A Safety and Efficacy Evaluation of Hemoglobin-Based Oxygen Carrier HBOC-201 in a Randomized, Multicenter Red Blood Cell Controlled Trial in Noncardiac Surgery Patients

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BACKGROUND: We present the results of a previously unpublished hemoglobin-based oxygen carrier (HBOC) study conducted in 1998–1999.

METHODS: In a multicenter, randomized, single-blind, comparative study of HBOC-201 versus allogeneic red blood cell (RBC) transfusions, no-cardiac surgery patients received HBOC-201 to a maximum of 7 units ($n = 83$) or RBCs ($n = 77$). Patients could be switched to RBCs for safety or any other reason. The efficacy end points were elimination and/or reduction of allogeneic RBC transfusions for 28 days.

RESULTS: The proportion of patients in the HBOC-201 group that avoided RBC transfusion was 0.427 (95% confidence interval, 0.321–0.533). Subjects in the HBOC-201 group received on average 3.2 units of RBCs versus 4.4 units in the control arm ($P = 0.004$). Seventy-nine (95.2%) subjects in the HBOC-201 group and 72 (93.5%) in the RBC group experienced adverse events (AEs), judged to be associated with study treatment in 59 (71.1%) and 18 (23.4%) subjects, respectively. Thirty-day mortality, 5 (6.0%) vs 4 (5.2%) patients ($P = 1.00$), incidence of serious AEs, 24 (28.9%) vs 20 (26.0%) ($P = 0.73$), or time to intensive care unit (log-rank $P = 0.15$) or hospital discharge (log-rank $P = 0.53$) were similar for the HBOC-201 and RBC groups, respectively.

CONCLUSIONS: Up to 7 units of HBOC-201 infused over the course of 6 days resulted in RBC transfusion avoidance in 43% of patients. There were no notable differences in mortality and serious AEs incidence. The use of HBOC-201 was associated with a notable excess of nonserious AEs. (Anesth Analg 2014;119:766–76)

During the 1970s and 1980s, many began to voice concern over the safety of blood transfusions. This led to the development of a number of hemoglobin-based oxygen carriers (HBOCs), which by the mid-1980s and 1990s were being touted as potential alternatives to red blood cell (RBC) transfusions. The present clinical trial was the culmination of a number of smaller phase II studies and served as the basis for the design of a subsequent phase III study investigating HBOC-201 as an alternative to blood transfusion in elective orthopedic surgery.[1] Since the completion of this clinical trial, transfusion medicine in general and HBOC therapy in particular have evolved considerably.[2,3] Before that the topic of "transfusion trigger" appeared in a number of publications.[4,5] Large clinical trials were conducted with different HBOCs, producing controversial results that ultimately led to a 2008 *Journal of the American Medical Association* meta-analysis[6] that questioned the safety of all HBOCs. The publication of these study results was delayed because the product developer and study Sponsor (Biopure Corporation, Cambridge, MA) filed for bankruptcy. OPK Biotech LLC (Cambridge, MA) acquired the former Biopure Corporation's assets and resumed both the manufacture and clinical development of HBOC-201.

The intent of this publication was to provide an objective evaluation of this randomized RBC-controlled clinical trial with HBOC-201. The Methods, Results, and Conclusions are presented as in original research report. An interpretation of findings in the context of contemporary transfusion medicine is included in the Discussion.

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Although RBC transfusions are an integral part of severe perioperative anemia treatment, logistical difficulties,[7,8] perception of risk, and past concerns regarding the safety and quality of the blood supply led to the development of oxygen-carrying solutions previously often referred to as "blood substitutes."

A comprehensive preclinical program in the 1990s established the ability of HBOC-201 to successfully deliver oxygen to the tissues[9–13] and treat severe anemia in a variety of animal models.[14–18] This was followed by a number of phase I and II clinical studies to evaluate the product's safety in the treatment of anemia.[19–21] Product safety and hemodynamic effects were also evaluated in patients undergoing cardiac procedures.[22,23] Presented in this article is the first of 2 phase III trials designed to evaluate safety and efficacy of HBOC-201 as a treatment for surgical anemia.

Currently, HBOC-201 is being clinically evaluated in 2 separate investigations: an Englewood Hospital and Medical Center, NJ, investigator-sponsored study, expanded access for patients with life-threatening anemia for whom RBC transfusion is not an option (ClinicalTrials.gov Identifier: NCT01881503), and a prehospital, randomized control (normal saline) trauma study in subjects with hemorrhagic shock, cosponsored by Alfred Hospital, Royal Melbourne Hospital and Ambulance Victoria, in Melbourne, Australia.

METHODS

Objectives

The study was conducted in accordance with the ethical principles stated in the Good Clinical Practices regulations of the U.S. Food and Drug Administration (FDA) (21 CFR, Part 56, and Informed Consent regulations, 21 CFR, Part 50). The protocol, informed consent form, and protocol amendment were approved in writing by each participating center's ethics committee. Written informed consent was obtained from each participant or their legal representative in a language they understood before undergoing any study-related procedures.

This was a multicenter therapeutic confirmatory, randomized, single-blind, RBC-controlled, comparative, parallel-group study of HBOC-201 administered to noncardiac surgery patients in place of RBC transfusions. It was primarily designed to estimate the proportion of patients who did not receive any transfusions of allogeneic RBCs during the study after initiation of treatment with HBOC-201 treatment, which was up to 7 units in the first 6 days.

The secondary objectives were to compare treatment arms for the number of RBC units transfused during the study and time to convalescence milestones. The safety and tolerability of HBOC-201 when used in place of allogeneic RBC transfusion was also evaluated. This study was conducted in 1997–1999, preceding the clinical trial registration requirement by several years.[a]

Product Characteristics

HBOC-201 (OPK Biotech LLC) is a cell-free purified, gluteraldehyde, cross-linked, and polymerized bovine hemoglobin (Hb) in a modified lactated Ringer's solution and is stable at room temperature (2–30°C) for 3 years. HBOC-201 requires no crossmatching and has a circulatory half-life of approximately 19 to 24 hours.[24] HBOC-201 is an isosmotic solution and its oxygen release is independent of 2,3-diphosphoglycerate concentrations. It does not require reconstitution and can be administered directly into a peripheral or central vein. The volume of 1 unit is 250 mL with a Hb concentration of 13 g/dL.

Trial Design

The study population comprises male or female patients older than 18 years in noncardiac surgery settings, for whom the decision to transfuse allogeneic blood had already been made. Patients with underlying medical conditions that would either put them at undue risk or preclude meaningful study-related evaluations were excluded. These included organ transplant recipients, anyone undergoing cardiopulmonary bypass, or partial pancreatectomy. Also excluded were major acute neurological disorders, uncontrolled hypertension, hematological disorders, acute or chronic hepatic disease, renal disease, severe carotid artery stenosis, coronary artery disease, congestive cardiac failure, a predisposition to systemic mast cell degranulation or hypersensitivity reactions, and anyone who was expected to require an allogeneic blood transfusion of >6 units.

Patient screening was conducted and informed consent obtained up to 1 month before surgery. During the perioperative period (within 24 hours of surgery until enrollment), the investigators were required to estimate a discharge Hb for each patient. After the first transfusion decision, patients were enrolled and randomly assigned (1:1) to 1 of 2 treatment groups (HBOC-201 or RBC).

Each subject's randomization assignment was provided in a sealed and sequentially numbered envelope corresponding to enrollment order for each participating site. Patients for whom the transfusion decision was reached after postoperative day 3 were ineligible for enrollment.

Clinical trial material (CTM) was defined for both treatment arms as 6 transfusions over the course of 6 days. If study participants in either treatment arm needed a transfusion subsequent to the first 6 transfusions or after day 6, they would be given RBCs, which would be considered non-CTM.

The first HBOC-201 infusion was 2 units (60 g Hb) to match the Hb content of 1 unit of RBC. Therefore, up to 7 units could be infused when compared to a maximum of 6 units in the RBC treatment arm. To assure patient safety, the principal investigator could at any time and for any reason switch subjects in the HBOC-201 group to RBCs. To avoid bias, investigators were also not obligated to disclose their reasons for switching (Fig. 1).

Baseline measurements were collected immediately before the first CTM administration. The follow-up period began at the end of the treatment period and included a final evaluation 3 to 4 weeks postoperatively.

Transfusion Decision

If a study subject's total Hb level was at least 2 g/dL less than the estimated discharge Hb or <6 g/dL, then a transfusion was required. If the total Hb was >10 g/dL, a transfusion was not permitted. Otherwise, a transfusion was allowed if one or more of the following was present: heart rate > 100 beats per minute; systolic blood pressure < 90 mm Hg; metabolic acidosis (base excess −4 or worse); acute blood loss

[a]Food and Drug Administration Amendments Act of 2007 (FDAAA; U.S. Public Law 110–85, Title VIII).

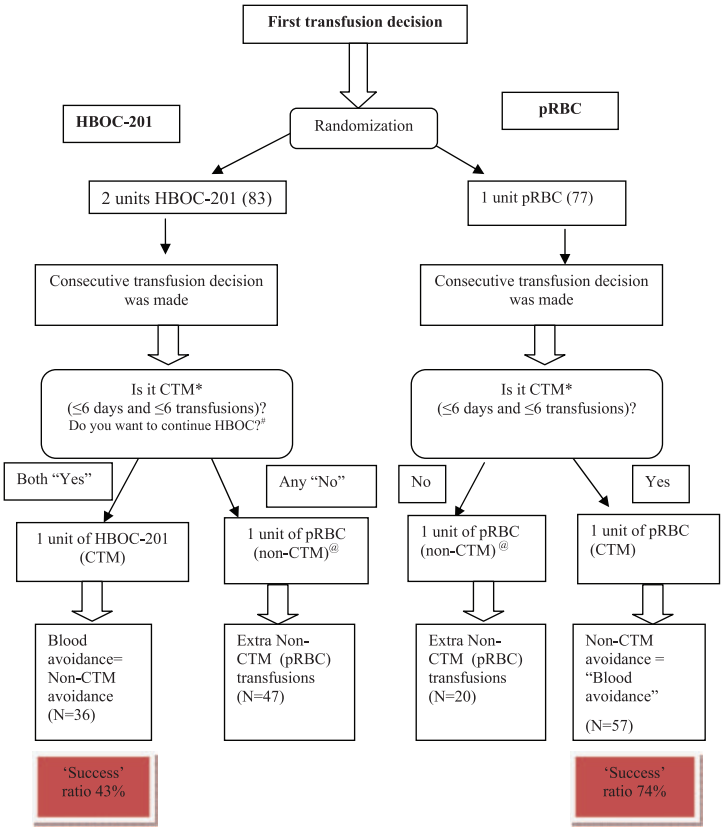

Figure 1. Schedule of CTM Administration. *CTM was defined for both arms as 6 transfusions over the course of 6 days. #Principal investigators were not obliged to provide reason for switching to RBC. @Non-CTM transfusions in both arms were RBC. HBOC = hemoglobin-based oxygen carrier; CTM = clinical trial material.

> 7 mL/kg within a period of 2 hours; oliguria with urine output < 0.5 mL/kg/h for ≥ 2 hours; and restricted activity due to weakness or dizziness. The HBOC-201 infusion rate was left to the discretion of each investigator.

Statistical Methods and Determination of Sample Size

The intention-to-treat (ITT) population included in the analyses was defined per protocol as all patients who had received at least 1 dose of CTM. The proportion of subjects in the HBOC-201 group who did not receive any allogeneic RBCs during the study was calculated as a function of time in hours using the Kaplan–Meier (product limit) method. A generalized linear model using a log-link function and Poisson distribution, using treatment and treatment center as fixed effects, analyzed the total number of units of RBCs. In addition, a Friedman test was performed for a treatment difference stratified by center. Intensive care unit (ICU) and hospital discharge were analyzed with Kaplan–Meier curves. Between-group differences in serious adverse event (SAE) incidence were analyzed by the Cochran–Mantel–Haenzel test. Where appropriate, other data were listed and described with summary statistics.

The target sample size in this study (80 enrolled per treatment group) was chosen according to the primary objective: the study was powered to detect 20% blood avoidance rate (power = 0.8, $\alpha = 0.05$) with 10% lower confidence limits.

The data-monitoring committee conducted an interim safety review of the first 40 enrolled subjects, and the trial was allowed to proceed. No interim efficacy analysis was performed.

RESULTS

Demographic

Twenty-one investigator sites participated: 12 in Europe and 9 in South Africa. Overall, there were 161 patients screened and 160 enrolled. All enrolled participants received the treatment to which they were randomized: 83 in the HBOC-201 group and 77 in the RBC group. The only enrollment deviation was that subjects 1004 and 1005 were randomized out of order; subject 1004 was randomized using envelope

Table 1. Study Demographics and Type of Surgery

	HBOC-201 *n* = 83 *n* (%)	RBC *n* = 77 *n* (%)
Age (years)		
Mean (SD)	61.1 (13.2)	60.9 (14.0)
Median (min/max)	63.0 (21 to 86)	62.0 (22 to 85)
Gender		
Male	46 (55.4)	44 (57.1)
Female	37 (44.6)	33 (42.9)
Race		
Caucasian	75 (90.4)	72 (93.5)
Black	4 (4.8)	4 (5.2)
Oriental/Asian	3 (3.6)	1 (1.3)
Other	1 (1.2)	0
Type of surgery		
Gastrointestinal	14 (16.9)	13 (16.9)
Genitourinary	4 (4.8)	6 (7.8)
Orthopedic	35 (42.2)	31 (40.3)
Vascular	27 (32.5)	24 (31.2)
Other	3 (3.6)	3 (3.9)

HBOC = hemoglobin-based oxygen carrier.

5; and subject 1005 randomized using envelope 4. During analyses, since these patients were treated "as randomized," ITT and "as treated" populations were considered equivalent.

Based on ITT analysis, 10 (12%) subjects in the HBOC-201 group and 4 (5%) subjects in the RBC group discontinued the study prematurely (P = 0.16) due to adverse events (AEs) (HBOC-201 4 [5%], RBC 2 [3%]); investigator discretion (HBOC-201 2 [2%]); lost to follow-up (HBOC-201 1 [1%], RBC 1 [1%]); and refusal to continue (HBOC-201 2 [2%], RBC 1 [1%]). The 2 treatment groups were similar for mean and median age, gender, and race composition. Demographic data are summarized in Table 1.

Compliance

There were initially 27 entry criteria violations identified, all related to level of total Hb at the time of the first transfusion decision, 13 (16%) in the HBOC-201 group and 14 (18%) in the RBC group (P = 0.68). On review of all these patients, 18 were noted to have had medical records substantiating Hb < 10 g/dL or significant blood loss. There were 3 subjects in the HBOC-201 arm and 6 subjects in the RBC arm for whom no documentation was available to justify study enrollment. Primary efficacy analyses were repeated with each of the subsets excluding different violations and yielded similar results (not presented).

Efficacy

The primary efficacy end point was the proportion of patients in the HBOC-201 arm who did not receive any allogeneic RBCs during the study. The lower confidence limits of proportion estimate over all analyzed subsets were in the range of 0.26 to 0.32. Thus, with a Kaplan–Meier proportion estimate of 0.427, it was concluded that HBOC-201 successfully eliminated the need for blood transfusions in a clinically and statistically significant proportion of patients. Primary efficacy analysis for the ITT population is provided in Table 2.

On average, 3.2 (SD = 5.9) RBC units were administered to HBOC subjects compared to the 4.4 (SD = 4.1) units in the control group (Wald confidence interval for parameter treatment in log-linear model adjusted by centers was (−0.22, −0.56) with Poisson P < 0.001; Friedman P = 0.004). The median was 2.0 (range 0–45) and 3.0 (range 1–27), respectively. The extent of CTM exposure and breakdown of non-CTM avoidance are summarized in Table 3.

Table 2. Primary Efficacy Analysis

Analysis group	Intent to treat (n = 83)
Subjects who had no RBC transfusions in the 28 days following first CTM infusion, n (%)	36 (43.4%)
Median time (95% CI) to first allogeneic RBC transfusion,[a] days	7.4 (5.5, —[b])
Kaplan–Meier proportion estimates and 95% CI at	
Day1	0.819 (0.737–0.902)
Day 7	0.553 (0.446–0.660)
Day 28	0.427 (0.321–0.533)[c]

CTM = clinical trial material; CI = confidence interval.

[a]Allogeneic transfusions were defined as packed red blood cells (RBCs) or whole blood only.

[b]No upper interval as not enough patients received allogeneic RBC transfusions.

[c]Kaplan–Meier estimates deviate from observed proportions due to censoring.

Per protocol non-CTM transfusions for either treatment arm were defined as RBCs given after the first 6 transfusions or later than day 6. The N (%) column for both arms shows how many subjects received 1 to 6 CTM transfusions, the CTM only/non-CTM column breaks this number into 2, those who avoided non-CTM (RBC) transfusions and those who did not. All patients are accounted for in the totals: 83 HBOC and 77 RBC subjects with overall rates of non-CTM avoidance 43% and 74%, respectively. The last column describes full exposure to blood products in the RBC arm and (despite expectation of enrollment) shows that 17% of RBC subjects needed >6 units of RBC.

Time to Convalescence Milestones

A secondary objective was to assess time to convalescence milestones. There were no statistically significant differences between treatment groups (HBOC-201 versus RBC) in time to ICU (Kaplan–Meier estimates for median were 3.2 [2.1–6.6] and 2.1 [1.3–3.7], log-rank P = 0.15) or hospital discharge (Kaplan–Meier estimates for median were 14 [12–16] and 13 [11–14], log-rank P = 0.53). Although the estimates (in days) for hospital discharge are very close, a notable trend toward longer time for HBOC-201 to ICU discharge might be attributed to lower efficacy of HBOC-201 in comparison with RBC (see Analysis of Major Hematology Markers).

Safety

Overall, 79 (95.2%) subjects in the HBOC-201 group and 72 (93.5%) subjects in the RBC group experienced AEs (P = 0.74). Fifty-nine (71.1%) subjects in the HBOC-201 group and 18 (23.4%) in the RBC group had AEs judged to be associated with study treatment (P < 0.001) (see safety assessment in the Discussion). The majority of AEs were mild to moderate in severity. The most common all-cause AEs were hypertension and fever, experienced by 48 (30%) subjects and 47 (29%) subjects, respectively. Hypertension may have been reported more commonly in the HBOC-201 (29 [35%] vs 19 [25%], P =0.17) group and fever (23 [28%] vs 24 [31%], P =0.73) more commonly in the RBC group.

In general, by Coding Symbols for a Thesaurus of Adverse Reaction Terms (COSTART), body system totals for all-cause AEs were higher in the HBOC-201 group than in the RBC group. Cardiovascular system AEs, however, were evenly distributed between the 2 groups. The only cardiovascular term with higher incidence in the HBOC arm was hypertension (transient increase in arterial blood pressure). The most common AEs are summarized in Table 4.

The greatest difference between the 2 groups was in the incidence of jaundice, reported in 31 (37.3%) HBOC-201 subjects and in 1 (1.3%) RBC subject (P < 0.001). All treatment-associated instances of jaundice in the HBOC-201 group were resolved, and approximately 50% of these had a duration of 3 days or less. There were no reports of liver failure in anyone with treatment-associated jaundice.

Transient jaundice was associated with higher doses of HBOC-201. Of the 29 subjects who experienced

Table 3. Extent of Exposure to Clinical Trial Material (CTM) and Avoidance of Non-CTM Transfusions

	HBOC-201 group (by CTM transfusions)			RBC group[a] (by CTM transfusions)		RBC group[b] (all RBC units)
Number of CTM transfusions	Total HBOC-201 hemoglobin (g)	*N* (%)	Breakdown of non-CTM avoidance CTM only/non-CTM	*N* (%)	Breakdown of non-CTM avoidance CTM only/non-CTM	*N* (%)
1	60[c]	10 (12.0)	9 (90)/1 (10)	11 (14.3)	9 (82)/2 (18)	9 (11.7)[d]
2	90	5 (6.0)	3 (60)/2 (40)	24 (31.2)	21 (88)/3 (12)	22 (28.6)[d]
3	120	18 (21.7)	9 (50)/9 (50)	11 (14.3)	10 (91)/1 (9)	11 (14.3)
4	150	6 (7.2)	2 (33)/4 (67)	10 (13.0)	8 (80)/2 (20)	10 (13.0)
5	180	12 (14.5)	4 (33)/8 (67)	5 (6.5)	4 (80)/1 (20)	6 (7.8)
6	210	32 (38.6)	9 (28)/23 (72)	16 (20.8)	5 (31)/11 (69)	6 (7.8)
Total		83 (100)	36 (43)/47 (57)	77 (100)	57 (74)/20 (26)	64 (83)

HBOC = hemoglobin-based oxygen carrier.
[a]Each red blood cell (RBC) transfusion = 1 unit packed RBC or 1 unit whole blood (<5%).
[b]When counting all RBC units given in RBC arm 13 (16.9%) patients received >6 units.
[c]First CTM transfusion in HBOC arm was 2 units of HBOC-201, all next were 1 unit.
[d]When counting all RBC units given in RBC arm number of patients that received 1 to 2 units decreased.

treatment-associated jaundice, 22 subjects received 5 or more units (17 subjects received the maximum 7 units). The skin pigmentation observed in most cases was a pre-hepatic jaundice based on increased bilirubin load and was consistent with physiological processing of HBOC-201 to bilirubin.

Hypertension was the AE with the next highest difference in incidence (Table 4). Thirteen of the treatment-associated

Table 4. Summary of Patients with All Causality Adverse Events[a]

	All cause (≥10%)		Treatment related (≥2.5%)	
Body system	HBOC-201 group	RBC group	HBOC-201 group	RBC group
COSTART preferred term	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Digestive system	63 (75.9%)†	49 (63.6%)	39 (47.0%)	5(6.5%)
Constipation	13 (15.7%)*	2 (2.6%)	*0*	*0*
Diarrhea	9 (10.8%)	11 (14.3%)	*1 (1.2%)*	*0*
Dysphagia	*4 (4.8%)*†	*0*	3 (3.6%)	0
Jaundice	31 (37.3%)*	1 (1.3%)	29 (34.9%)	1 (1.3%)
Nausea	17 (20.5%)	20 (26.0%)	9 (10.8%)†	2 (2.6%)
Nausea and vomiting	9 (10.8%)	7 (9.1%)	3 (3.6%)	1 (1.3%)
Vomiting	10 (12.0%)	8 (10.4%)	5 (6.0%)	2 (2.6%)
Body as a whole	61 (73.5%)*	41 (53.2%)	26 (31.3%)	8 (10.4%)
Abdominal pain	11 (13.3%)†	4 (5.2%)	8 (9.6%)	1 (1.3%)
Asthenia	16 (19.3%)*	6 (7.8%)	4 (4.8%)†	0
Fever	23 (27.7%)	24 (31.2%)	5 (6.0%)	5 (6.5%)
Pain	21 (25.3%)	19 (24.7%)	*1 (1.2%)*	*0*
Cardiovascular system	49 (59.0%)	45 (58.4%)	21 (25.3%)	3 (3.9%)
Atrial fibrillation	5 (6.0%)	8 (10.4%)	*1 (1.2%)*	*0*
Hemorrhage	9 (10.8%)	11 (14.3%)	3 (3.6%)	1 (1.3%)
Hypertension	29 (34.9%)†	19 (24.7%)	14 (16.9%)	0
Tachycardia	12 (14.5%)	12 (15.6%)	*1 (1.2%)*	*0*
Metabolic and nutritional disorders	47 (56.5%)*	29 (37.7%)	13 (15.7%)	2 (2.6%)
Edema	9 (10.8%)	7 (9.1%)	4 (4.8%)†	0
Healing abnormal	15 (18.1%)	10 (13.0%)	*0*	*1 (1.3%)*
Hypokalemia	10 (12.0%)	5 (6.5%)	*1 (1.2%)*	*0*
Hemic and lymphatic system	21 (25.3%)†	11 (14.3%)	7 (8.4%)	0
Methemoglobinemia	*4 (4.8%)*†	*0*	4 (4.8%)	0
Nervous system	35 (42.2%)	25 (32.5%)	6 (7.2%)	2 (2.6%)
Confusion	13 (15.7%)	7 (9.1%)	3 (3.6%)	0
Insomnia	11 (13.3%)	8 (10.4%)	1 (1.2%)	2 (2.6%)
Respiratory system	31 (37.3%)	21 (27.3%)	*4 (4.8%)*	*1 (1.3%)*
Dyspnea	4 (4.8%)	8 (10.4%)	*0*	*1 (1.3%)*
Skin and appendages	23 (27.7%)†	12 (15.6%)	15 (18.1%)	3 (3.9%)
Pruritus	*3 (3.6%)*	*2 (3.6%)*	3 (3.6%)	1 (1.3%)
Rash	13 (15.7%)†	6 (7.8%)	8 (9.6%)	2 (2.6%)
Urogenital system	33 (39.8%)	27 (35.1%)	6 (7.2%)	2 (2.6%)
Hematuria	14 (16.9%)†	5 (6.5%)	3 (3.6%)	1 (1.3%)
Oliguria	19 (22.9%)	12 (15.6%)	*2 (2.4%)*	*1 (1.3%)*

HBOC = hemoglobin-based oxygen carrier.
[a]Adverse events (AEs) experienced by ≥10% of patients in either treatment group) or treatment-associated (including unknown association) AEs (experienced by ≥2.5% of patients in either treatment group). Italics were used while reporting incidences that did not make a cut for 1 category.
**P* value (Fisher exact 2-tail test) <0.05.
†*P* value (Fisher exact 2-tail test) between 0.05 and 0.2.

Table 5. Arterial Blood Pressure (BP) After First Infusion[a]: Summary of Changes

	Preinfusion	Change	Preinfusion	Change
	Under anesthesia		Pre- or postanesthesia	
Parameter	Mean (SD, *n*)	Mean (SD, *n*)	Mean (SD, *n*)	Mean (SD, *n*)
HBOC-201 group				
Supine systolic BP (mm Hg)	112.4 (25.1, 51)	19.3 (27.7, 36)	121.3 (18.6, 32)	28.0 (23.7, 22)
Supine diastolic BP (mm Hg)	59.5 (12.0, 51)	12.2 (13.7, 36)	62.9 (11.8, 32)	14.8 (10.5, 22)
Red blood cell group				
Supine systolic BP (mm Hg)	108.7 (19.9, 44)	14.2 (33.1, 20)	121.0 (25.8, 33)	10.7 (35.0, 17)
Supine diastolic BP (mm Hg)	54.9 (12.1, 44)	4.8 (13.9, 20)	63.7 (13.8, 33)	7.8 (19.8, 17)

HBOC = hemoglobin-based oxygen carrier.
[a]Transfusions 2 to 6 are not shown due to insufficient sample sizes.

instances of hypertension in the HBOC-201 group were resolved. One instance judged to have an unknown association with study treatment was reported as ongoing. In 7 of the 14 subjects, the duration of hypertension was 2 days or less.

A more detailed analysis of increases in arterial blood pressure after the first study infusion appears in Table 5. Changes in systolic and diastolic blood pressure were larger in the HBOC-201 group but not clinically significant.

All treatment-associated cases of nausea in the HBOC-201 group resolved, and all occurred in subjects who received 3 or more infusions. There were no clinically significant changes in temperature, respiration rate, or heart rate after CTM administration. Mean heart rates increased from baseline in both groups. In the HBOC-201 group, heart rate increased from 73.7 bpm at baseline to 84.2 bpm at postoperative day 7. At follow-up, heart rates were 79.2 bpm for the HBOC-201 group and 79.7 bpm for the RBC group.

SAEs are reported in Table 6. Thirty-day mortality in the trial was 5 (6.0%) subjects in the HBOC-201 group and 4 (5.2%) subjects in the RBC group ($P = 1.00$). None of the deaths in either group was considered to be CTM related. Overall, 24 (28.9%) subjects in the HBOC-201 group and 20 (26.0%) subjects in the RBC group experienced SAEs. There were no notable differences in incidence reported by COSTART by either body system or preferred terms. All incidences were <5%. The difference between the 2 groups in the incidence of all SAEs (% of patients) was not statistically significant ($P = 0.69$; 95% confidence interval, −11.02, 16.6).

Analysis of laboratory variables indicates that mean blood urea nitrogen, creatinine, lipase, serum glutamic-pyruvic transaminase, and serum glutamic oxaloacetic transaminase values increased at postoperative day 7 and decreased toward baseline levels at follow-up (Table 7). At follow-up, mean blood urea nitrogen, creatinine, lipase, and serum glutamic-pyruvic transaminase levels in both groups were still elevated when compared with baseline, but serum glutamic oxaloacetic transaminase was similar to baseline. Mean levels at follow-up were similar in both treatment arms. It appears that for all measurements, transient (at day 7 only) elevations in the HBOC-201 arm were observed in comparison with control, but (probably, due to small sample size) none of them reached statistical significance.

Table 6. Serious Adverse Events

Preferred term	HBOC-201 *n* (%)	RBC *n* (%)
Abscess	0	1 (1.3)
Acidosis	0	1 (1.3)
Anemia (1 vs 0)	1 (1.2)	0
Aspiration pneumonia	0	1 (1.3)
Atrial fibrillation	0	2 (2.6)
Biliary pain	1 (1.2)	0
Cerebrovascular accident (CVA)	2 (2.4)	0
Cholestatic jaundice	0	1 (1.3)
Colitis (1 vs 0)	1 (1.2)	0
Convulsion	0	1 (1.3)
Disseminated intravascular coagulation	1 (1.2)	0
Dyspnea	1 (1.2)	3 (3.9)
Edema	0	1 (1.3)
Fever	0	2 (2.6)
GI hemorrhage	1 (1.2)	1 (1.3)
Healing abnormal (0 vs 1)	1 (1.2)	2 (2.6)
Heart arrest	0	2 (2.6)
Hemorrhage (1 vs 0)	3 (3.6)	2 (2.6)
Hepatobiliary leakage	1 (1.2)	0
Hypothyroidism	1 (1.2)	0
Hypoxia (1 vs 0)	1 (1.2)	0
Ileus	1 (1.2)	0
Infection	1 (1.2)	1 (1.3)
Insufficiency of anastomosis	1 (1.2)	0
Insufficiency of collar anastomosis	0	1 (1.3)
Intestinal obstruction	1 (1.2)	0
Lung disorder	1 (1.2)	0
Lung edema	1 (1.2)	1 (1.3)
Lung function decreased	0	1 (1.3)
Myocardial infarct	1 (1.2)	0
Oliguria	0	1 (1.3)
Peritonitis	2 (2.4)	0
Pneumonia	1 (1.2)	2 (2.6)
Pneumothorax	0	1 (1.3)
Pulmonary embolism	0	1 (1.3)
Reaction unevaluable	0	1 (1.3)
Rectal hemorrhage	0	2 (2.6)
Respiratory disorders	0	1 (1.3)
Sepsis	2 (2.4)	0
Shock	1 (1.2)	1 (1.3)
Urinary retention	1 (1.2)	0
Urinary tract infection	0	1 (1.3)

P values were not provided since the smallest of them are >0.6. Incidences for SAEs of unknown association are given in italic.
HBOC = hemoglobin-based oxygen carrier.

Analysis of Major Hematology Markers

Table 8 presents pre- and postinfusion hematology markers, i.e., total Hb, hematocrit (HCT), and plasma Hb by infusion number. Despite the challenges of consistently collecting hematology data between back-to-back infusions, which led to the different subsets of patients for pre- and postmeasurements (thus limiting available data for the calculation of

Table 7. Selected Clinical Chemistry Parameters: Summary of Changes from Baseline

	Baseline	Change POD 1	Change POD 7	Change follow-up
HBOC-201 group: Mean (SD, *n*)				
BUN (mmol/L)	11.5 (9.3, 83)	1.6 (8.3, 68)	5.7 (18.7, 68)	1.9 (7.9, 73)
Creatinine (μmol/L)	82.6 (22.7,83)	14.2 (22.6, 78)	8.7 (30.9, 68)	8.9 (7.3, 73)
Lipase (U/L)	30.0 (23.9, 33)	N/A	23.6 (57.7, 29)	14.6 (28.4, 25)
SGOT (U/L)	27.1 (29.3, 83)	N/A	14.8 (40.2, 66)	−0.9 (26.4, 73)
SGPT (U/L)	22.7 (24.9, 83)	N/A	35.8 (91.8, 49)	10.0 (45.9, 73)
RBC group: Mean (SD, *n*)				
BUN (mmol/L)	11.9 (10.5, 76)	−0.9 (10.7, 72)	1.1 (13.1, 56)	2.1 (14.6, 70)
Creatinine (μmol/L)	88.7 (29.4, 74)	10.4 (26.0, 70)	4.7 (19.1, 52)	12.9 (35.7, 68)
Lipase (U/L)	30.1 (33.4, 31)	N/A	20.8 (58.1, 24)	12.8 (49.2, 27)
SGOT (U/L)	30.0 (56.2, 75)	N/A	10.4 (61.2, 54)	−2.9 (62.3, 69)
SGPT (U/L)	21.5 (24.5, 76)	N/A	25.9 (27.0, 34)	11.4 (36.4, 70)

BUN = blood urea nitrogen; HBOC = hemoglobin-based oxygen carrier; POD = postoperative day; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase.

Table 8. Absolute Pre- and Postinfusion Hematology Markers by Infusion Number

	HBOC-201: value (*n*, SE)			RBC: value (*n*, SE)		
Infusion no.	Total Hb (g/dL)	HCT (%)	Plasma Hb (g/dL)	Total Hb (g/dL)	HCT (%)	Plasma Hb (g/dL)
Preinfusion 1	8.76 (83, 0.18)	26.1 (83, 0.6)	0.0 (77, 0.0)	8.54 (77, 0.19)	25.6 (77, 0.6)	0.0 (70, 0.0)
Postinfusion 1	9.10 (57, 0.20)	23.9 (56, 0.6)	1.5 (54, 0.1)	9.81 (35, 0.34)	29.1 (35, 1.0)	0.0 (31, 0.0)
Preinfusion 2	8.00 (36, 0.18)	21.7 (36, 0.6)	0.9 (34, 0.1)	8.79 (11, 0.46)	25.8 (11, 1.4)	0.1 (11, 0.1)
Postinfusion 2	8.89 (38, 0.22)	22.5 (39, 0.7)	2.1 (35, 0.2)	9.91 (41, 0.22)	30.1 (47, 0.6)	0.0 (43, 0.0)
Preinfusion 3	8.15 (28, 0.25)	21.2 (28, 0.7)	1.0 (26, 0.2)	7.86 (13, 0.36)	24.0 (16, 0.8)	0.0 (13, 0.0)
Postinfusion 3	8.77 (49, 0.19)	21.0 (49, 0.6)	2.3 (45, 0.1)	9.86 (26, 0.31)	29.6 (26, 0.9)	0.1 (23, 0.0)
Preinfusion 4	7.96 (25, 0.22)	20.3 (25, 0.8)	1.3 (24, 0.2)	8.56 (10, 0.44)	25.7 (10, 1.4)	0.0 (10, 0.0)
Postinfusion 4	8.58 (31, 0.18)	19.3 (30, 0.7)	2.5 (27, 0.2)	9.54 (19, 0.29)	29.3 (19, 0.8)	0.1 (18, 0.0)
Preinfusion 5	7.91 (24, 0.18)	19.2 (24, 0.8)	1.7 (22, 0.2)	8.63 (9, 0.52)	27.0 (9, 1.0)	0.0 (8, 0.0)
Postinfusion 5	8.25 (28, 0.19)	18.5 (28, 0.9)	2.5 (25, 0.2)	10.07 (13, 0.37)	29.9 (13, 1.1)	0.1 (14, 0.0)
Preinfusion 6	7.97 (11, 0.33)	18.3 (11, 1.5)	2.3 (11, 0.3)	8.48 (5, 0.57)	25.1 (5, 1.8)	0.0 (4, 0.0)
Postinfusion 6	8.00 (30, 0.23)	16.6 (30, 0.8)	2.8 (28, 0.2)	9.76 (14, 0.37)	29.1 (13, 1.1)	0.0 (13, 0.0)

HBOC = hemoglobin-based oxygen carrier.

changes), the observed results illustrate the general dynamic of hematology markers in both arms during the treatment. Because of small sample sizes, hematologic markers are not reported on a per transfusion basis but rather by combining all available data for 3 major types of CTM administration, as per Table 9.

These results demonstrate the short-term impact of both HBOC-201 and RBC infusions. The HCT would decrease with transfusion of HBOC-201 and increase with RBCs. In both cases, total Hb would increase but more so with RBC transfusions. This reflects the dual hemodilution effect caused by the absence of RBCs (for HCT) and significantly lower Hb concentration in HBOC-201 compared to RBC (total Hb).

To assess the long-term impact of treatment in both arms, the data were analyzed using a strictly chronological approach (Table 10). These results suggest that the difference in total Hb between the HBOC-201 and RBC arms increased slowly through day 1 to day 4 (from 0.9 to 1.4 g/dL), while the difference in HCT was stable near 6%. The maximum total Hb difference was achieved when average plasma Hb in the HBOC-201 arm decreased to 0.5 g/dL. Even though discharge days varied and data collected around day 10 and at discharge time were sparse, it appears that the marker values between the HBOC-201 and the RBC groups started to converge from day 4 to day 10. Nevertheless, the discharge Hb was notably lower in the HBOC arm (see Evaluation of Efficacy in Discussion).

The observed trend in the first day reflects the relatively short half-life of HBOC-201, while the rebounding of HCT could be attributed to the restoration of RBC (native RBC for patients who received HBCO-201 and avoided blood transfusion and allogeneic RBC for patients who received blood transfusions).

Circa 2000 Conclusion

Administration of up to 7 units of HBOC-201 over the course of 6 days enabled the avoidance of RBC transfusion in approximately 43% of treated subjects who would have otherwise required such transfusion. No deaths or SAEs were adjudicated to be associated with CTM, and there were no statistically significant differences between groups in the incidence of SAEs and 30-day mortality. Therefore, a key conclusion reached at that time was that HBOC-201 was well-tolerated in this study by both groups of participants who received HBOC-201: those who totally avoided RBCs and those who eventually received allogeneic blood.

Although there were no CTM-associated SAEs, HBOC-201 administration was coupled with a mild but notable side effect profile that resulted in an excess of non-SAEs in all main body systems (as defined by COSTART), with exception of the cardiac organ class (no difference).

Table 9. Changes in Hematology Markers Following CTM Infusions by Infusion Number

		Changes in measurements: value (n, SE)		
	Number of infusions	Total Hb (g/dL)	HCT (%)	Plasma Hb (g/dL)
HBOC-201, 1 unit	240	0.15 (65, 0.07)	−1.1 (65, 0.2)	0.65 (60, 0.04)
HBOC-201, 2 units	83	0.45 (57, 0.11)	−1.8 (56, 0.4)	1.51 (49, 0.07)
RBC, 1 unit	219	0.89 (71, 0.12)	+2.7 (70, 0.4)	0.0 (63, 0.0)

HBOC = hemoglobin-based oxygen carrier.

Table 10. Absolute Hematology Markers by Treatment Period

	HBOC-201: value (*n*, SE)			RBC: value (*n*, SE)		
Time point	Total Hb (g/dL)	HCT (%)	Plasma Hb (g/dL)	Total Hb (g/dL)	HCT (%)	Plasma Hb (g/dL)
Postoperative day 1	9.31 (79, 0.15)	24.3 (78, 0.6)	1.4 (72, 0.1)	10.22 (71, 0.18)	30.6 (71, 0.5)	0.0 (70, 0.0)
Postoperative day 2	9.09 (79, 0.15)	24.3 (79, 0.5)	1.7 (75, 0.1)	10.19 (72, 0.19)	30.5 (72, 0.6)	0.0 (69, 0.0)
Postoperative day 4	9.37 (81, 0.17)	26.8 (81, 0.5)	0.5 (76, 0.1)	10.78 (72, 0.19)	32.2 (72, 0.5)	0.0 (66, 0.0)
Postoperative day 7	10.08 (66, 0.19)	29.7 (66, 0.6)	0.1 (66, 0.0)	11.07 (54, 0.21)	33.0 (54, 0.6)	0.0 (49, 0.0)
Postoperative day 10	10.29 (12,0.57)	30.2 (12, 1.7)	0.0 (12, 0.0)	11.68 (12, 0.52)	34.8 (12, 1.6)	0.1 (11, 0.1)
Discharge	9.84 (16, 0.36)	28.9 (16, 1.1)	0.1 (16, 0.1)	11.33 (16, 0.53)	33.6 (16, 1.7)	0.0 (12, 0.0)
Follow-up	11.94 (72, 0.17)	36.0 (72, 0.5)	N/A	11.98 (73, 0.19)	36.2 (73, 0.5)	N/A

HBOC = hemoglobin-based oxygen carrier.

DISCUSSION

The history of HBOCs' clinical development has not been without controversy, with proponents and detractors of the field disagreeing over safety, efficacy, and even the need for such products. Inconclusive or negative results from several clinical trials [1,25] have contributed to unfavorable perceptions of safety with some attributing these to intrinsic Hb toxicity,[6] while others assigned blame on clinical trial design and HBOC limitations when compared to RBCs. The understanding of HBOCs' limitations relating to lower Hb concentrations (4–13 g/dL), short half-lives (6–20 hours), and their effect on the biomarkers widely used in making transfusion decisions (e.g., arterial blood pressure, and oxygen saturation) has improved significantly these past few years. This recently acquired understanding makes the use of HBOCs as a "blood substitute" highly problematic, and continued HBOC clinical development will require focusing on indications where blood is not an option or is not immediately available.

Of all the HBOC studies conducted, this study and the other phase III study with the same product (HBOC-201 as an alternative to blood transfusion in elective orthopedic surgery)[1] are the only true head-to-head comparison between HBOCs and RBC transfusion. Because it is unlikely that additional direct comparison studies will be conducted anytime soon, data from this study provide valuable information. As extensive data collection typical for phase III, these 2 trials also provide valuable insights on the treatment of anemia with RBCs.

Although the data and their interpretation are reported in the context and understanding of transfusion medicine and HBOCs at the time that this study was conducted, the Discussion has been written in the context of current understanding of HBOCs and RBC transfusion practice. Although data from this trial seemingly supported the use of HBOC-201 in place of RBC, there are several limitations to the interpretation of these results.

Study Design

The impact of single-blind study design bias on study results is not clear and depends on investigator expectations. For example, if HBOC-201 subjects were undertreated based on the assumption that they did not need the same level of total Hb as RBC patients, this could have led to the overestimation of HBOC-201 efficacy. In addition, given limited knowledge of HBOC usage compared with RBCs, the same observed events were assessed differently in terms of association with treatment (Table 4) and led to over-reporting of HBOC-201–associated AEs.

Transfusion Trigger

One could argue that ambiguous transfusion recommendations (total Hb between 6 and 10 g/dL) impair the study's ability to assess the impact of HBOC-201 usage on blood reduction and avoidance. This is not a trial design issue, but instead is a long-standing issue within the field of transfusion medicine, where despite many attempts to do so over the past 20 years, "more precise" recommendations for transfusion have yet to be issued.[2,4] The transfusion trigger for South African participants was noticeably higher than in Europe and likely related to the altitude at the South African study sites (1 mile above sea level), and most of the protocol violations came from these sites (typical Hb reference ranges are 1–1.5 g/dL higher). Study recommendations were in line with blood transfusion guidelines, which were in effect at the time. Average total Hb at the moment of the first transfusion decision across the study was 8.17 g/dL (n = 101, SE = 0.12) for Europe and 9.46 (n = 59, SE = 0.26) for South Africa.

Data Collection and Analysis

In the setting of anemia, factors such as patient condition, time of treatment, and transfusion volume complicate data analysis and the interpretation of results. In this study, some of the important data for the assessment of both safety and efficacy of HBOC-201 were not collected. Troponin and creatin kinase-MB were not tested after infusion because of concerns that the deeply colored HBOC-201 would interfere with the spectrophotometric assays. Troponin and creatin kinase-MB interference testing was not performed at all sites. The question of possible cardiotoxicity was addressed by analyses of electrocardiogram data and reported cardiac

AEs. Methemoglobin, which may influence the efficacy of HBOC-201, was not measured.

Sample Size and Safety Assessment

The trial was powered to detect safety signals with an increased incidence of 5% to 10%. Accordingly, a mild but demonstrable side effect profile of HBOC-201 was established. The seemingly impressive misbalance in treatment-associated AEs is, probably, not very informative due to the subjectivity of assignments (principal investigator was given a choice between "associated," "no association," and "unknown") and combining treatment-associated events with events of unknown association (see for example "Nausea and Vomiting" in Table 4). It is generally accepted that current generation HBOCs have vasoactive properties possibly due to nitric oxide scavenging.[26,27]

In this study, gastrointestinal effects and arterial blood pressure increases were evident in the HBOC arm, and thus, there was a safety concern especially with respect to cardiotoxicity. The absence of cardiac AEs and safety signals (except for arterial blood pressure increases) is consistent with results from other HBOC-201 studies,[22,23,28,29] which reported the absence of cardiotoxicity. A definitive conclusion, however, is precluded because the study was not powered to detect safety signals with an incidence of 1% to 3%.

Evaluation of Efficacy

The results of the trial demonstrated that the ability of HBOC-201 to restore total Hb is less than that of packed RBCs. The analyses for HBOC-201 are in agreement with theoretical expectations and analyses of efficacy derived later from the larger phase III trial with similar design.[1] Because of the lower HBOC-201 Hb concentration (13 g/dL) in comparison with that of packed RBCs (≈ 20–30 g/dL), increases in total Hb on a per unit basis are about 4 times lower (approximately 0.2 vs 0.9 g/dL) and consequently the initial loading dose to achieve an immediate increase of 1 to 2 g/dL in total Hb would be virtually impossible for normovolemic patients. Interestingly, this bears a similarity to whole blood transfusions. Although the increase in total Hb with HBOC-201 may seem small, it should be compared to the effect of plasma volume expanders, which would result in a decrease in total Hb of 0.5 g/dL.

The results (Tables 8 and 9) are in line with the theoretical expectations from a simplistic 1-compartment model (Fig. 2). In summary, the short-term effect of HBOC-201 transfusion on HCT is similar to that of plasma volume expanders, and on Hb it is similar to that of whole blood. Furthermore, the relatively short (19–24 hours) half-life of HBOC-201 requires constant redosing so as to maintain the total Hb level. To put this in perspective, the rate of complete blood avoidance (43%) in the HBOC arm was just slightly higher than 40% of patients in the control arm who received only 1 to 2 units of RBCs (Table 3). Similarly, using per protocol criteria (6 units/6 days), avoidance of non-CTM transfusions in the RBC arm was 74% (Fig. 1, Table 3).

On average, patients in the HBOC-201 arm were maintained with a total Hb concentration approximately 1 g/dL lower and a HCT approximately 6% lower (in absolute terms) than patients in the RBC arm. The clinical significance and impact of this difference on study outcome are not known, but patients having preexisting cardiac disease have been shown to be at risk when maintained at conservative Hb concentrations that were otherwise safe in healthier patients.[4,5,30]

The question of whether patients in the HBOC-201 arm were undertreated or patients in the RBC arm were overtreated with oxygen carriers compared with modern standards remains open; difference in discharge Hb between HBOC and RBC arms probably represents both factors. The most important question is whether this difference could

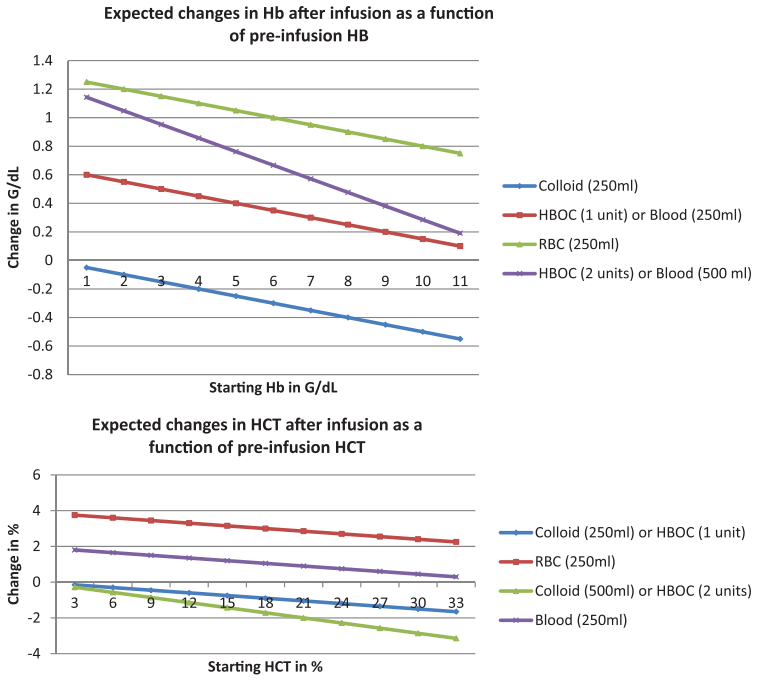

Figure 2. Impact on hematology markers: one-compartment model predictions. Calculations are based on formula $\delta = \Delta/k^*$, where δ is expected change in concentration, Δ is a difference between concentration in solution and in circulation (could be negative!), and k is the ratio of "volume in circulation after infusion" (V_{post}) to "volume of infusion" (v_{inf}). Assumptions: (1) No major fluid shifts: $V_{post} = V_{pre} + v_{inf}$; (2) Almost normovolemic average patient: V_{pre} = 4750 mL (v_{inf} = 250 mL, k = 20; v_{inf} = 500 mL, k = 10.5); (3) HB concentrations: colloid 0, HBOC and blood 13, packed RBC 26 (G/dL). (4) HCT: colloid, HBOC 0, Blood 39, RBC 78 (%). *Note: While this formula requires some algebra to be derived, the interpretation is very clear—the only driving force for changes is the difference in concentrations (with equal concentrations there will be no changes): the bigger difference the bigger change is. The magnitude of impact depends on ratio between resulting volume and infused volume, because the difference that exists in infused volume will be spread over entire resulting volume, thus the bigger the ratio the smaller effect of infusion is. HBOC = hemoglobin-based oxygen carrier; HCT = hematocrit.

lead to untoward safety consequences (as was suggested by HBOC-201 as an alternative to blood transfusion in elective orthopedic surgery[1]) or is it purely an efficacy issue that could not be answered by this trial.

RBC-Controlled Trials with HBOCs

This study and the later phase III study (multicenter randomized single-blinded randomized–controlled trial in elective orthopedic surgery settings)[1] are the only true RBC-controlled clinical trials among all HBOC trials. Although the efficacy outcomes of these 2 trials were similar, the safety outcomes were distinctly different, particularly with respect to cardiac AEs. These 2 trials were similar in design, settings, and patient populations and differed only by number of allowed CTM units (7 vs 11). A detailed comparison of these 2 trials may be instructive in determining best practices for the administration of HBOC-201 for anemia treatment, optimizing efficacy and safety.

Apart from the comparative effects of HBOC-201 and RBCs on total Hb concentration, this study provides an insight into the effect of RBC transfusions on hematologic variables.

Final Note

The results of this trial lend support to continued clinical development for indications where RBC transfusions are not an option. A similar conclusion was reached by a majority of panelists at the 2008 National Institutes of Health FDA workshop on HBOCs.[31,32] A recently published commentary further discusses situations where the use of HBOCs could be beneficial.[33] Data on hematologic markers gathered from this study and other RBC-controlled trials should serve as a basis for the development of optimal treatment regimens in any new indications. ■

DISCLOSURES

Name: Jan Van Hemelrijck, MD, PhD.
Contribution: This author helped design the study, conduct the study, analyze the data, and prepare the manuscript.
Attestation: Jan Van Hemelrijck attests to the integrity of the original data and the analysis and is the archival author.
Conflicts of Interest: The author participated in this trial as a paid clinical investigator.
Name: Lewis J. Levien, MB BCh, PhD.
Contribution: This author helped design the study, conduct the study, analyze the data, and prepare the manuscript.
Attestation: Lewis J. Levien attests to the integrity of the original data and the analysis.
Conflicts of Interest: The author was a paid consultant to Biopure and also participated in this trial as a paid clinical investigator. This author acknowledges receiving funding as an ad hoc consultant to Biopure and participating in the trial as a paid clinical investigator.
Name: Luc Veeckman, MD.
Contribution: This author helped design the study, conduct the study, analyze the data, and prepare the manuscript.
Attestation: Luc Veeckman attests to the integrity of the original data and the analysis.
Conflicts of Interest: The author participated in this trial as a paid clinical investigator.
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Conflict of Interest: This author was previously employed by Biopure Corporation and its successor OPK Biotech LLC.
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Attestation: Zafiris Zafirelis attests to the integrity of the original data and the analysis.
Conflicts of Interest: This author was previously an employee of Biopure Corporation and its successor, OPK Biotech LLC.
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Contribution: This author helped design the study, conduct the study, analyze the data, and prepare the manuscript.
Attestation: Thomas Standl attests to the integrity of the original data and the analysis.
Conflicts of Interest: The author participated in this trial as a paid clinical investigator.
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