolyHeme patients and 174 (48%) control patients did not require RBCs ($p < 0.001$). In addition, the time to first exposure to RBCs in patients who received RBCs was markedly different. The median time to first unit of RBCs in the PolyHeme group was 7.6 hours (453.0 minutes) compared with 1.5 hours (88.5 minutes) in the control group. In the per-protocol cohort, the median time to first unit of RBCs in the PolyHeme group was

Table 2. 30-Day Mortality by Subgroups

Characteristic	As randomized (n = 714)		As treated (n = 714)		Per protocol (n = 590)		Protocol violations (n = 124)	
	PolyHeme (n = 350)	Control (n = 364)	PolyHeme (n = 349)	Control (n = 365)	PolyHeme (n = 279)	Control (n = 311)	PolyHeme (n = 71)	Control (n = 53)
Mortality by mechanism of injury								
Blunt, deaths, n (%)	30/166 (18.1) [†]	18/174 (10.3)	30/165 (18.2) [†]	18/175 (10.3)	22/138 (15.9)	17/154 (11.0)	8/28 (28.6)	1/20 (5.0)
Penetrating, deaths, n (%)	17/184 (9.2)	17/186 (9.1)	16/183 (8.7)	18/187 (9.6)	9/141 (6.4)	12/156 (7.7)	8/43 (18.6)	5/30 (16.7)
Mortality by ISS category*								
Mild/moderate (1–8), deaths n (%)	1/56 (1.8)	1/60 (1.7)	0/51 (0)	2/65 (3.1)	0/47 (0)	0/53 (0)	1/9 (11.1)	1/7 (14.3)
Serious (9–15), deaths, n (%)	4/107 (3.7)	2/101 (2.0)	4/107 (3.7)	2/101 (2.0)	3/87 (3.4)	2/90 (2.2)	1/20 (5.0)	0/11 (0)
Severe (16–24), deaths, n (%)	8/61 (13.1)	5/74 (6.8)	8/60 (13.3)	5/75 (6.7)	6/51 (11.8)	4/67 (6.0)	2/10 (20.0)	1/7 (14.3)
Critical (25–75), deaths, n (%)	34/126 (27.0)	25/123 (20.3)	33/129 (25.6)	26/120 (21.7)	22/94 (23.4)	22/99 (22.2)	12/32 (37.5)	3/24 (12.5)
Mortality regression model interaction terms (p value)								
By randomization SBP	0.687		0.751		0.919		ND	
By APACHE age category	0.333		0.236		0.462		ND	
By mechanism of injury	0.187		0.146		0.350		ND	
By Injury Severity Score	0.981		0.645		0.863		ND	
By Glasgow Coma Scale	0.536		0.703		0.172		ND	
By prerandomization crystalloid	0.194		0.106		0.101		ND	
By gender	0.312		0.914		0.779		ND	

*Six patients did not have an ISS calculated: 4 were patients with nontrauma-related blood loss; 2 were trauma patients who died early postinjury and had inadequate documentation of their injuries.

[†]p < 0.05 compared with control.

ISS, Injury Severity Score; SBP, systolic blood pressure.

14.1 hours (848.0 minutes) compared with 1.5 hours (89.0 minutes) in the control group.

The incidence of MOF was low in this study and was not significantly different between groups: 26 of 350 (7.4%) in the PolyHeme group versus 20 of 364 (5.5%) in the control group. Of patients in whom MOF developed, a similar proportion from each group received at least 6 U of RBCs (PolyHeme, 23 of 26; control, 18 of 20). There was a strong association in both groups between receiving 6 U or more of RBCs in the first 12 hours posttrauma and MOF (PolyHeme odds ratio = 6.76; p < 0.001; control odds ratio = 4.83; p = 0.002), as we found previously.¹⁰

Safety analyses

AEs, were reported in virtually all: 93% (324 of 349) of PolyHeme patients and 88% (322 of 365) of control patients (p = 0.041), as expected in seriously injured patients. Investigator-reported AEs occurring in ≥ 20% of patients included anemia, pyrexia, hypocalcemia, hypokalemia, hyperglycemia, thrombocytopenia, leukocytosis, and tachycardia. SAEs were reported in 40% (141 of 349) of PolyHeme patients and 35% (126 of 365) of control patients (p = 0.122, Table 3).

Hypertension (Table 4) was reported more frequently as an AE in the PolyHeme group compared with the control group (18% versus 12%, respectively; p = 0.028). But the overall incidence of substantially abnormal episodes of systolic and diastolic hypertension on arrival at the hos-

pital or through 6 hours postinjury was low (5% PolyHeme versus 3% control and 7% PolyHeme versus 7% control, respectively). The lack of significant evidence of vasoconstriction is consistent with our previous work.⁴⁶

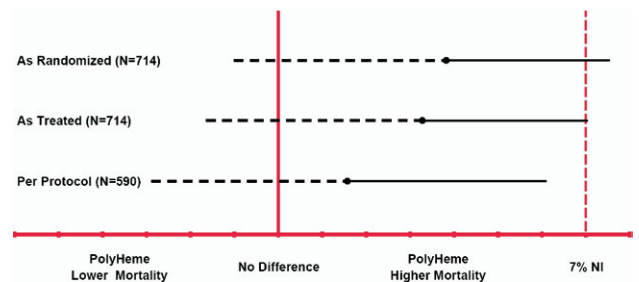


Figure 3. Noninferiority (NI) and inferiority mortality analyses. The solid circle represents the point estimate for the observed mortality difference between the PolyHeme group and the control group for each cohort. The dashed black line represents the one-sided inferiority analysis using a final critical α (α_{crit}) level of 0.0144, having split the 0.05 starting α level using the Pocock spending function approach between four planned interim analyses and one final analysis. The solid black line represents the one-sided noninferiority analysis using a final α_{crit} level of 0.044, having split the 0.05 starting α level using the O'Brien-Fleming spending function approach, as described by Lan and DeMets,⁴⁰ between one planned interim analysis for superiority after 500 patients had been enrolled and one final analysis. The "7%NI" term and the vertical red dashed line refer to the noninferiority boundary at 7% higher mortality in the PolyHeme group compared with the control group. The as-randomized cohort included 714 patients (350 PolyHeme, 364 control), the as-treated cohort included 714 patients (349 PolyHeme, 365 control), and the per-protocol cohort included 590 patients (279 PolyHeme, 311 control).

Table 3. Investigator-Reported Adverse Events

Event*	As treated (n = 714)			
	PolyHeme (n = 349)		Control (n = 365)	
	n	%	n	%
Adverse events	324	93 [†]	322	88
Serious adverse events	141	40	126	35
Most common SAEs (> 2%)				
Pneumonia	27	8	21	6
Hemorrhagic shock	20	6	16	4
Respiratory failure	21	6	17	5
Hypercoagulable state	18	5	12	3
Coagulopathy	13	4 [†]	4	1
Sepsis	12	3	11	3
Myocardial infarction	10	3 [†]	2	1
Myocardial infarction adverse events [‡]	11	3 [†]	3	1
Myocardial infarction	7		2	
NSTEMI	3		0	
Non Q wave MI	0		1	
Acute traumatic MI	1		0	
Requiring intervention	1		1	
Death within 30 d	3		1	
Cardiovascular events				
Heart failure/CHF/PE/fluid overload/hypervolemia	20	6	20	5
Cardiac arrest/EMD/V-fibrillation/V-arrhythmia/V-tachycardia	15	4	9	2
CVA/cerebral ischemia/cerebral infarction	3	1	1	1
Multiple organ failure in 30 d (adjudicated)				
Renal (creatinine > 1.8 mg/dL)	13/26	50	9/20	45
Hepatic (total bilirubin > 2.0 mg/dL)	20/26	77	15/20	75
Cardiac (inotropes)	9/26	35	4/20	20
Pulmonary (PaO ₂ /FiO ₂ < 240)	24/26	92	19/20	95

Adverse events and serious adverse events are defined according to International Committee on Harmonization Guidelines. Multiple organ failure was defined according to Moore FA and colleagues.¹⁰

*Expressed as: number of patients with an event divided by the total number of patients in each treatment group.

[†]p < 0.05 compared with control.

[‡]Two myocardial infarction events were not considered “serious” by the investigator.

CHF, congestive heart failure; EMD, electromechanical disassociation; NSTEMI, non-ST-segment elevation myocardial infarction; PaO₂/FiO₂, arterial O₂ partial pressure/fractional concentration of O₂ in inspired air; PE, pulmonary embolism; SAE, serious adverse event.

The incidence of renal failure was also low and comparable between groups (3% PolyHeme, 2% control). There was also no difference in the incidence of nausea (17% PolyHeme, 15% control) or vomiting (13% PolyHeme, 12% control). Hyperamylasemia was reported in one PolyHeme patient versus none in the control group,

and acute pancreatitis occurred in one PolyHeme patient and two control patients.

The risk of adverse myocardial-related events associated with HBOCs is particularly germane in light of a recent metaanalysis suggesting an increased risk of MI with HBOC in clinical trials.⁴⁷ There was no difference between groups in the incidence of combined cardiovascular events related to cardiac failure, malignant dysrhythmias, or cerebral ischemic or thrombotic complications (Table 3). There were 11 investigator-reported MIs in the PolyHeme group versus 3 in the control group; none was considered by the investigator to be “possibly” or “probably” related to PolyHeme. Three MIs in the PolyHeme group did not fit the classic clinical profile: two were not substantiated by ECGs, enzymes, or autopsy reports, and a third followed ligation of a coronary artery during repair of a ventricular stab wound. Cardiologists recommended cardiac catheterization for one patient in each group; otherwise no interventions were necessary. Three PolyHeme patients and one control patient who had MIs died by day 30; the cause of death was MI in two patients, on day 1 (man, age = 55) and day 19 (man, age = 56), cardiac arrhythmia in one patient on day 8 (woman, age = 88), and MOF in one on day 30 (man, age = 63). This overall low incidence of MI (2%) contrasts with the high rate of abnormal ECGs (75% PolyHeme, 78% control), markedly abnormal creatine kinase-MB isoenzymes (65% PolyHeme, 68% control), and markedly abnormal troponin I (26% PolyHeme, 19% control).

Because of the disparity between high rates of ECG abnormalities and troponin and creatine-kinase-MB elevation, but low MI incidence, a posthoc independent Cardiac Event Subcommittee of experts in cardiology and resuscitation medicine, blinded to treatment, was convened to adjudicate these results. The committee developed a standardized decision algorithm to classify study patients as to the likelihood of infarction (Fig. 4). Patients were stratified by chest trauma (defined as chest Abbreviated Injury Score [AIS] scores ≤ 2 or > 2. Patients with AIS ≤ 2 were classified as having “absent infarction,” “indeterminate infarction,” “possible infarction,” or “probable infarction,” and “physiologic stress or possible infarction.” Patients with AIS > 2 were classified as “absent infarction or injury,” “indeterminate infarction or injury,” “possible infarction or injury,” or “probable infarction or injury” and “physiologic stress or possible infarction or injury.” The addition of the term *injury* to the possible and probable classifications of patients with AIS > 2 was done in recognition of the fact that chest trauma in and of itself can cause elevations of cardiac enzymes and biomarkers and abnormal ECGs.^{48,49} Standardized methods of assessing ischemia or infarction

Table 4. Systemic Blood Pressure

Characteristic	As treated (n = 714)		Per protocol (n = 590)	
	PolyHeme (n = 350)	Control (n = 364)	PolyHeme (n = 279)	Control (n = 311)
Markedly abnormal high SBP (≥ 180 mmHg)				
At admission*	6 (2)	5 (1)	5 (2)	4 (1)
At 6 h postinjury*	11 (3)	5 (1)	10 (4)	4 (1)
At admission OR 6 h postinjury*	16 (5)	10 (3)	14 (5)	8 (3)
Markedly abnormal high DBP (≥ 105 mmHg)				
At admission*	23 (7)	18 (5)	17 (6)	14 (5)
At 6 h postinjury*	3 (1)	8 (2)	3 (1)	5 (2)
At admission OR 6 h postinjury*	26 (7)	25 (7)	20 (7)	18 (6)
Mean SBP at admission [†]	115 (2)	113 (2)	115 (2)	113 (2)
Mean SBP at 6 h postinjury [†]	129 (2) [‡]	122 (2)	129 (2) [‡]	122 (2)
Mean DBP at admission [†]	71 (1) [‡]	68 (1)	71 (1)	68 (1)
Mean DBP at 6 h postinjury [†]	73 (1) [‡]	66 (1)	73 (1) [‡]	66 (1)

*Expressed as n (%); percentages are based on the number of patients in each treatment group divided by number of patients with nonmissing values.

[†]Expressed as mean (SE) in mmHg.

[‡]p < 0.05 compared with control.

DBP, diastolic blood pressure; SBP, systolic blood pressure.

on ECG recordings using ST elevation, ST depression, and T-wave changes, ECG changes associated with earlier myocardial ischemia, and left bundle branch block were used. If a single abnormal ECG or biomarker was available before the patient died, the patient was classified as having “possible infarction.” If a patient had a cardiac arrest before laboratory tests or ECG were obtained, the patient was classified as having “indeterminate infarction,” with a footnote that the patient died before testing. Each case was reviewed initially by two subcommittee members. In the event that classifications by these two members differed, all members reviewed the case. The final classification was determined by consensus.

The committee found a higher incidence of “probable infarction” in patients with and without chest trauma than was reported by the investigators (Table 5). More patients were designated as having “probable infarction” in the PolyHeme group. The committee also reported the incidence of “possible infarction” in patients with and without chest injury. When all of the categories of “probable” and “possible” infarction are grouped together, more than half of the patients in each group had some evidence of MI. There were more patients with these combined designations in the control group (193 [55.3%] patients versus 190 [52.1%] patients). There were more patients with “absent infarction” in the PolyHeme group (84 versus 64) and more patients with “indeterminate infarction” in the control group (72 versus 111). In summary, a relationship of PolyHeme to elevated cardiac biomarkers and abnormal ECG findings was not supported by the data. A relationship of PolyHeme to MI could not be clearly ascertained from the totality of the cardiac data in

this study, despite the higher number of investigator-reported MIs.

DISCUSSION

Mortality rates at days 1 and 30 were not statistically different between injured patients in shock who received up to 6 U of PolyHeme in lieu of blood for up to 12 hours after injury and control patients, who received the standard of care, including early blood transfusion. Mortality is best understood in the context of the novel study design and conduct of this trial, which presented multiple challenges. The protocol specified enrollment of patients who were bleeding and in shock, so it was necessary to conduct the study under federal regulation, 21§CFR50.24, allowing an exception from informed consent.³⁷ Additionally, the choice of the control group and the basis for statistical assessment of efficacy were complex. The ideal control group to assess efficacy of an oxygen carrier when blood is not available would comprise severely injured, massively bleeding patients with critically low Hb levels, substantially delayed access to blood, and the resulting high mortality. Earlier clinical experience with PolyHeme in hospitalized patients^{1,9,21-26} showed that PolyHeme can provide life-sustaining oxygen-carrying capacity at otherwise life-threatening hemoglobin levels.⁹ Because of the possibility that the control group would include patients with mixed injury severity, variable volumes of blood loss, and early access to blood, dual primary end points of superiority and noninferiority were used. So, control patients without crit-

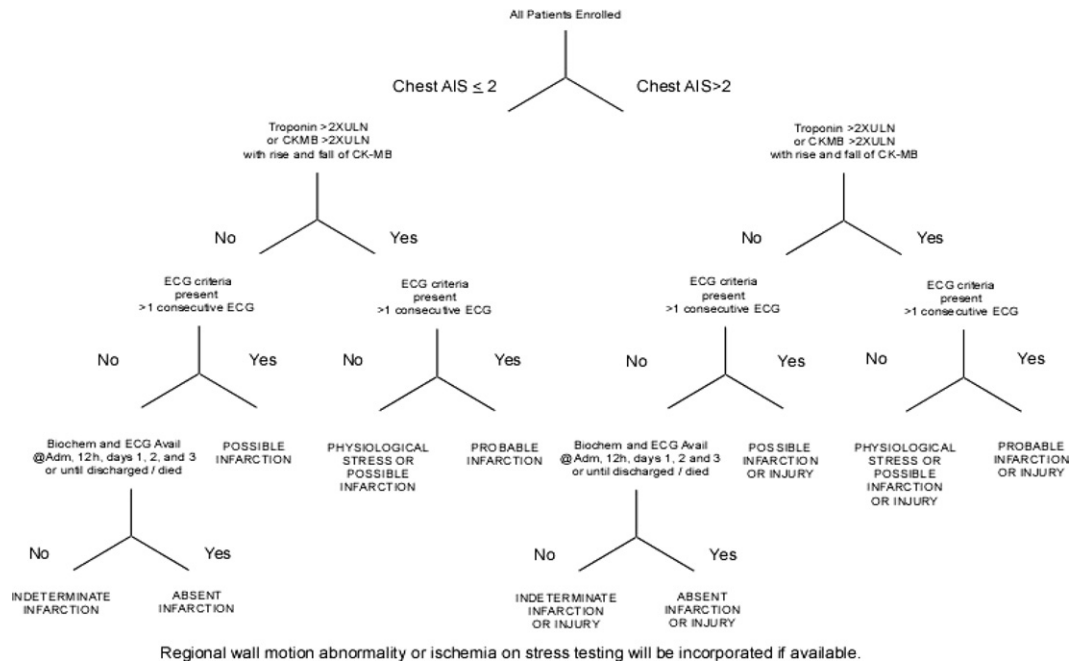


Figure 4. Independent Data Safety Monitoring (IDMC) Cardiac Subcommittee myocardial infarction algorithm. An IDMC Cardiac Subcommittee reviewed all patient records in the modified intent-to-treat (MITT) as-randomized cohort in a treatment-blinded fashion. Patients were assigned an MI category according to the algorithm based on chest Abbreviated Injury Score (AIS), troponin, and creatine kinase-MB data, and ECG criteria.

ical anemia would not represent the scientifically appropriate control in which statistical superiority would be anticipated. This was the basis for extrapolating the observed results to the intended population.

Superiority in day 30 survival was not observed, likely because of several factors: short transport times, enrollment of patients who were not severely injured (only 34% of the control group required more than 6 U of RBCs in the first 12 hours), and the occurrence of protocol violations. In the per-protocol population, the results fell within the 7% noninferiority boundary, with a mortality difference of two patients between groups. The per-protocol population represented the clearest opportunity to assess potential treatment effects because all other variables were well matched. The difference between the per-protocol and as-randomized outcomes was influenced by patients with major prespecified protocol violations (17%) related to eligibility or treatment regimen in this open-label study. Tables 1 and 2 illustrate that more PolyHeme patients had indicators of poor prognosis on enrollment, indicating potential futile resuscitation efforts. This is most evident in the subset of protocol violation patients with blunt injury, in whom close review highlighted an imbalance in critical variables between the PolyHeme and control groups. The protocol-violation patients receiving PolyHeme were characterized by higher Injury Severity Score (31 versus 26),

lower randomization SBP (80 mmHg versus 97 mmHg), lower randomization Glasgow Coma Scale (12 versus 14), and more severe acidosis on arrival to the hospital (base deficit -8.8 mEq/L versus -6.5 mEq/L), all of which correlate with a higher mortality. PolyHeme administration cannot influence Injury Severity Score, randomization SBP, or Glasgow Coma Scale because these are obtained before infusion. In addition, more PolyHeme patients had markedly abnormal coagulation status (prothrombin time > 17 seconds) on enrollment (29% versus 20%). This is an important clinical observation and another potential indication of difference in injury severity. The imbalances in these critical variables alone could potentially account for the observed mortality difference between PolyHeme and control groups in the blunt injury protocol-violation patients.

Transfusion avoidance is particularly relevant to the intended use of PolyHeme for treatment until definitive care and blood become available. Transfusion of 6 U or more of allogeneic blood in the first 12 hours has been invoked as the dominant risk factor in the development of postinjury MOF.^{10,11} PolyHeme has been shown to attenuate the immunoinflammatory effects of stored RBCs,²⁷⁻²⁹ forming the basis for infusing up to 6 U of PolyHeme in 12 hours. PolyHeme patients had a statistically significant reduction in the incidence of blood use during the 12- and 24-hour

Table 5. Independent Data Safety Monitoring Cardiac Subcommittee Assignment of Myocardial Infarction

Cardiac committee determination	As treated (n = 714)			
	PolyHeme (n = 349)		Control (n = 365)	
	n	%	n	%
Chest Abbreviated Injury Score ≤ 2				
Probable infarction	20	5.7	11	3.0
Physiologic stress or possible infarction	62	17.8	67	18.4
Possible infarction	5	1.4	6	1.6
Total possible or probable	87	24.9	84	23.0
Absent infarction	62	17.8	52	14.2
Indeterminate infarction	43	12.3	79	21.6
Chest Abbreviated Injury Score > 2				
Probable infarction or injury	23	6.6	19	5.2
Physiologic stress or possible infarction or injury	82	23.5	81	22.2
Possible infarction or injury	1	0.3	6	1.6
Total possible or probable	106	30.4	106	29.0
Absent infarction or injury	22	6.3	12	3.3
Indeterminate infarction or injury	29	8.3	32	8.8

Percentages are based on the number of patients in each treatment group divided by number of patients with nonmissing values.

intervals. Although there was no difference in MOF between the groups, 23 of 26 PolyHeme patients and 18 of 20 control patients in whom MOF developed also received ≥ 6 U of banked blood. Conceivably, higher allowed doses of PolyHeme within the 12-hour therapeutic window, in lieu of blood, might have reduced the incidence of MOF by averting the adverse immunoinflammatory effects of stored RBCs.

Safety of HBOCs remains a topic of considerable debate.⁴⁷ The early history with HBOCs included efforts to remove the stromal contaminants believed to be responsible for the observed safety concerns. But Savitsky and coworkers³³ described vasoconstriction and organ dysfunction in healthy volunteers with a stroma-free tetrameric hemoglobin preparation. Subsequent efforts have focused on removal of small molecular weight tetrameric hemoglobin as a way of improving safety.^{50,51} In addition, there has been considerable attention to the adverse experiences with HBOCs and the potential mechanisms of nitric oxide scavenging,^{52,53} generation of reactive oxygen species,⁵⁴ and reflex autoregulation.⁵⁵ PolyHeme is polymerized to improve intravascular persistence and then purified to removal virtually all unpolymerized tetramer (< 1%) and to decrease interactions with nitric oxide that could lead to vasoconstriction.¹²⁻²⁰

Safety data in Table 3 show a higher incidence of AEs and SAEs in the PolyHeme group. Of particular concern was that investigators reported more MIs in the PolyHeme

group in this open-label study, although the overall low incidence of MI contrasted the high incidence of abnormal ECGs, enzymes, and biomarkers. Because of the disparity between high numbers of reported ECG abnormality and troponin and creatine kinase-MB elevation but low reported MI rates, an independent Cardiac Event Subcommittee was convened to adjudicate these results. The committee found a higher incidence of “probable infarction” in patients with and without chest trauma than was reported by the investigators. More patients were designated as having “probable infarction” in the PolyHeme group. The committee also reported the incidence of “possible infarction” in patients with and without chest injury. When all of the categories of “probable” and “possible” infarction were grouped together, more than half of the patients in each group had some evidence of MI. Troponin elevations have been reported in 29% to 44% of critically ill and trauma populations.^{48,49} Because blood samples and ECGs were obtained serially in this study after admission, the high rates of abnormal ECGs, creatine kinase-MB, and troponin may reflect the transitory sequelae of chest trauma in many of these patients, rather than myocardial ischemia from coronary artery disease. In addition, analysis of a comprehensive grouping of cardiovascular AEs showed no difference between the groups in the incidence of events related to ischemia, pump failure, or dysrhythmias. Incidence of significant hypertensive episodes and renal failure were low and comparable between groups.

Safety must be assessed in the context of the potential benefit of PolyHeme, ie, improved survival in patients with life-threatening hemoglobin levels, when blood is not available or not an option. This benefit was demonstrated in earlier clinical experience with PolyHeme in hospitalized patients^{1,9,22-24} and multiple emergency, compassionate treatments.^{21,25,26} The results from this study suggest some increase in frequency of adverse events compared with that with the use of blood. Consequently, PolyHeme would not be used interchangeably with RBCs, but rather when the likelihood of dying without oxygen-carrying replacement is so great that the potential life-sustaining benefit would exceed any potential risks. A recent metaanalysis pooled data from multiple different products used in various different clinical settings and concluded that there was no role for any of the HBOCs currently in clinical development.⁴⁷ Such a statistical tool of metaanalysis comparing varied products under the umbrella of an entire class can be a useful tool to raise questions that merit answers with respect to the class. But metaanalysis is not designed to provide answers about specific products or to examine fully the risk-to-benefit ratio of any particular product, particularly for the intended clinical use.⁵⁶ Rather, that is the role of

clinical trials, such as this study with PolyHeme, which are focused and conducted to assess the safety and efficacy in relation to a proposed indication; ie, in this case, an indication that addresses a critical, unmet need when there is no available alternative. In this setting, it is possible for an HBOC such as PolyHeme to provide a clinically meaningful benefit even though the outcomes may be less favorable than those seen with stored blood.

There is an undisputed need for a universally compatible, oxygen-carrying product with reduced risk of disease transmission and longterm storage capability for use when RBCs are not available or not an option. Military battlefield casualties,⁵⁷ disaster scenarios,⁵⁸ blood incompatibility and shortages, and religious objection⁵⁹ represent additional situations in which PolyHeme can address this critical unmet medical need. The combination of PolyHeme's life-sustaining capability, the logistic benefits, and the acceptable benefit-to-risk calculus for the intended indication represent an opportunity to provide an alternative to transfusion for patients at high risk of death when stored RBCs are not available.

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