Blood Products Advisory Committee
88th Meeting, December 14, 2006
Silver Spring, Maryland

Issue Summary

Topic : Review of a Proposed Clinical Trial of HBOC-201 in Trauma

Issue:

FDA seeks the advice of the Committee on a Phase 3 clinical trial proposed by the Naval Medical Research Center (NMRC) using HBOC-201, a hemoglobin-based oxygen carrier manufactured by Biopure® Corporation, for the treatment of post-traumatic hemorrhagic shock, with or without concomitant traumatic brain injury, in an urban-ambulance setting under the provisions of 21 CFR 50.24, waiver from the requirements for informed consent.

Background:

The NMRC proposes to evaluate use of HBOC-201 under IND for immediate treatment of life-threatening traumatic hemorrhage in an urban-ambulance setting based on a proposed 15% relative reduction of all cause mortality at 28 days compared with use of a standard crystalloid solution as the initial resuscitation fluid. Specifically, subjects in hypovolemic shock in the absence of penetrating head injury will receive either HBOC-201 or lactated Ringer's solution. As soon as subjects arrive at the hospital, standard of care measures, including transfusion of allogeneic blood (when indicated), will be instituted and no additional HBOC-201 will be administered. The protocol proposes waiver of informed consent based on FDA's regulation, exception from informed consent requirements for emergency research, 21 CFR 50.24, (Appendix 1).

Studies evaluated under 21 CFR 50.24 present important questions to be addressed by IRBs reviewing the study, including issues of community consultation and public disclosure. For this BPAC meeting we are not seeking advice on these important issues, but rather to advise FDA on certain issues related to our scientific evaluation of information submitted in support of the study.

To permit the RESUS trial to proceed, FDA must determine that:

a. Human subjects would not be exposed to an unreasonable and significant risk of illness or injury (a safety standard applicable to all INDs under 21 CFR 312.42(b)(1)(iv), (2)(i))
b. Participation in the research holds out the prospect of direct benefit to the subjects (21 CFR 50.24) because:

   (i) Appropriate animal and other non-clinical studies have been conducted, and the information derived from those studies and related evidence support the

potential for use of HBOC-201 to provide a direct benefit to the individual subjects; and

(ii) Risks associated with the investigation are reasonable in relation to what is known about the medical condition of the potential class of subjects, the risks and benefits of standard therapy, if any, and what is known about the risks and benefits of the proposed intervention or activity.

In considering the RESUS IND, FDA assessed relevant information from a Biologics License Application (BLA) for HBOC-201 submitted to FDA in 2002 by Biopure Corporation. Biopure had previously performed Phase 2 and 3 studies of HBOC-201 to pursue an indication for avoidance of allogeneic red cell transfusion in elective orthopedic surgery. The BLA was reviewed and a Complete Review (CR) letter identifying a number of serious deficiencies was issued in 2003. Biopure has informed the agency that it is no longer pursuing this indication.

FDA has placed the RESUS IND (BB-IND 12504) on Clinical Hold for three primary reasons:
(A) Safety concerns based on i) the adverse events seen in previous clinical studies, and ii) potential risks related to product dosing and patient monitoring limitations
(B) Heterogeneity in the expected mortality of individual subjects meeting the inclusion criteria for RESUS
(C) Insufficient basis for estimating the effect size of HBOC-201 for possible reduction in mortality.

FDA seeks the advice of the Committee on the scientific issues that impact on these reasons.

Discussion:

At the December 14, 2006 BPAC meeting, FDA will present a scientific evaluation of the pre-clinical and clinical data submitted to FDA by Biopure Corporation and NMRC in support of the proposed treatment and monitoring of subjects in the planned RESUS trial under BB-IND 12504.

Protocol for the RESUS trial

The RESUS protocol assumes that because urban ambulance transportation times are usually short, most subjects will receive only two units of HBOC-201, based on the proposed rate of infusion. The enrollment criteria for the RESUS trial are summarized below:

Inclusion

1. Adults ≥ 18 - < 70 years old
2. Injury with obvious/suspected massive bleeding
3. Absence of known or suspected penetrating traumatic brain injury (TBI)
4. Systolic Blood Pressure (SBP) < 90 mm Hg
5. Expected transport to a participating hospital
6. Secured IV access
7. Revised Trauma Score (RTS) 1 to <5

Exclusion:

1. Penetrating Traumatic Brain Injury (TBI)
2. Paralysis
3. Pregnancy (known or suspected)
4. Cardiac Arrest
5. Known allergy to HBOC-201
6. Known opposition to receiving HBOC-201
7. Burns > 20% body surface area (partial or full thickness)
8. Blood transfusion available (guideline: expected < 10-15 minutes to hospital arrival)

A total of 1130 field-trauma subjects will be enrolled into this randomized, single-blind, multicenter ambulance trial. The proposed study also includes a separate 50 subject Phase 2 sub-study prior to initiation of the full RESUS study to evaluate the logistical feasibility of conducting the full RESUS trial. Subjects initially will receive 2 units (1 unit = 250 mL and contains ~30 g of hemoglobin), i.e., 500 mL HBOC-201 or 1000 mL lactated Ringer's (LR) generally over 10 minutes. The assigned solution will be infused as needed up to a maximum of 6 units (1,500 mL of HBOC-201) or 1,500 mL of lactated Ringer's for SBP < 90 mmHg, or for SBP 90-99 mmHg and a heart rate ≥100 bpm; otherwise, LR will be infused. Both HBOC-201 and LR will be stopped for SBP ≥120 mm Hg, and

standard IV fluids (crystalloid and/or colloid solutions) will be administered for other persistent signs of hemorrhagic shock and occult hypoperfusion when clinical trial material (CTM) re-infusion criteria have not been met. At least two abnormal parameters in the appropriate setting should be present for a diagnosis of persistent or recurrent hemorrhagic shock requiring fluid reinfusion to be made. The default infusion rate is 50 mL/min.

Transport and resuscitation of trauma subjects from the field to the hospital emergency room will be performed by EMTs. After the first dose of HBOC-201 or lactated Ringer's solution has been infused, the decision to infuse additional units will be based on two parameters: blood pressure cuff systolic blood pressure measured approximately every 5 minutes and heart rate measured using an ECG monitor.

Upon arrival in the ER, all subjects will receive standard of care, i.e., allogeneic blood products, LR, and/other replacement fluids at the discretion of the treating physician.

The primary endpoint is 28-day survival. The effect size chosen by the NMRC is a relative mortality risk reduction of 15%, i.e., from 58.1% (estimated historical mortality rate for hypotensive patients with RTS 1 to < 5) to 49.4%.

An independent Data Monitoring Committee (DMC) will monitor the trial.

The dosing and administration scheme is presented below.

Study Schematic

FIELD

Population

Trauma subjects in hypovolemic shock, 18 − <70 y/o with RTS 1 to <5,
with or without non-penetrating traumatic brain injury

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AMBULANCE

Initial Dose of Clinical Trial Material

HBOC-201 (2 units=500 mL) or LR (2 units=1000 mL) over 10 min titrated to maintain SBP=90 mmHg

↓ **HBOC-201** ↓ **Lactated Ringer's**

Re-Infusion of Clinical Trial Material

1. Repeat regimen for SBP <90 mmHg (or SBP 90-99 mmHg + HR ≥100 bpm) UP TO 1500 mL of CTM

2. Standard IV fluids administered if
   a. SBP>100 mmHg or SBP>90 mmHg and HR<100 bpm
   b. 2 or more signs of HS present

3. Stop all CTM if SBP ≥ 120 mmHg

HBOC-201 ↓ **Lactated Ringer's** ↓

HOSPITAL

↓

STANDARD OF CARE INCLUDING ALLOGENEIC BLOOD

1° ENDPOINT: Survival status at 28 days
2° ENDPOINTS: Superiority in various markers of organ damage and/or physiological impairment

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Signs of persistent/recurrent hemorrhagic shock:

Weak and/or thready pulse
Narrow pulse pressure (e.g. < 40 mm Hg)
Bradycardia (HR < 60 bpm)
Tachypnea
Decreased oxygen saturation
Oximeter failure to obtain reading
Skin pallor
Cool and clammy skin
Decreased capillary refill
Mental status changes (in absence of TBI)

A. Safety Concerns

Framework for review under 21 CFR 312.42(b)(1)(iv), (2)(i)

Under the provisions of 21 CFR 312.42(b)(1)(iv) and (2)(i), FDA may place an IND on clinical hold if it determines that human subjects are or would be exposed to an unreasonable and significant risk of illness or injury. A clinical hold is an order issued by FDA to the sponsor to delay a proposed clinical investigation or to suspend an ongoing investigation. The standard is based on the premise, articulated in the preamble to the 1983 Proposed New Drug, Antibiotic, and Biologic Drug Product Regulations (48 FR 26720) and in the preamble to the 1987 Final Rule (52 FR 8798), that safety concerns will be considered to ensure that research subjects are not exposed to unreasonable risks. This standard implies that a new drug or biologic must be "safe enough" to be studied in humans in the manner proposed, contingent also on other regulations to protect the rights and safety of subjects. These other regulations include 21 CFR Part 56, provisions for oversight by an Institutional review Board, and 21 CFR Part 50, provisions for Informed Consent.

The word "unreasonable" is a relative standard. Risks associated with the clinical investigation and with the use of the investigational drug must be weighed against the potential benefit. In RESUS, the proposed benefit is an increase in survival (decrease in mortality). The assumptions for the RESUS trial are that the underlying mortality rate for traumatic hemorrhagic shock among subjects with an RTS of 1 to < 5 is 58.1% and that the potential benefit is a relative reduction in mortality of 15% (i.e., 49.4% mortality rate in the HBOC-201 arm and a 58.1% mortality rate in the control arm).

Clinical Experience with HBOC-201

HBOC-201 was studied in a pivotal Phase 3 elective orthopedic surgery trial for avoidance of allogeneic transfusion in consenting subjects. HEM-0115 was a multicenter, randomized, single-blind, RBC-controlled, parallel-group study to evaluate the effect of HBOC-201 on allogeneic blood use. Subjects undergoing elective orthopedic surgery were randomized to receive either test product or RBC as soon as a prospectively-defined transfusion trigger was reached. A total of 693 subjects were enrolled, 353 HBOC-201: 340 RBC (5 subjects, 3 HBOC-201 and 2 RBC], dropped out).

HBOC-201 was initially infused as a 2 unit (500 mL or ~ 60 g) loading dose, followed by additional unit-by-unit administration, to a maximum of 10 units (~300 g) at protocol-specified transfusion triggers. RBCs were administered unit-by-unit at the same protocol-specified transfusion triggers.

Primary Endpoint: Proportion of subjects in the HBOC-201 group who did not receive any transfusion of allogeneic RBC during the study following the initial treatment with HBOC-201. Statistically, the primary endpoint would be met if ≥ 35% (lower bound of the 95% confidence interval ≥ 30%) of subjects avoided an allogeneic RBC transfusion during the 6 week study period.

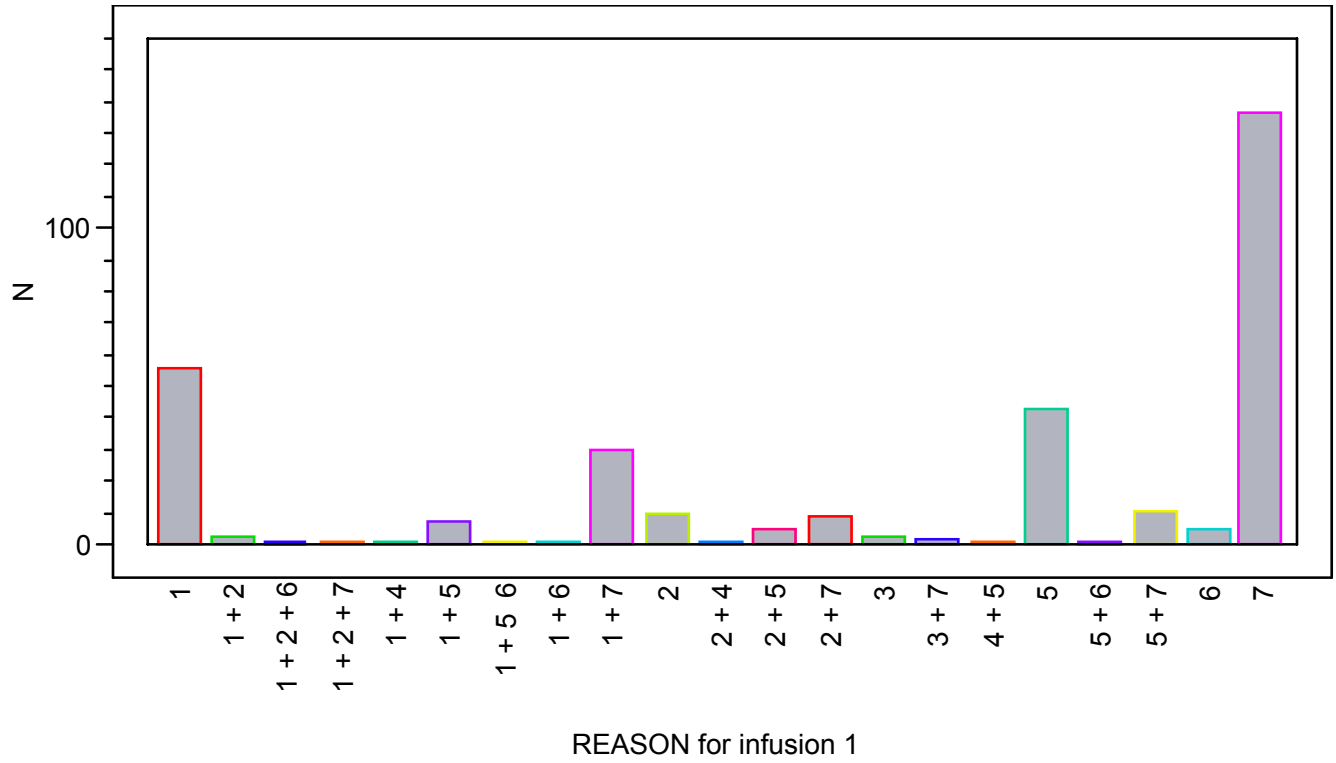
Outcome: Of 353 subjects treated with HBOC-201, 213 (~60%) avoided allogeneic transfusion whereas 140 (~40%) received at least 1 unit of allogeneic RBCs during the 6-week post-operative period.

The protocol permitted infusion/transfusion if total hemoglobin was between 6 g/dL and 10.5 g/dL AND there was at least one of the following findings:

1. Heart rate ≥ 100 bpm
2. Systolic BP < 90 mm Hg or < 70% of preoperative screening value
3. ECG evidence of myocardial ischemia
4. Metabolic acidosis (base excess -4 or worse)
5. Acute blood loss > 7 mL/kg within a period of 2 hours or less
6. Oliguria with urine output < 0.5 mL/kg/hr for at least 2 hours
7. Restricted subject activity (e.g. ambulation) due to weakness (asthenia) or dizziness

The HEM-0115 protocol specified that subjects could be randomized within 24 hours before or 72 hours after start of surgery and that HBOC-201 could be administered up to 9 times (10 units) within 6 days after randomization. Of subjects who received HBOC-201, the mean time to randomization was 24.8 hours (median 20.8 hours) after the start of surgery. 68% of the HBOC-201 subjects (241/353) received less than 4 units (3 administrations, ~120 grams) of product. The total hemoglobin at randomization was 8.8 ± 1.2 g/dL (median 8.9 g/dL) for HBOC-201 vs 8.8 ± 1.1 g/dL (median 8.9 g/dL) for RBC. The predominant reason for the initial infusion of HBOC-201 or transfusion of RBCs was "restricted patient activity" with or without associated tachycardia.

REASONS FOR THE INITIAL INFUSION OF HBOC-201



1. **Tachycardia**
2. **Hypotension**
3. **ECG ischemia**
4. **Acidosis**
5. **Bleeding**
6. **Oliguria**
7. **Restricted activity due to weakness or dizziness**

One hundred ten subjects received less than ~ 300 g of HBOC-201 (9 units, 8 infusions). Of these, 29 were no longer eligible to receive HBOC-201 and received RBC instead. The remaining 81 who were eligible to receive at least one additional infusion of HBOC-201 yet were administered allogeneic RBCs instead. Among these 81 subjects, the investigator-recorded reasons for not continuing with the administration of HBOC-201 were predominantly related to total hemoglobin concentrations or hematocrit with/without additional signs or symptoms of anemia. In 6 subjects, maintenance of intravascular volume was listed as the reason for not continuing with the product. Adverse events were noted in 15 subjects, of whom 7 were discontinued from HBOC-201 because of ischemic adverse events (cardiac, CNS, renal).

The 110 subjects who received allogeneic red blood cells received a median dose of 2 units (mean approximately 3 units) of red blood cells, regardless of the total dose of

HBOC-201 received. This dose of red blood cells was comparable to the dose received by subjects randomized to the control group.

Subjects in the HBOC-201 test arm had 1g/dL lower Hb levels on each post-randomization day , and lower total hemoglobin and hematocrit at discharge (28% vs. 31%) compared to the control arm,. These findings suggest that the clinical threshold for ordering RBCs was higher in the HBOC-201 treatment arm resulting in avoidance of red cell transfusion. Even though the primary transfusion avoidance endpoint was met:

(i) Randomization occurred late for most subjects
(ii) The volume of HBOC-201 used was low
(iii) Red blood cell transfusions were generally ordered as "back-to-back" units regardless of transfusion need
(iv) HBOC-201 and red blood cells were used predominantly in subjects with post-operative "asthenia"
(v) There was premature switching from HBOC-201 to RBCs in 81 subjects
(vi) Hb levels in the HBOC-201 recipients was 1 g/dL lower Hb than the RBC control subjects throughout hospitalization and on discharge. Transfusion/infusion decisions were predominantly for post-operative asthenia, which suggests that most transfusion/infusions were not physiologically required in most subjects.

Adverse Events Seen with HBOC-201:

The protocol for HEM-0115 specified that adverse event data would be reviewed after completion of the study by a blinded adjudication committee (SEEC). FDA has raised serious concerns about the implementation of the SEEC safety evaluation in response to the BLA filing. However, as Biopure has elected not to pursue the orthopedic surgery indication in the United States the SEEC review of safety has not been the basis for any further discussions among Biopure, NMRC, and FDA.

Adverse events reported for this Issue Summary are based on a conditional assessment agreed to by Biopure and FDA during recently held meetings. These tables do not reflect a final assessment of adverse events, but rather represent information as currently understood by FDA and Biopure. Biopure and FDA do not agree on the assessment of a few cases captured in the categories of cardiac arrest, myocardial infarction, and dialysis. FDA will present an analysis based on its own assessment of the cases in those categories. FDA believes that the data provided in this summary provide a credible minimum estimate of the adverse event information from the clinical studies of HBOC-201.

In Phase 1, Phase 2, and Phase 3 studies, 797 subjects were infused with HBOC-201 and 661 subjects received control transfusions/infusions, i.e., red blood cells or asanguinous solutions. Appendix 3 summarizes the studies conducted using HBOC-201, extent of exposure, and number of subjects by age cohorts ≤ 50, 51-75, > 75. Conditional tables 3-10 contain a summary of FDA analyses of safety data supplied by Biopure.

The studies with HBOC-201 were not designed, and had insufficient power to determine statistical differences in adverse events between groups, thus statistical differences were not usually found. Nevertheless the safety signals consistently showed imbalances against the product and need to be considered in any risk:benefit calculation.

The combined incidence of serious adverse events for HBOC-201 (N = 797) vs. control (N = 661) (Conditional Tables 3A, 3B, 4, 5, 8, 9) for:

- Death was 25 vs. 14 (3.1% vs. 2.1%)
- Heart failure/CHF/pulmonary edema/fluid overload was 54 vs. 22 (6.8% vs. 3.3%)
- Cardiac arrest/cardiopulmonary arrest/ventricular fibrillation was 17 vs. 6 (2.1% vs. 0.9%)
- Myocardial infarction was 14 vs. 4 (1.8% vs. 0.6%)
- Pneumonia was 36 vs 24 (4.5% vs 3.6%)
- CVA and cerebral ischemia/infarction was 16 vs. 3 (2% vs. 0.5%)
- Necessity for dialysis was 7 vs 2 (0.9% vs. 0.3%)

Troponin was measured in only about one-third of the study population in Biopure's Phase 3 HEM-0115 trial. The incidence of elevated serum troponin was 18/146 (12.4%) for HBOC-201 vs. 2/130 (1.5%) for control.

For crystalloid/colloid controlled surgery studies in a variety of surgical settings (N=177 for HBOC-201 vs. 131 for control), the imbalances noted above persisted (Conditional Tables 3A and 3B, 5, 6, 8, and 9). The combined incidence of HBOC-201 vs. control for:

- Death was 4 vs. 1 (2.3% vs. 0.8%)
- Heart failure/CHF/pulmonary edema/fluid overload was 16 vs. 9 (9% vs. 6.9%)
- Cardiac arrest/cardiopulmonary arrest/ventricular fibrillations was 2 vs 1 (1.1% vs. 0.8%)
- Myocardial infarction was 7 vs. 1 (3.9% vs 0.8%)
- Pneumonia was 7 vs. 3 (3.9% vs. 2.3%)
- Acute renal failure was 2 vs. 0 (1.1% vs 0%)
- Necessity for dialysis was 2 vs. 0 (1.1% vs 0%) .

The imbalances noted above for the overall population are also present when the results are analyzed for surgery studies by age ≤ 69 (N=476 vs 422, HBOC-201 vs control). (Conditional Table 9)

- Death was 9 vs 3 (1.9% vs 0.7%)
- Heart failure/CHF/pulmonary edema/fluid overload was 26 vs 10 (5.5% vs. 2.4%)
- Cardiac arrest/cardiopulmonary arrest/ventricular fibrillation was 6 vs 2 (1.3% vs. 0.5%)
- Myocardial infarction was 6 vs 2 (1.3% vs 0.5%)
- Pneumonia was 16 vs. 6 (3.4% vs 1.4%)

- CVA and cerebral ischemia/infarction was 3 vs 0 (0.6% vs 0%)
- Acute renal failure was 6 vs 3 (1.3% vs 0.7%)
- Necessity for dialysis was 4 vs 0 (0.8% vs 0%)

For HEM-0115, the imbalances noted above remained for *post-hoc* analyzed subgroups, although sample sizes were too small to evaluate with a test of statistical significance. These subgroups included subjects < 70 years old, subjects hypotensive at randomization, and subjects with antecedent trauma. These trauma subjects in HEM-0115 (N = 32 vs. 28, HBOC-201 vs. control, respectively) are not comparable to the proposed trauma subjects in the RESUS trial as they were treated at least 24-48 hours after the trauma event and were hemodynamically stable, euvolemic, and normothermic at the time of randomization)(Conditional Tables 7 and 10).

In its BLA, Biopure reported the following results:

Number of subjects experiencing ≥ 1 AE:

Overall roster: 740/797 (93%) for HBOC-201 vs. 581/661 (88%) for control

Number of subjects experiencing ≥ 1 SAE:

HEM-0115: 88/350 (25.1%) for HBOC-201 vs. 59/338 (17.5%) for RBC
Overall roster: 173/797 (22%) for HBOC-201 vs. 109/661 (16%) for control

Subjects discontinuing CTM due to adverse events:

Overall roster: 36/740 (5%) for HBOC-201 vs. 5/581 (1%) for control for those subjects experiencing ≥ 1 AE.

The BLA, documented 25 deaths (3.1%) among recipients of HBOC-201 vs 14 deaths (2.1%) among recipients of control solutions. Cardiac deaths accounted for 9 of 25 deaths for HBOC-201 (1.1%, N = 797) vs. 3 of 14 deaths for control (0.4%; N = 661). As stated in the RESUS Investigator Brochure, mortality in Biopure clinical trials was higher in the HBOC-201 arm compared to controls for the following categories: all patients (odds ratio 1.50), > 75 year old cohort (odds ratio 3.53), ≤ 75 year old cohort (odds ratio 1.1), > 50 year old cohort (odds ratio 1.45), and ≤ 50 year old cohort (3 vs. 0, indeterminate). As noted above, for subjects ≤ 69 years old, there were 9 deaths among 476 subjects (1.9%) vs 3 deaths among 422 control subjects (0.7%) for an absolute difference in mortality of 1.2%.

A European study of outpatients (N=45) undergoing percutaneous coronary intervention (PCI) was completed by Biopure in 2005. Preliminary information submitted by the sponsor indicates that two HBOC-201 subjects had SAEs after infusion of 1 unit of product: one experienced cardiac arrest complicated by myocardial infarction and stroke, and the second had a prolonged episode of uncontrollable hypertension necessitating treatment and monitoring in an intensive care unit over several days. The sponsor

concluded that HBOC-201 had the potential for "unpredictable blood pressure responses." A final study report has not yet been submitted to FDA.

Biopure proposed three reasons why SAEs were observed more frequently in the HBOC-201 arm: i) total hemoglobin levels were lower in the HBOC-201 arm than in the RBC arm, thereby placing the test group at greater risk of tissue ischemia, ii) there were more test subjects than control subjects with a history of cardiac disease who experienced SAEs, and iii) the trial dosing guidelines were inaccurate, leading investigators to over-infuse the product. However,

i. The difference (mean) between the HBOC-201 cohort and the RBC cohort in terms of lowest recorded total hemoglobin at any time in the trial was only 1.23 g/dL. This small difference may be the result of fluid shifts into the intravascular space due to the infusion of HBOC-201, an oncotically active product.

The proportion of subjects who had a nadir total hemoglobin <8 g/dL was significantly higher for the HBOC-201 cohort than for the RBC cohort (47% vs. 18%). In a *post hoc* analysis of the data, however, NMRC found there were no between-group differences in the incidence of hypertension, troponin elevations, or oliguria in HBOC-201 subjects stratified by a nadir total hemoglobin < 8 g/dL or ≥ 8 g/dL,

ii. Also in a *post hoc* analysis, NMRC found there were no between-group differences in the incidence of AEs in HBOC-201 subjects when stratified by the presence or absence of a history of heart disease.

One of the sponsor's assumptions is that the age and presence of co-morbid conditions of the subjects in the surgery trials resulted in a higher SAE and AE rate than is likely to be encountered in the RESUS study. The Biopure database does not contain data to support an assumption that younger victims of acute trauma who are in hemorrhagic shock in the field will have a physiologic status post-injury that is superior to that in older subjects undergoing elective surgical procedures in the operating room

<u>Safety Concerns Regarding Proposed Dosing of HBOC-201</u>

The RESUS study calls for administration of up to 6 units (250 mL/unit) of HBOC-201 at rates up to 50 mL/min (0.7 mL/kg/min for a 70 kg adult), with the default RESUS rate at 50 mL/min. However, there are very limited clinical data on dose and rate of administration using HBOC-201 to support the RESUS dosing guidelines.

1. In study HEM-0115, the mean infusion rate for the first unit of HBOC-201 was 5.5 mL/min, and only 4 (~1%) HBOC-201 subjects received product with a maximum infusion rate ≥ 40 mL/min. When measured in pre-clinical studies, HBOC-201 was administered at rates ranging from gravity infusion (not otherwise quantified) to 10 mL/kg/min.
2. A total of 36 subjects, all in study HEM-0115 (N = 353) received 10 units (~300 g) of HBOC-201. In all, a total of 108 subjects have received

HBOC-201 in doses greater than 6 units or ~180 g over periods of up to 6 days.

3. No studies of deliberate dose escalation coupled with deliberate escalation of rate of administration have been performed.

Safety Concerns Regarding Limitations to Patient Monitoring in RESUS

The response of patients with traumatic hemorrhagic shock to fluid therapy in the ambulance is primarily monitored by BP. The classic paradigm for fluid resuscitation uses blood pressure as a surrogate for tissue perfusion. When the resuscitation fluid is vasoactive, it is difficult to know whether the increase in SBP is due to adequate fluid replacement and/or vasoconstriction associated with administration of the fluid.

Non-clinical and clinical studies indicate that HBOC-201 is vasoactive, i.e., its use results in arteriolar vasoconstriction. In Biopure's Phase 3 orthopedic elective surgery trial (N = 353 vs. 340), 28% (N = 7) of subjects with a SBP < 90 mm Hg (N= 25) who were then randomized to receive HBOC-201 had a peak SBP of 141-160 mm Hg following infusion of the first unit. In the group with a SBP <90 mmHg randomized to receive RBCs (N = 20), 5% (N = 1) had a peak SBP of 141-160 mm Hg with infusion of the first unit. Data from the overall population in study HEM-0115 indicate that 36% (9/25) of hypotensive subjects with initial SBP ≤ 90 mm Hg who received HBOC-201 had increases in SBP above 130 mm Hg whereas only 10% (2/20) of similar subjects who received allogeneic red blood cells experienced SBP increases to greater than 130 mm Hg.

Based on these data, FDA has expressed a concern that the vasopressor effects of HBOC-201 could result in under-resuscitation of hypovolemic trauma patients, and/or aggravate hemorrhage. In response to FDA's concerns, the sponsor has proposed stopping rules for administration of CTM (HBOC-201 or lactated Ringer's solution) while the subject is being treated in the ambulance and proposes stopping infusion of CTM once SBP reaches ≥ 120 mm Hg. Administration of standard IV fluids is specified for other persistent signs of hemorrhagic shock.

Additionally, to reduce the risk of fluid under-resuscitation, the sponsor has incorporated heart rate (presence or absence of tachycardia) as well as additional signs of tissue hypoperfusion into the dosing and administration guidelines. These include heart rate <60 bpm, altered mentation, decreased and/or failure to obtain pulse oximetry readings, weak or thready pulse, skin pallor, cool and clammy skin, and decreased capillary refill. However, these parameters are non-specific or poor indicators of tissue hypoperfusion in field trauma subjects. In addition, data are lacking to support their validity to detect underperfusion when a vasoactive HBOC is used, since they are derived from data in hypovolemic patients receiving conventional volume expanders. RESUS also instructs EMTs to discontinue infusion of Clinical Trial Material (CTM) if SBP reaches ≥ 120 mm Hg, and not to restart the infusion, but to administer standard IV fluids for other persistent signs of hemorrhagic shock.

However, FDA remains concerned that the hazards of a vasopressor effect of HBOC-201 remain uncertain and may not be mitigated under the RESUS protocol. The sponsor has not submitted any clinical or preclinical data to support stopping infusion at a SBP of 120 mm Hg (or any other SBP). There are no protocol guidelines for the pharmacologic treatment of subjects who experience elevated SBP in the ambulance, and acute and unexpected increases in SBP to 220 mm Hg have been noted with HBOC-201 administration in settings where SBP was being invasively monitored in real time, e.g., elective percutaneous coronary intervention. Stopping HBOC-201 at a SBP ≥ 120 mm Hg could limit potential increases in oxygen content (resulting in a decreased benefit, and a worse benefit:risk ratio). On the other hand, risks (elevated SBP resulting in clot disruption and increased hemorrhage) might remain the same or even intensify if SBP continued to rise in the ambulance.

Limitations to In-hospital Trauma Care Draft Guidelines

The RESUS In-Hospital Trauma Care Draft Guidelines summarize current trauma care but do not address two important concerns associated with infusion of a vasoactive HBOC. First, the Guidelines do not address the critical importance of immediate and effective treatment of elevated SBP in any subject who has received HBOC-201, especially trauma subjects with uncontrolled arterial bleeding. Hypertension has been seen consistently in preclinical and clinical studies. The second concern not addressed is the danger of fluid under-resuscitation. HBOC-201 has a half-life of approximately 19 hours. Since both systemic and pulmonary pressures are increased by the vasoactive properties of HBOC-201, use of "pressures" as surrogates for "volumes" may seriously mislead clinicians to administer too little fluid and thereby to under-resuscitate trauma patients.

Sponsor's summary of Risks from HBOC-201

The Investigator Brochure summarizes risks particularly related to the RESUS trial and notes that HBOC-201 may induce transient and reversible smooth muscle constriction in the GI tract resulting in dysphagia and abdominal pain, and vascular smooth muscle contraction resulting in mild-moderate transient reversible increases in blood pressure and decreases in cardiac output and heart rate. The document notes that vasoconstriction may explain the excess of cardiovascular (ACS and CHF), pulmonary (pulmonary edema and respiratory failure), renal (acute renal failure), and CNS (CVA, TIA, and confusion) AEs/SAEs in the HBOC-201 arm of HEM-0115. "Vasoconstriction-induced increased BP may theoretically increase bleeding in subjects with uncontrolled hemorrhage, especially when adequate CPP (cerebral perfusion pressure) is required in the setting of concomitant TBI (traumatic brain injury)," but notes that this effect has not been seen with hypotensive resuscitation. Other laboratory findings with HBOC-201 include "transient and usually asymptomatic hyperbilirubinemia and elevations of hepatic transaminases (hepatitis), amylase/lipase (chemical pancreatitis), BUN (azotemia), mild hemoglobin desaturation with normal pO_2, methemoglobinemia, and persistently low hemoglobin levels due to intravascular fluid shifts secondary to the oncotic properties of HBOC-201." "Administration of HBOC-201 will result in a reduction of hematocrit

proportional to the volume of HBOC-201 infused." The Investigator Brochure notes that ongoing RBC transfusion requirements should be managed taking these findings and the 19 hour half-life of the product into account.

B. Heterogeneity in the expected mortality of individual subjects meeting the inclusion criteria for RESUS

1. Expected mortality in RESUS has been estimated from the National Trauma Database (NTDB-in hospital data) and from trauma registries from the University of Alabama and the University of Maryland (prehospital data). In addition to entry criteria for SBP, the sponsor proposes to use the weighted Revised Trauma Score (RTS; full range 0-7.84), with enrollment criteria ranging from 1 to <5, with no additional criteria for the Glasgow Coma Score (GCS) portion of the RTS. The average mortality for subjects in RESUS is estimated at approximately 55-58% from analysis of the two data bases (range: 27-70%, NTDB; 42-89%, UAB/UMD prehospital databases).

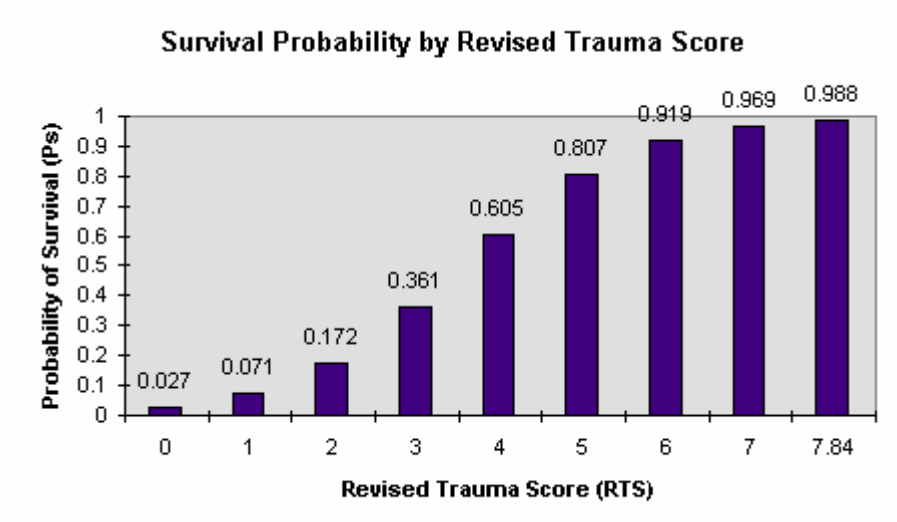
2. The Revised Trauma Score is a calculation based on three parameters, Glasgow Coma Score, systolic blood pressure, and respiratory rate using the following formula to weight each component:

Glasgow Coma Scale (GCS)	Systolic Blood Pressure (SBP)	Respiratory Rate (RR)	Coded Value
13-15	>89	10-29	**4**
9-12	76-89	>29	**3**
6-8	50-75	6-9	**2**
4-5	1-49	1-5	**1**
3	0	0	**0**

RTS = 0.9368 GCS + 0.7326 SBP + 0.2908 RR

Based on the proposed entry criteria for RESUS, this information suggests that the patient population is likely to be heterogeneous. Using the RTS criterion rather than the data from the trauma registries from the University of Alabama and the University of Maryland cited above (section B.1.), the probability of survival ranges from 7.1% (92.9 % mortality) (RTS = 1) to approximately 81% (19% mortality) (RTS = 5). Thus, while ranges for mortality differ in the three available databases, all indicate a very wide range of survival probabilities.

The Revised Trauma Score is not a linear scale but appears to be sigmoidal, with the steepest portion of the curve occurring between scores of 2 and 5. The following graph is found at www.trauma.org.



Small, imbalances in this RTS entry criterion could impact the primary mortality outcome more significantly than any difference attributable to the effect of the product, either positive or negative. Given the high probability of survival at higher RTS scores, and based on information from the 2 pre-hospital trauma registries for distribution of subjects by RTS scores, it is quite likely that the overall average 58% mortality will be derived from deaths among patients with RTS scores well below 5, rather than from the group of patients with RTS scores closer to the upper limit of 5.

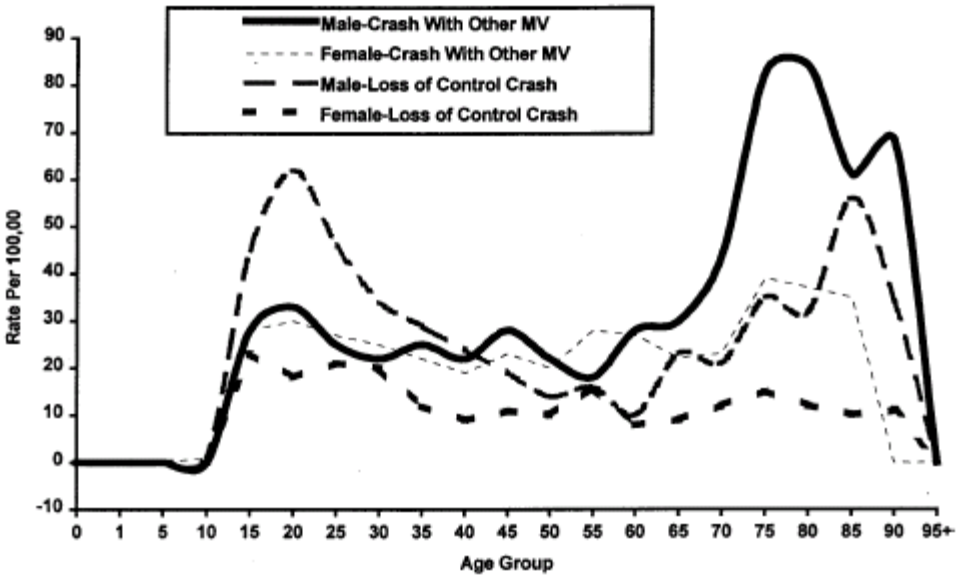
These considerations raise a major concern. Many subjects who ordinarily would survive and do well without HBOC-201 will be exposed to its risks. This is a central concern for a trial performed with waiver from informed consent where there must be a likelihood of benefit to individual subjects.

Additionally, FDA is concerned about the proposed exclusion of subjects age 70 years and older. FDA notes that:

1. The fastest growing segment of the civilian trauma population is the population over 50 years old, and more particularly, over 70 years of age (Donald Trunkey MD, personal communication).
2. Distinguishing subjects above or below a particular age under field conditions is difficult and probably cannot be done with certainty.
3. Older subjects may be at greater risk of ischemic consequences of severe hemorrhage and therefore might also potentially benefit more from administration of a safe and effective oxygen-carrying resuscitation fluid than younger individuals.

According to the National Trauma Databank, motor vehicle traffic-related injuries account for almost 50% (48.5%, approximately 285,000) of cases in the NTDB, and the largest number of deaths is caused by motor vehicle traffic-related injuries (14,000). The average trauma patient is now older, and, as shown in figure 6 (taken from an article published in Accident Analysis and Prevention, 2001; 33: 167-172), there is now a

bimodal age distribution of hospitalizations for automobile injuries by type of crash and gender (information for Wisconsin, 1997).



Thus, older persons constitute a large segment of the expected trauma population, undermining the clinical relevance of a study that would exclude them. The sponsor has argued against inclusion of subjects ≥70 years based on an analysis of the orthopedic studies of HBOC-201 in which the adverse event rate appeared higher in the older age group, and a conjecture that younger trauma victims would have fewer adverse events from HBOC-201 than older subjects in that trial.

While it is likely that younger individuals experiencing trauma will have a better survival rate than older individuals experiencing the same degree of trauma, it should not be assumed *a priori* that

> a) the adverse event rate observed in stable, elective surgery patients who have been medically cleared for that procedure and closely monitored in the hospital will be worse than in unstable trauma subjects with uncontrolled hemorrhagic shock in the field, or
>
> b) younger trauma victims will necessarily have a better outcome than older subjects undergoing elective surgery. This is not only because definitive control of surgical bleeding in the operating room is usually achieved rapidly, but also because there are many new drugs and devices --- unavailable in the ambulance but used routinely in the hospital --- that allow rapid detection and early correction of acidosis, hypoxemia, and hypothermia.

C. Insufficient Basis for Estimation of the Treatment Effect Size:

FDA Review of Pre-clinical Data

In the preamble to the 1983 proposed rule (48 FR 26720) to revise the regulations governing the review of investigational new drug applications and the monitoring of the progress of investigational drug use, FDA commented on the role of pharmacology and toxicology information: "The results of such tests serve primarily to support FDA's assessment of the safety of proposed clinical investigations. These studies are directed toward defining the drug's safety, toxicity, and pharmacological action rather than its efficacy. They are meant to predict effects which might be expected when the drug is administered to human subjects." Except in unusual circumstances of evaluating safety and efficacy of drugs intended to ameliorate or prevent serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic, biological, chemical, radiological, or nuclear substances where definitive studies in humans cannot ethically be conducted, the so-called "Animal Rule" (21 CFR 601.90 ff, "Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible"), results of preclinical tests are not intended to supplant data derived from adequate and well-controlled trials in humans, nor is safety information derived from animal studies intended to supplant safety data derived from clinical trials performed in humans.

With these thoughts in mind, FDA notes that several porcine models have been studied by Biopure, independent investigators, and U.S. Navy contractors. Appendix 4 summarizes the highlights of the various experiments performed in porcine models of hemorrhagic shock with or without traumatic brain injury that compare HBOC-201 to various other resuscitation fluids. The sponsor has hypothesized that the survival outcomes of widely disparate models of hemorrhagic shock, generally with military rather than civilian relevance, with or without traumatic brain injury can be combined into one analysis from which an estimate of survival benefit can be made. FDA is concerned that this analysis overemphasizes the efficacy results of preclinical studies and assumes that efficacy information derived from preclinical studies can offset safety signals in humans. Nevertheless, some general conclusions can be drawn from the various models studied.

1. As a proof of concept, HBOC-201 can sustain life in narrowly defined lethal models of hemorrhagic shock, generally with military rather than civilian relevance.

2. As a proof of concept, an oxygen-carrying resuscitation fluid can sustain life better than a non-oxygen carrying asanguinous solution in models of overwhelming, rapidly fatal hemorrhage. Individuals having these characteristics would generally die at the scene prior to medical intervention and would not meet the RESUS inclusion criteria.

3. No single animal model replicates all of the conditions of RESUS.

4. Results of animal studies are highly model-dependent. Combining data from heterogeneous animal models is problematic.

    a. Parameters measured in different models show outcomes that raise safety concerns:

- All models employed young, healthy animals
- Used anesthesia (necessarily) in the form of potent anesthetics (e.g., isoflurane) that reduce cardiovascular preload and afterload, and protect the heart against subsequent ischemic injury
- Used invasive hemodynamic monitoring not available in the field situation
- Were unblinded
- In many instances, modeled prolonged delay to definitive care rather than rapid delay as would occur in the urban ambulance setting proposed for RESUS.

These confounding procedures and study designs in different models may preclude direct extrapolation to the clinical situation of RESUS.

b. Ability to sustain life in narrowly defined (experimental) situations does not necessarily translate into ability to sustain life in other situations.

Approach to the Assessment of Benefit:Risk:

In 1999, FDA, NIH, and DoD held a workshop to discuss which criteria should be considered in the safety and efficacy evaluation of oxygen therapeutics when used as red blood cell substitutes and as resuscitation fluids in trauma. In 2004, FDA published a draft guidance outlining a clinical development program for these two indications.

In the guidance, FDA recommended a hierarchical approach to evaluate safety in situations where adverse events were expected to be uncommon, so that discovery of product-associated safety signals would be facilitated. Subjects would be medically cleared, carefully monitored, and medically managed according to in-hospital standard of care guidelines, with red blood cells serving as the control. Demonstration of an adequate safety profile might then lead to an evaluation in less stable trauma subjects, or unstable trauma subjects unable to provide consent. The ethical justification for exposing subjects without their consent to an investigational product in a setting where monitoring and medical management are basic and extremely limited (i.e., the ambulance) would largely depend on the safety profile generated in earlier clinical trials.

In the draft guidance document, FDA notes that an oxygen therapeutic product for use in field trauma should have a superior survival outcome when compared to an asanguinous solution. It is quite possible for an oxygen therapeutic product to have an inferior safety profile when compared with blood and yet reduce mortality in a trauma trial and have clinical utility as a resuscitation fluid in situations where blood is not available. However, in such situations, it becomes very difficult to design a clinical trial because it is not easy to weigh the relative importance of safety signals and adverse events observed in earlier clinical trials in terms of increased risk of death, as opposed to the potential benefit in terms of lives saved, particularly if findings suggesting clinical benefit have not been observed in other settings.

FDA and NMRC differ in their interpretation of the importance of safety signals arising out of previous studies using HBOC-201 in surgical subjects. It is very difficult to state reliably that the adverse events and safety signals noted in the clinical trial roster for HBOC-201, and particularly for pivotal study HEM-0115, will or will not be informative in the context of the proposed RESUS trial. However, subjects enrolled in HEM-0115 were medically cleared to undergo the surgical procedures in question, were judged not to be at particular cardiovascular risk, and were monitored and treated according to standard of care. Moreover, the adverse event rate was expected to be low, and it was assumed *a priori* that a rigorous statistical assessment of differences between HBOC-201 and control for a particular adverse event would not be possible due to the relatively small sample size of the trial.

Table 1

NUMBER	MORTALITY RATE	EFFECT SIZE	POTENTIAL LIVES SAVED	NUMBER NEEDED TO TREAT TO SAVE ONE LIFE	LIVES SAVED AS A % OF THOSE TREATED
565	0.80	0.25	113	5	20
565	0.80	0.15	68	8	12
565	0.80	0.10	45	13	8
565	0.80	0.05	23	25	4
565	0.70	0.25	99	6	18
565	0.70	0.15	59	10	11
565	0.70	0.10	40	14	7
565	0.70	0.05	20	29	4
565	0.58	0.25	82	7	15
565	0.58	0.15	49	11	9
565	0.58	0.10	33	17	6
565	0.58	0.05	16	34	3
565	0.40	0.25	57	10	10
565	0.40	0.15	34	17	6
565	0.40	0.10	23	25	4
565	0.40	0.05	11	50	2
565	0.20	0.25	28	20	5
565	0.20	0.15	17	33	3
565	0.20	0.10	11	50	2
565	0.20	0.05	6	100	1

Table 2

Δ DEATH RATE DUE TO SAEs	POTENTIAL ADDITIONAL DEATHS DUE TO SAEs	NUMBER NEEDED TO TREAT TO HARM ONE LIFE
0.01	6	100
0.02	11	50
0.03	17	33
0.04	23	25
0.05	28	20
0.06	34	17
0.07	40	14
0.08	45	13
0.09	51	11
0.10	57	10
0.11	62	9
0.12	68	8
0.13	73	8
0.14	79	7
0.15	85	7
0.20	113	5

These tables may be used to assess the impact of SAEs leading to death on the overall benefit:risk calculation. For the overall roster (HBOC-201 vs control), there were 25 (3.1%) vs 14 (2.1%) deaths for an absolute difference in mortality of 1%. Among subjects < 70 years old, there were 9 deaths among 476 subjects (1.9%) vs. 3 deaths among 422 control subjects (0.7%) for an absolute difference of 1.2%.

The following examples serve to illustrate the sensitivity of the benefit:risk assessment to the underlying assumptions. The stated effect size is the decrease in mortality from the beneficial effects of the product. The stated increase in mortality due to product-related SAEs is based on the difference in observed mortality rate for the entire clinical trial roster, study HEM-0115 alone, and for the subgroup of subjects < 70 years old.

Example 1 most closely resembles the assumptions made for RESUS.

1. 58% mortality in control group, 15% relative effect size, alpha = 0.05, two-sided, 1% excess deaths due to product-associated SAEs. Sample size = 1130.

For a study of 1130 subjects, 565 of whom receive HBOC-201, the number of potential lives saved is 49, the number of additional deaths due to SAEs is 6, and the number of lives saved is net 43 (49 saved – 6 lost = 43). The drug appears to have a favorable benefit:risk profile and the power of the study to detect the effect size of approximately 13% is about 80%.

However, if both the effect size estimate and the estimate of the difference in mortality associated with use of the drug are changed, the benefit:risk calculation is altered.

2. 58% mortality in the control group, 5% relative effect size, alpha = 0.05, two-sided,, no excess deaths due to product-associated SAEs. Sample size = 1130.

Given the sample size of 1130, the power of the study to detect the difference is only 17%. Because the curve for power is sigmoidal and steep, any error in the underlying assumption for effect size can result in a study severely underpowered to detect a difference should a difference actually exist. Thus, although the study might detect 16 lives saved, it has very little power to do so. To preserve the power of the study at 80% to detect a 5% relative reduction in mortality, the sample size would have to be 9094.

3. 58% mortality, 15% relative effect size, alpha = 0.05, two-sided, 3% excess deaths due to product-related SAEs. Sample size = 1130.

Given the sample size of 1130, the number of potential lives saved is 16, the number of additional deaths due to SAEs is 17, and the net number of lives saved is -1 (16 saved -17 lost = -1). The drug has at best an equivocal benefit:risk profile.

The sensitivity of the benefit:risk calculations to the underlying assumptions may be lowered somewhat by enrolling subjects with a higher underlying mortality rate.

4. 80% mortality in the control group, 15% relative effect size, alpha = 0.05, two-sided, no excess deaths due to product-associated SAEs. Sample size = 1130.

If there are no deaths due to product-associated clinically serious AEs, the power of the study to detect the difference is 99.8% at 5% level of significance. The sample size for 80% power is 349.

5. 80% mortality in the control group, 5% relative effect size, alpha = 0.05, two-sided, no excess deaths due to product-associated SAEs. Sample size = 1130.

If the effect size is 5% and there are no deaths due to product-related clinically serious adverse events, the power of the study is only 39%. To preserve the power of the study at 80% with 5% level of significance to detect a 5% relative reduction in mortality when there are no excess product-related deaths would require a sample size of 3140.

The power of the study to detect a difference in mortality due to use of HBOC-201 is further reduced if the number of deaths due to product-associated clinically serious AEs is greater than 0. The following two examples illustrate the point.

6. 80% mortality in the control group, 15% relative effect size, alpha = 0.05, two-sided, 3% excess deaths due to product-related SAEs. Sample size = 1130.

From the table, the net number of lives saved is 51 (68 lives saved – 17 lives lost = 51), the true relative effect size is 11.3%, and the power of the study to detect the difference is 96.7% at 5% level of significance. The sample size for 80% power is 569.

7. 80% mortality in the control group, 5% relative effect size, alpha = 0.05, two sided, 3% excess deaths due to product-related SAEs. Sample size = 1130.

From the table, the net number of lives saved is 6 (23 lives saved – 17 lives lost = 6), the true relative effect size is approximately 1.25%, and the power of the study is only 7.5% with two-sided 5% level of significance. To preserve 80% power, the sample size would have to increase to 32149.

Questions to the Committee:

1. Do the available data on HBOC-201 raise significant safety concerns based on:
   a. safety signals and adverse events in previous clinical studies
   b. demonstrated vasoactivity of the product
   c. limited safety data for higher doses and rates of administration?

2a. Do the studies of HBOC-201 in animals indicate that HBOC-201 has the potential to provide clinical benefit in the RESUS trial?

2b. Please discuss whether the available pre-clinical and clinical data are sufficient to estimate a treatment benefit of 15% reduction in relative mortality in the RESUS trial.

3.a. On considering all the available data, do the potential benefits outweigh the likely risks of the RESUS study for individual subjects?

3.b. If not, would a trial targeting a group with higher predicted mortality for all subjects merit consideration?

4. Please comment whether any additional modifications of RESUS should be considered that might improve the benefit:risk ratio in individual subjects.

1) Appendix 1:

Sec. 312.42 Clinical holds and requests for modification.

(a) General. A clinical hold is an order issued by FDA to the sponsor to delay a proposed clinical investigation or to suspend an ongoing investigation. The clinical hold order may apply to one or more of the investigations covered by an IND. When a proposed study is placed on clinical hold, subjects may not be given the investigational drug. When an ongoing study is placed on clinical hold, no new subjects may be recruited to the study and placed on the investigational drug; patients already in the study should be taken off therapy involving the investigational drug unless specifically permitted by FDA in the interest of patient safety.

(b) Grounds for imposition of clinical hold--(1) Clinical hold of a Phase 1 study under an IND. FDA may place a proposed or ongoing Phase 1 investigation on clinical hold if it finds that:

(i) Human subjects are or would be exposed to an unreasonable and significant risk of illness or injury;

(ii) The clinical investigators named in the IND are not qualified by reason of their scientific training and experience to conduct the investigation described in the IND;

(iii) The investigator brochure is misleading, erroneous, or materially incomplete; or

(iv) The IND does not contain sufficient information required under Sec. 312.23 to assess the risks to subjects of the proposed studies.

(v) The IND is for the study of an investigational drug intended to treat a life-threatening disease or condition that affects both genders, and men or women with reproductive potential who have the disease or condition being studied are excluded from eligibility because of a risk or potential risk from use of the investigational drug of reproductive toxicity (i.e., affecting reproductive organs) or developmental toxicity (i.e., affecting potential offspring). The phrase ``women with reproductive potential'' does not include pregnant women. For purposes of this paragraph, ``life-threatening illnesses or diseases'' are defined as ``diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted.'' The clinical hold would not apply under this paragraph to clinical studies conducted:

(A) Under special circumstances, such as studies pertinent only to one gender (e.g., studies evaluating the excretion of a drug in semen or the effects on menstrual function);

(B) Only in men or women, as long as a study that does not exclude members of the other gender with reproductive potential is being conducted concurrently, has been conducted, or will take place within a reasonable time agreed upon by the agency; or

(C) Only in subjects who do not suffer from the disease or condition for which the drug is being studied.

(2) Clinical hold of a Phase 2 or 3 study under an IND. FDA may place a proposed or ongoing Phase 2 or 3 investigation on clinical hold if it finds that:

(i) Any of the conditions in paragraphs (b)(1)(i) through (b)(1)(v) of this section apply; or

(ii) The plan or protocol for the investigation is clearly deficient in design to meet its stated objectives.

(3) Clinical hold of a treatment IND or treatment protocol.

(i) Proposed use. FDA may place a proposed treatment IND or treatment protocol on clinical hold if it is determined that:

(A) The pertinent criteria in Sec. 312.34(b) for permitting the treatment use to begin are not satisfied; or

(B) The treatment protocol or treatment IND does not contain the information required under Sec. 312.35 (a) or (b) to make the specified determination under Sec. 312.34(b).

(ii) Ongoing use. FDA may place an ongoing treatment protocol or treatment IND on clinical hold if it is determined that:

(A) There becomes available a comparable or satisfactory alternative drug or other therapy to treat that stage of the disease in the intended patient population for which the investigational drug is being used;

(B) The investigational drug is not under investigation in a controlled clinical trial under an IND in effect for the trial and not all controlled clinical trials necessary to support a marketing application have been completed, or a clinical study under the IND has been placed on clinical hold:

(C) The sponsor of the controlled clinical trial is not pursuing marketing approval with due diligence;

(D) If the treatment IND or treatment protocol is intended for a serious disease, there is insufficient evidence of safety and effectiveness to support such use; or

(E) If the treatment protocol or treatment IND was based on an immediately life-threatening disease, the available scientific evidence, taken as a whole, fails to provide a reasonable basis for concluding that the drug:

(1) May be effective for its intended use in its intended population; or

(2) Would not expose the patients to whom the drug is to be administered to an unreasonable and significant additional risk of illness or injury.

(iii) FDA may place a proposed or ongoing treatment IND or treatment protocol on clinical hold if it finds that any of the conditions in paragraph (b)(4)(i) through (b)(4)(viii) of this section apply.

(4) Clinical hold of any study that is not designed to be adequate and well-controlled. FDA may place a proposed or ongoing investigation that is not designed to be adequate and well-controlled on clinical hold if it finds that:

(i) Any of the conditions in paragraph (b)(1) or (b)(2) of this section apply; or

(ii) There is reasonable evidence the investigation that is not designed to be adequate and well-controlled is impeding enrollment in, or otherwise interfering with the conduct or completion of, a study that is designed to be an adequate and well-controlled investigation of the same or another investigational drug; or

(iii) Insufficient quantities of the investigational drug exist to adequately conduct both the investigation that is not designed to be adequate and well-controlled and the investigations that are designed to be adequate and well-controlled; or

(iv) The drug has been studied in one or more adequate and well-controlled investigations that strongly suggest lack of effectiveness; or

(v) Another drug under investigation or approved for the same indication and available to the same patient population has demonstrated a better potential benefit/risk balance; or

(vi) The drug has received marketing approval for the same indication in the same patient population; or

(vii) The sponsor of the study that is designed to be an adequate and well-controlled investigation is not actively pursuing marketing approval of the investigational drug with due diligence; or

(viii) The Commissioner determines that it would not be in the public interest for the study to be conducted or continued. FDA ordinarily intends that clinical holds under paragraphs (b)(4)(ii), (b)(4)(iii) and (b)(4)(v) of this section would only apply to additional enrollment in nonconcurrently controlled trials rather than eliminating continued access to individuals already receiving the investigational drug.

(5) Clinical hold of any investigation involving an exception from informed consent under Sec. 50.24 of this chapter. FDA may place a proposed or ongoing investigation involving an exception from informed consent under Sec. 50.24 of this chapter on clinical hold if it is determined that:

(i) Any of the conditions in paragraphs (b)(1) or (b)(2) of this section apply; or

(ii) The pertinent criteria in Sec. 50.24 of this chapter for such

an investigation to begin or continue are not submitted or not satisfied.

(6) Clinical hold of any investigation involving an exception from informed consent under Sec. 50.23(d) of this chapter. FDA may place a proposed or ongoing investigation involving an exception from informed consent under Sec. 50.23(d) of this chapter on clinical hold if it is determined that:

(i) Any of the conditions in paragraphs (b)(1) or (b)(2) of this section apply; or

(ii) A determination by the President to waive the prior consent requirement for the administration of an investigational new drug has not been made.

(c) Discussion of deficiency. Whenever FDA concludes that a deficiency exists in a clinical investigation that may be grounds for the imposition of clinical hold FDA will, unless patients are exposed to immediate and serious risk, attempt to discuss and satisfactorily resolve the matter with the sponsor before issuing the clinical hold order.

(d) Imposition of clinical hold. The clinical hold order may be made by telephone or other means of rapid communication or in writing. The clinical hold order will identify the studies under the IND to which the hold applies, and will briefly explain the basis for the action. The clinical hold order will be made by or on behalf of the Division Director with responsibility for review of the IND. As soon as possible, and no more than 30 days after imposition of the clinical hold, the Division Director will provide the sponsor a written explanation of the basis for the hold.

(e) Resumption of clinical investigations. An investigation may only resume after FDA (usually the Division Director, or the Director's designee, with responsibility for review of the IND) has notified the sponsor that the investigation may proceed. Resumption of the affected investigation(s) will be authorized when the sponsor corrects the deficiency(ies) previously cited or otherwise satisfies the agency that the investigation(s) can proceed. FDA may notify a sponsor of its determination regarding the clinical hold by telephone or other means of rapid communication. If a sponsor of an IND that has been placed on clinical hold requests in writing that the clinical hold be removed and submits a complete response to the issue(s) identified in the clinical hold order, FDA shall respond in writing to the sponsor within 30-calendar days of receipt of the request and the complete response. FDA's response will either remove or maintain the clinical hold, and will state the reasonsfor such determination. Notwithstanding the 30-calendar day response time, a sponsor may not proceed with a clinical trial on which a clinical hold has been imposed until the sponsor has been notified by FDA that the hold has been lifted.

(f) Appeal. If the sponsor disagrees with the reasons cited for the clinical hold, the sponsor may request reconsideration of the decision in accordance with Sec. 312.48.

(g) Conversion of IND on clinical hold to inactive status. If all

investigations covered by an IND remain on clinical hold for 1 year or more, the IND may be placed on inactive status by FDA under Sec. 312.45.

Appendix 2.

Sec. 50.24 Exception from informed consent requirements for emergency research.

(a) The IRB responsible for the review, approval, and continuing review of the clinical investigation described in this section may approve that investigation without requiring that informed consent of all research subjects be obtained if the IRB (with the concurrence of a licensed physician who is a member of or consultant to the IRB and who is not otherwise participating in the clinical investigation) finds and documents each of the following:

(1) The human subjects are in a life-threatening situation, available treatments are unproven or unsatisfactory, and the collection of valid scientific evidence, which may include evidence obtained through randomized placebo-controlled investigations, is necessary to determine the safety and effectiveness of particular interventions.

(2) Obtaining informed consent is not feasible because:

(i) The subjects will not be able to give their informed consent as a result of their medical condition;

(ii) The intervention under investigation must be administered before consent from the subjects' legally authorized representatives is feasible; and

(iii) There is no reasonable way to identify prospectively the individuals likely to become eligible for participation in the clinical investigation.

(3) Participation in the research holds out the prospect of direct benefit to the subjects because:

(i) Subjects are facing a life-threatening situation that necessitates intervention;

(ii) Appropriate animal and other preclinical studies have been conducted, and the information derived from those studies and related evidence support the potential for the intervention to provide a direct benefit to the individual subjects; and

(iii) Risks associated with the investigation are reasonable in relation to what is known about the medical condition of the potential class of subjects, the risks and benefits of standard therapy, if any, and what is known about the risks and benefits of the proposed intervention or activity.

(4) The clinical investigation could not practicably be carried out without the waiver.

(5) The proposed investigational plan defines the length of the potential therapeutic window based on scientific evidence, and the investigator has committed to attempting to contact a legally authorized representative for each subject within that window of time and, if feasible, to asking the legally authorized representative contacted for consent within that window rather than proceeding without consent. The investigator will summarize efforts made to contact legally authorized representatives and make this information available to the IRB at the time of continuing review.

(6) The IRB has reviewed and approved informed consent procedures and an informed consent document consistent with 50.25. These procedures and the informed consent document are to be used with subjects or their legally authorized representatives in situations where use of such procedures and documents is feasible. The IRB has reviewed and approved procedures and information to be used when providing an opportunity for a family member to object to a subject's participation in the clinical investigation consistent with paragraph (a)(7)(v) of this section.

(7) Additional protections of the rights and welfare of the subjects will be provided, including, at least:

(i) Consultation (including, where appropriate, consultation carried out by the IRB) with representatives of the communities in which the clinical investigation will be conducted and from which the subjects will be drawn;

(ii) Public disclosure to the communities in which the clinical investigation will be conducted and from which the subjects will be drawn, prior to initiation of the clinical investigation, of plans for the investigation and its risks and expected benefits;

(iii) Public disclosure of sufficient information following completion of the clinical investigation to apprise the community and researchers of the study, including the demographic characteristics of the research population, and its results;

(iv) Establishment of an independent data monitoring committee to exercise oversight of the clinical investigation; and

(v) If obtaining informed consent is not feasible and a legally authorized representative is not reasonably available, the investigator has committed, if feasible, to attempting to contact within the therapeutic window the subject's family member who is not a legally authorized representative, and asking whether he or she objects to the subject's participation in the clinical investigation. The investigator will summarize efforts made to contact family members and make this information available to the IRB at the time of continuing review.

(b) The IRB is responsible for ensuring that procedures are in place to inform, at the earliest feasible opportunity, each subject, or if the subject remains incapacitated, a legally authorized representative of the subject, or if such a representative is not reasonably available, a family member, of the subject's inclusion in the clinical

investigation, the details of the investigation and other information contained in the informed consent document. The IRB shall also ensure that there is a procedure to inform the subject, or if the subject remains incapacitated, a legally authorized representative of the subject, or if such a representative is not reasonably available, a family member, that he or she may discontinue the subject's participation at any time without penalty or loss of benefits to which the subject is otherwise entitled. If a legally authorized representative or family member is told about the clinical investigation and the subject's condition improves, the subject is also to be informed as soon as feasible. If a subject is entered into a clinical investigation with waived consent and the subject dies before a legally authorized representative or family member can be contacted, information about the clinical investigation is to be provided to the subject's legally authorized representative or family member, if feasible.

(c) The IRB determinations required by paragraph (a) of this section and the documentation required by paragraph (e) of this section are to be retained by the IRB for at least 3 years after completion of the clinical investigation, and the records shall be accessible for inspection and copying by FDA in accordance with 56.115(b) of this chapter.

(d) Protocols involving an exception to the informed consent requirement under this section must be performed under a separate investigational new drug application (IND) or investigational device exemption (IDE) that clearly identifies such protocols as protocols that may include subjects who are unable to consent. The submission of those protocols in a separate IND/IDE is required even if an IND for the same drug product or an IDE for the same device already exists. Applications for investigations under this section may not be submitted as amendments under 312.30 or 812.35 of this chapter.

(e) If an IRB determines that it cannot approve a clinical investigation because the investigation does not meet the criteria in the exception provided under paragraph (a) of this section or because of other relevant ethical concerns, the IRB must document its findings and provide these findings promptly in writing to the clinical investigator and to the sponsor of the clinical investigation. The sponsor of the clinical investigation must promptly disclose this information to FDA and to the sponsor's clinical investigators who are participating or are asked to participate in this or a substantially equivalent clinical investigation of the sponsor, and to other IRB's that have been, or are, asked to review this or a substantially equivalent investigation by that sponsor.

Appendix 3: Age by Trial										
Trial	Type	Control	HBOC-201			Dose (grams)	Surgery	Control		
			≤ 50	51-75	> 75			≤ 50	51-75	> 75
			Number of subjects					Number of subjects		
0053	Normal volunteer	HSA	32	0	0	3.25-45	None	23	0	0
0059		LR	18	0	0	≤45	None	6	0	0
0062		None	6	0	0	45	None	0	0	0
0070		None	8	0	0	45-90	None	0	0	0
Total			**64**	**0**	**0**			**29**	**0**	**0**
0072	Non-surgical	NS	12	0	0	10.8-42.9	Sickle- no crisis	7	0	0
0088		LR	0	0	1	90.2	Ventilator	0	0	0
0099		LR	12	0	0	14.0-78.3	Sickle-crisis	7	0	0
Total			**24**	**0**	**1**			**14**	**0**	**0**
0061	Surgical-crystalloid or colloid control	LR	0	16	0	0.5-45	Rad. Prostatectomy	2	9	0
0063		LR	8	2	0	23.4-45	GYN	6	2	0
0068		LR	0	13	0	21.8-45	Orthopedics	1	6	3
0071		LR	1	0	0	27	Emergency GYN	1	0	0
0100		LR	12	40	3	0.7-244.9	Elective Surgery	2	23	1
0101		LR	4	21	0	0.7-165.8	Elective Surgery	2	12	0
0118		LR	5	19	2		Elective-No cardiac	7	16	2
0144		HES	0	19	0	28.5-97.8	AAA Resection	1	19	0
0149		HES	2	4	0	22.4-36.4	Liver transplant	1	7	0
0153		HES	0	6	0	43.2-66.6	Orthopedics	2	6	0
Total			**32**	**140**	**5**			**25**	**100**	**6**
0075	Surgical-RBC control	RBC	6	35	9	60-120	Elective cardiac	1	35	12
0107		RBC	1	38	9	60-150	Elective AAA Res.	1	16	7
0114		RBC	21	51	11	60-210	Elect. Surg.	19	47	11
0115		RBC	97	193	60	60-300	Elective Ortho	69	216	53
Total			**125**	**317**	**89**			**90**	**314**	**83**

Appendix 4: Summary of Animal Studies (Source: CBER review of study reports and literature articles)			
MODEL	**GROUPS (citation)**	**SURVIVAL**	**COMMENTS**
	Study #1 McNeil, J.D., et al.. J Trauma 2001;50 (6):1063-1075 Phase 1 HBOC-201 to MAP 60 mm Hg (N=6) LR to MAP 80 mm Hg (N=6) LR/shed blood to MAP 80 mm Hg (N=6) Phase 2 HBOC-201 to MAP 50 mm Hg (N=6) HBOC-201 to MAP 60 mm Hg (N=6) Shed blood to MAP 60 mm Hg (N=6) LR to MAP 80 mm Hg (N=6) LR/shed blood to MAP 60 mm Hg (N=6)	6/6 at 4 h 6/6 at 4 h 6/6 at 4 h 5/6 at 4 h 6/6 at 4 h 6/6 at 4 h 6/6 at 4 h 6/6 at 4 h	1. Invasive monitoring, anesthesia, fixed BP controlled hemorrhage. 2. Survival ≠ primary endpoint 3. Mean PAP lower in HBOC-201 groups and Blood-60 group than LR alone or LR/shed blood 4. Resuscitation volumes lowest in HBOC-201 groups 5. HBOC 60 group returned to baseline values for arterial pH, lactate, and base excess earlier in resuscitation phase than LR alone 6. HBOC 50 group hemodynamically similar to HBOC 60 group, but metabolically inferior 7. SVO_2 lowest in HBOC-201 groups 8. HBOC 60 returned to baseline values earlier in resuscitation phase; LR alone had ↑lactate and ↓ pH during experiment

	Study #2 Sampson, JB, et al. J Trauma 2003;55 (4) :747 LR to MAP 60 mmHg HBOC-201 to MAP 60 mmHg Hextend to MAP 60 mmHg Pentastarch to MAP 60 mmHg Hypertonic saline dextran to 60 mmHg Hypertonic saline to 60 mmHg	 6/6 at 4 h 6/6 at 4 h 6/6 at 4 h 6/6 at 4 h 5/6 at 4 h 0/6 at 4 h	1. Invasive monitoring, anesthesia, fixed BP controlled hemorrhage. 2. Survival ≠ primary endpoint 3. resuscitation volume lowest in HBOC-201 group 4. Urine output significantly lower in HBOC-201 group 5. Volume to achieve target MAP lowest in HBOC-201 group 6. Cardiac output lowest in HBOC-201 group 7. Trend toward lower PAOP and increased PAP in HBOC-201 group 8. Lactate levels equivalent during resuscitation 9. SVO_2 increase after resuscitation, but not to baseline; HBOC-201 significantly lower than any other group during resuscitation 10. VO_2 equivalent among groups during hemorrhage and resuscitation. 11. Effectiveness of HBOC-201 not evident in some traditional markers of resuscitation including CO, urine output, and SVO_2 12. All groups survived to 4 h except hypertonic saline animals due to hypernatremia and hyperchloremic acidosis 13. Laboratory markers of cellular ischemia and global oxygen consumption suggest satisfactory cellular oxygenation 14. No comparison of HBOC-201 vs LR followed by blood in time frame of RESUS. 15. Lactate ↑ and pH ↓ equally in all groups during hemorrhage, then resolved similarly in all groups during resuscitation. 16. 4-8 h before definitive care is not relevant to RESUS.

	Study #3 York, GB, et al. J Trauma 2003;55 (5):873-885, 2003 Survival model HBOC-201 to MAP 60 mm Hg (N=6) Shed blood to MAP 60 mm Hg (N=6) Shed blood to baseline MAP (N=6) LR (40 mL/kg) to MAP baseline (N=6) After 4 hours, shed blood 60 resuscitated to baseline MAP using remaining shed blood; HBOC-201 60 to baseline MAP using LR. Additional observation for 60 minutes	 6/6 at POD 3 6/6 at POD 3 6/6 at POD 3 5/6 at POD 3	1. Fixed BP endpoint of resuscitation 2. HBOC-201 volume of resuscitation = shed blood 60 volume 3. C.O. HBOC-201 significantly lower than shed blood 60, returning to baseline only with LR administration in hour 5 4. After fluid resuscitation to baseline in hour 5, lactate levels for HBOC-201 animals higher than for other 3 groups; all groups returned to baseline at end of experiment. 5. Urine output lower with HBOC-201, increasing in hour 5 with LR infusion. 6. Creatinine levels trended higher in HBOC-201 groups; all groups returned to baseline creatinine by POD 1. 7. Jejunal pO_2 higher in HBOC-201 group during resuscitation. 8. Multifocal, mild/moderate hepatic necrosis/degeneration 4/6 HBOC-201 animals and 1/5 LR +blood animals- ? ischemic, ? NO scavenging. 9. HBOC-201 pH ↑ to baseline during resuscitation

	Study #4 Fitzpatrick, CM, et al. JACS 2004;199:693-701 Survival ≠ primary endpoint HBOC-201- baseline MAP (N=8) LR (40 mL/kg + shed blood to baseline MAP or until remaining blood used) (N=8) Shed blood to baseline MAP or until remaining blood used (N=8) Control (no hemorrhage)	 8/8 at 4 h 8/8 at 4 h 8/8 at 4 h	1. Purpose = to determine if HBOC-201 alters endothelial function and NO physiology 2. Resuscitation volumes lowest in HBOC-201 group 3. CO remained low in HBOC-201 group with resuscitation 4. MAP and PAP returned to baseline in all groups 5. Lactate levels corrected with resuscitation in all groups, but were higher in HBOC-201 group 6. SVO_2 significantly lower for HBOC-201 group than for other groups throughout resuscitation 7. Absence of vasodilatation with nitroprusside at hour 4 indicates persistently altered vasomotor tone in HBOC-201 animals. 8. Hypothesis that NO scavenging is solely responsible for the vasoactive properties of HBOC-201 not confirmed.

	Study #5 Fitzpatrick, CM, et al. J Trauma 2005;59 (2) :273- 283) Survival model Hextend to MAP 60 mmHg HBOC-201 to MAP 60 mmHg At 4 hours, Hextend group given 4L LR + shed blood; HBOC-201 animals given 4L LR.	4/8 at POD 5 7/8 at POD 5	1. HBOC-201 returned MAP to target; Hextend did not 2. CO returned to normal with Hextend, but remained depressed with HBOC-201 3. SVR increased with hemorrhage. Resuscitation with Hextend returned SVR to below baseline.. HBOC-201 animals decreased to baseline values gradually 4. Resuscitation resulted in similar metabolic parameters for lactate, base excess, and pH. 5. Urine output in both groups was low with hypotensive resuscitation but increased in both groups with definitive resuscitation 6. Hextend treated animals had larger increases in amylase, creatinine, AST, and ALT levels early in resuscitation.
Carolina Resuscitation Research Model Severe uncontrolled bleeding from liver laceration "controlled" by palpation; liver crush injury, or exsanguinating cardiac arrest + prolonged delay to definitive care	**Study #1** Manning, et al. Shock 2000;13;152-159 Short term survival model LR to MAP 60 mmHg for 2 h HBOC-201 to MAP 60 mmHg for 2 h	1/9 at 2 h 4/5 at 2 h	1. Improved survival with lower lactate levels for HBOC-201 vs controls 2. Lower volume required for HBOC-201/unit time to achieve resuscitation 3. Model of Class IV shock demonstrates what is already known: a) untreated massive hemorrhage is rapidly fatal and b) ability of an asanguinous solution to resuscitate from massive fatal hemorrhage is very low.

Hydrated Warmed	**Study #2** Katz, et al. Resuscitation 2002;54:77-87 Survival model 6% HES at 6 mL/kg/min X 15 min followed by 3 mL/kg x 30 minutes (N=8) HBOC-201 at 6 mL/kg/min x 15 min followed by 3 mL/kg x 30 min (N=8) Sham- no fluid during resuscitation (N=6) Followed by reinfusion of collected blood over 20 minutes, and surgical repair and ICU care and transfusion to maintain hct > 18%.	0/8 at 24 h 8/8 at 24 h; 7/8 at 96 h 0/8 at 24 h	See comments for Study 1. 1. Higher MAP and CI seen with HBOC-201 than with Hetastarch 2. BD less severe with HBOC-201 than with Hetastarch 3. Whole blood used to develop model showed that an average 4 mL/kg/min needed to maintain MAP = 60 mm Hg during 45 minute resuscitation phase and allow 96 hour survival. 4. MAP, SVR, MPAP increased significantly with HBOC-201, possibly as a result of aggressive infusion 5. ? about potential to increase bleeding 6. Oxygenation, ventilation, perfusion not compromised by HBOC-201 in this model 7. Oozing from cut-down sites after resuscitation suggest need to determine effect of HBOC-201 on coagulation pathways
	Study #3 Crit Care Med 2001;29:2067) LR HBOC-201	0/6 at 60 min 5/6 at 60 min	1. Improved survival with HBOC-201 2. HBOC-201 restored viable cardiovascular function after experimental cardiac arrest. 3. Not relevant to RESUS because these patients will be excluded from the study

Knudson and Manley Model Controlled, moderate, low-severity, fixed-BP hemorrhagic shock to MAP 40 mm Hg in splenectomized animals, with short delay to definitive care Warmed Hydrated	**Study #1** Knudson, MM, et al. J Trauma 2003; 54(2): 242-252 HBOC-201 6 mL/kg (N=10) HSD 4 mL/kg (N=10) LR 12 mL/kg (N=10) 2 hour observation	10/10 at 2 h 9/10 at 2 h 10/10 at 2 h	1. Blood volume loss = 33% 2. Liver pO_2 return to baseline with HSD and LR; HBOC-201 animals had liver pO2 significantly below baseline at 2 h. 3. Fixed volume resuscitation: HBOC-201 returns MAP closest to baseline with more sustained response than HSD or LR 4. More HBOC-201 animals achieved SBP > 90 mm Hg than HSD or LR (9/10 HBOC-201 vs 1?10 LR vs 4/9 HSD) 5. C.O. lowest in HBOC-201 group 6. DO_2 for HBOC-201 same as for LR but less than for HSD 7. Study evaluated use of invasive tissue oxygenation monitoring, not proposed for RESUS
	Study # 2 Lee, SK, et al. Acad. Em. Med. 2002 9(10): 969-976 HBOC-201 6 mL/kg bolus(N=7) 2 hour observation and evaluation of brain tissue oxygenation	No control	1. Fixed volume bolus administration- no SVR data 2. HR decreased with HBOC-201 3. MAP and C.O. ↑ (83% and 84% of baseline) 4. $PBrO_2$ exceeded baseline by 66% 5. Mild increase in PAP with HBOC-201 6. Preliminary study with rapid infusion of HBOC-201 through distal port on thermodilution CO catheter resulted in immediate hemodynamic collapse
Proctor Model Controlled hemorrhagic shock + TBI	**Study # 1** King, DR et al. J Trauma 2005; 59(3): 553-562 Part 1- nonsurvivor LR+mannitol + RBC to MAP 70 mm Hg (N=5) HBOC-301 to MAP 70 mm Hg (N=5) LR + HBOC-301 to MAP 70 mm Hg (N=5)	HBOC-301 used, not HBOC-201	Part 1: 1. Decreased fluid and blood transfusion needs for HBOC-301 group 2. No elevation in ICP with HBOC-301 groups 3. Brain tissue oxygenation maintained by HBOC-301 groups 4. CPP maintained in HBOC-301 groups 5. OER "persistently" elevated with HBOC-301 but not with LR alone or LR + HBOC-301 6. "Lactate clearance initially impaired with HBOC alone; however, all groups corrected appropriately and converged…by end of experiment." Part 2- survivor:

	Part 2- survivor LR + mannitol + RBC (N=3) HBOC-301 (N=3) Two pre-randomization deaths	 0/3 3/3	1. LR/mannitol/RBC animals unable to wean from ventilator within 6 hours- therefore euthanized 2. 3/3 HBOC-301 animals weaned from ventilator and survived to POD 3 3. Similar data to Part 1
NMRC Model Moderate Controlled Hemorrhage Muscle crush injury and controlled vascular hemorrhage- 40% with extended BP/HR-determined 4 hour resuscitation Water deprived 12-14 hours before study start Room Temperature solutions Animals warmed	Philbin, N., et al. Resuscitation 2005; 66: 367-378 No treatment (N=8) Hextend 10 mL/kg over 10 minutes (N=8) HBOC-201 10 mL/kg over 10 minutes (N=8) Additional 5 mL/kg at 30, 60, 120, and 180 minutes for MAP < 60 mm Hg or tachycardia (HR > baseline) In-hospital phase- 10 mL/kg shed blood for hb < 7 g/dL; 10 mL/kg NS for hypotension without anemia	Controlled as opposed to uncontrolled hemorrhage 7/8 at 4 h//5/8 at 3d 8/8 at 4 h// 7/8 at 3d 8/8 at 4 h// 8/8 at 3d	Resuscitation phase: 1. Higher MAP and MPAP, lower CI than for HEX (but at baseline) 2. Marked increase in SVRI with initial infusion of HBOC-201; differences diminished over time 3. Decreased fluid requirements at 60, 120, 180 minutes in HBOC-201 group 4. VO_2 significantly higher for HBOC-201 animals than other groups; DO_2 and OER not significantly different but trended higher in HBOC-201 group 5. Lactate levels not significantly different among the groups 6. Urine output similar between HBOC-201 and HEX 7. Transcutaneous tissue oxygenation higher for HBOC-201 over HEX 8. SVO_2 trended higher for HBOC-201 than for HEX animals In-hospital phase 9. Fluid infusion and transfusion requirements lower for HBOC-201 animals Critique: 1. Less severe controlled hemorrhage does not show particular survival advantage 2.Hextend not relevant to RESUS 3. 4 hour delay not relevant to RESUS 4.

NMRC Model Severe Controlled Hemorrhage Muscle crush injury and controlled vascular hemorrhage- 55% with extended BP/HR-determined 4 hour resuscitation Water deprived 12-14 hours before study start Room Temperature solutions Animals warmed Survival model	Rice, et al. Shock 26(3):1-10, 2006 No treatment (N=8) Hextend 10 mL/kg over 10 minutes (N=8) HBOC-201 10 mL/kg over 10 minutes (N=8) Additional 5 mL/kg at 30, 60, 120, and 180 minutes for MAP < 60 mm Hg or tachycardia (HR > baseline) In-hospital phase- 10 mL/kg shed blood for hb < 7 g/dL; 10 mL/kg NS for hypotension without anemia	Controlled as opposed to uncontrolled hemorrhage 2/8 at 4 h// 2/8 at 72 h 7/8 at 4h// 6/8 at 72 h 8/8 at 4 h and 72 h	1. Higher MAP, MPAP, and SVRI 2. CI and HR not different from HEX treated animals 3. Higher tissue oxygenation for HBOC-201 4. Similar BD, lactate, DO_2, VO_2, and OER 5. Fluid requirements similar in prehospital phase 6. Lower transfusion requirements 7. Decreased urine output for HBOC-201 animals compared with HEX treated animals, but higher than for untreated animals Critique: 1. 4 hour delay to definitive care not relevant to estimate of effect size for RESUS which is short-term arrival to definitive care 2. Use of Hextend not relevant to RESUS which proposes use of LR 3. Controlled hemorrhage experiment, not relevant to uncontrolled HS with/without TBI
NMRC Model Severe Controlled Hemorrhage	Philbin, N., et al. manuscript in preparation		1. MAP, MPAP higher with HBOC-201,> HEX; MAP HBOC-201 and HEX increase similar until 60 minutes

Muscle crush injury and controlled vascular hemorrhage- 55% with extended BP/HR-determined 24 hour resuscitation Water deprived 12-14 hours before study start Room Temperature solutions Animals warmed Animals preassigned to the various groups, not randomized after hemorrhage Survival model	No treatment (N=8) Hextend 10 mL/kg over 10 minutes (N=8) HBOC-201 10 mL/kg over 10 minutes (N=8) Additional 5 mL/kg at 30, 60, 120, and 180 minutes for MAP < 60 mm Hg or tachycardia (HR > baseline) In-hospital phase at 24 hours- 10 mL/kg shed blood for hb < 7 g/dL; 10 mL/kg NS for hypotension without anemia Additional infusions at 48 hours as needed.	Non: 3/8 at 4 hours, 2/8 at 72 hours Hextend: 8/8 at 4 hours; 7/8 at 72 hours HBOC-201: 8/8 at 4 hours; 7/8 at 72 hours	when HEX plateaus and HBOC-201 continues to increase 2. CI intermediate between HEX and Non; HR lower than HEX and NON 3.SVRI not significantly different from HEX and NON although higher Lactate non different from HEX; lower than NON SVO(2) not different from HEX Oxygen delivery higher but not significantly different form HEX Oxygen consumption not different from other solutions Urine output and creatinine not different; BUN higher with HBOC-201 at 24 and 48 hours Critique: 1. 4 hour delay to definitive care not relevant to estimate of effect size for RESUS which is short-term arrival to definitive care 2. Use of Hextend not relevant to RESUS which proposes use of LR 3. Controlled hemorrhage experiment, not relevant to uncontrolled HS with/without TBI 4.% EBV estimated very closely: 54.8± 0.2%, 55.0±0%, and 54.9

NMRC Model- Uncontrolled hemorrhagic shock from liver laceration Extended BP/HR-determined Resuscitation after 15 minutes hemorrhage from lacerated liver At 4 hours, abdomen opened and residual blood removed Water deprived 12-14 hours before study start Room Temperature solutions Animals warmed	Gurney, J, et al. J Trauma 2004; 57(4): 726-738 No treatment (N=8) Hextend 10 mL/kg over 10 minutes (N=8) HBOC-201 10 mL/kg over 10 minutes (N=8) Additional 5 mL/kg at 30, 60, 120, and 180 minutes for MAP < 60 mm Hg or tachycardia (HR > baseline) After 4 hours, transfusion for Hb < 7 gm/dL 20 mL/kg NS Data from 2 animals unable to regain spontaneous breathing before resuscitative fluids were excluded	1/8 at 4 h/ 1/8 at 3 d 3/8 at 4 h/ 1/8 at 3 d 7/8 at 4 h/ 7/8 at 3 d	1. 4 hour resuscitation- HEX deaths at 25, 60, 75, 105, and 240 minutes 2. After 4 hours, abdomen opened and blood removed for quantitation 3. 3 day survival model 4. **HBOC-201 animals had less hemorrhage volume (25-26%) vs 35-38% for other groups- confounds survival, hemodynamic, and metabolic data- FDA non-clinical reviewer questioned robustness of the model and concluded that the Carolina Resuscitation model was more reliable in terms of assessing EBL with a vasoactive HBOC.** 5. Hemorrhage volumes not correspond with EBV % loss 6. Higher MAP and MPAP and lower HR with HBOC-201 7. Lower prehospital fluid requirements 8. SVRI higher during prehospital phase for HBOC-201 animals 9. Increased tissue oxygenation 10. Similar cardiac index and blood loss post-resuscitation 11. DO_2 higher with no effect on VO_2 and OER to HEX treated animals 12. Urine output similar among 3 groups in prehospital phase but resumed more rapidly with HBOC-201. 13. Trend toward more rapid lactate clearance in HBOC-201 animals- data confounded by early deaths in HEX and No treatment groups 14. Not fully simulate RESUS with short prehospital transit times and blood transfusion availability. Critique: Shows what is already known which is that if delay to definitive care is prolonged for severe HS, an oxygen carrier is better than asanguinous solutions for resuscitation. Also, that less hemorrhage results in less damage than larger volume hemorrhage.

NMRC model Uncontrolled hemorrhagic shock from liver laceration + TBI Initial hemorrhage 20-25 mL/kg (approximately 30-37% EBV) Feed withheld 12-14 hours prior to experiment Protocol silent on whether water also withheld Protocol silent on whether animals were warmed	<u>Study #1: 30 minute delay to definitive care</u> No fluid, then up to 3 RBC transfusions LR 20 mL/kg/infusion, then up to 3 RBC transfusions HBOC-201 10 mL/kg/infusion, then up to 3RBC transfusions <u>Study #2: 75 minute delay to definitive care</u> No fluid, then up to 3 RBC transfusions for Hb < 7 g/dL LR, then up to 3 RBC transfusions for Hb < 7 g/dl HBOC-201, then up to 3 RBC transfusions for hb < 7 g/dl	16/16 at 30 min; 3/3 at 6 h 28/28 at 30 min; 4/9 at 6 h 31/31 at 30 min; 5/9 at 6 h 3/10 at 75 min; 1/8 at 6 h 10/14 at 75 min; 1/11 at 6 h 15/17 at 75 min; 8/14 at 6 h	No final study report submitted to IND. Results from both time-cohorts confounded by ketorolac administration at time of RBC transfusion (blunting PHT associated with HBOC-201 infusion but not with LR) and under-transfusion/over hemodilution of non-HBOC-201 animals. <u>Study # 1</u> 1. Survival not improved over no fluid 2. Higher left brain and transcutaneous tissue oxygenation with HBOC-201 vs LR 3. Tissue oxygenation monitoring not proposed for RESUS 4. Similar blood loss to LR 5. Survival may be confounded by limitation of transfusion to 3 regardless of hb level 6. <u>Study # 2</u> 1. Survival significantly improved at 6 hours with HBOC-201 2. MAP, CPP, and sagittal sinus oxygen saturation higher with HBOC-201 3. Sagittal sinus and arterial lactate lower with HBOC-201 vs LR 4. Similar blood loss to LR 5. Survival may be confounded by limitation of transfusions to 3 regardless of Hb level Critique:

			1. 58 animals originally proposed (30 added with amendment 3 1/28/05) 2. Total 88 animals- 75 included in analysis, of which 54 received ketorolac. 8 animals were excluded, 4 were culled, and 1 was a developmental animal. 3. 31 HBOC (8 without ketorolac, 23 with), 28 LR (8 without ketorolac, 20 with), 16 NON animals (5 without ketorolac/11 with). 4. Non ketorolac animal survival (30 MINUTE COHORT): HBOC-201 (N=5)- 2.83 hours LR (N=5) 4.2 HOURS Non (n=3)- 1.3 HOURS 5. Ketorolac animals (30 minute cohort): HBOC-201 (N=9)- 3.99 hours LR (N=9)- 3.22 hours Non (N=3) 6 hours 5. Non ketorolac survival (75 minute cohort): HBOC-201 (N=3)- 3.42 hours LR (N=3)- 1.4 hours Non (N=2)- 4.5 hours 6.Ketorolac animals (75 minute cohort): HBOC-201 (N=14)- 4.4 hours LR (N=11)- 1.53 hours NON (N=8)- 1.31. hours 7. Note that blood requirement chart includes 14, not 13 animals, and does not comport with figure 4 of the study report which shows only 13 animals. 8. Transfusion data and total hemoglobin data must be

			clarified and reconciled- It is not clear what the relationship is between total hemoglobin and transfusion. Further, it is not clear from the tables when transfusion were actually given in the 75 minute cohort. The table for transfusion indicates transfusions at 30 minutes for the 75 minute cohort. Also, one animal is duplicated and data from one animal are missing. 9. Amendment 4 of the protocol, dated 9/16/05 and approved in 9/05 indicates a request for 10 additional animals to be added to the NON cohort for the 30 minute cohort in order to allow for statistical analysis and comparison to the other two groups. This statement and this intent do not comport with statements made that no statistical analysis on survival was intended for the short delay (30 minute) cohort. Where are the data from the 10 additional animals, and what statistical analyses were planned? Where are the results of the analyses?
USUHS model Soft tissue injury and severe 50% vascular hemorrhage with additional, ongoing removal of approximately 5% EBV (3.25 mL/kg)/hr Exclusively BP-controlled resuscitation 4 hour hemorrhage followed by definitive surgical repair. Autologous shed blood for hct < 20%, persistent MAP < 50 mm Hg, or			BP-controlled resuscitation led to severe under-resuscitation due to inadequate volume infusion HBOC-201 and HEX animals received 12-19 mL/kg (hypotensive) or 26-40 mL/kg (normotensive) resuscitation. In contrast to 3 other NMRC studies (see 40% controlled hemorrhage, 55% controlled hemorrhage, and liver laceration/uncontrolled hemorrhage) in which animals received 18-30 mL/kg, > 30 mL/kg, and 20 mL/kg fluid infusion respectively. Total blood loss (initial injury, rebleeding volume, and catheter withdrawn blood loss) was similar in all hypotensive groups, which were comparable to untreated

tachycardia (not prespecified) At 300 minutes, remaining shed blood administered at 20 mL/kg/hr until all shed blood used or until 360 min Feed withheld for 12 hours prior to experiment. No information about water Fluid resuscitation 30 minutes after injury 6 hour study	<u>Hypotensive</u> HBOC-201 5 mL/kg q 30 min to maintain MAP < 50 mm Hg (N=10) Hextend 5 mL/kg q 30 minutes to maintain MAP < 50 mm Hg (N=8) Saline 10 mL/kg q 30 minutes to maintain MAP < 50 mm Hg (N=7) <u>Normotensive</u> HBOC-201 10 mL/kg PRN to maintain baseline MAP (N=8) Hextend 10 mL/kg PRN to maintain baseline MAP (N=8) Saline 20 mL/kg to maintain baseline MAP (N=8) Non-treated (N=10)	5/10 at 6 h 7/8 at 6 h 5/7 at 6 h 4/8 at 6 h 3/8 at 6 h 6/8 at 6 h 1/10 at 6 h	animal blood loss. For normotensive group, HBOC-201 and HEX were not significantly different but trended higher than for NS treated animals. Hypotensive resuscitation: 1. Mean PAP higher in HBOC-201 animals- spiked to 38 mm Hg at time of first transfusion 2. Cardiac index low in HBOC-201 animals- lowest with hypotensive resuscitation 3. SVRI higher for HBOC-201 animals 4. Incidence of rebleeding higher for HBOC-201 treated animals, and trended to larger volumes for HBOC-201 treated animals 5. Urine output trended lower for HBOC-201 treated animals 6. DO_2lowest for HBOC-201 treated animals 7. VO_2 lowest in HBOC-201 treated animals 8. OER significantly higher than baseline in all groups with HBOC-201 = HEX = NS 9. Lactate increased early with HBOC-201 10. pH lowest with HBOC-201 11. No difference total hb concentration among groups 12. Cutaneous tissue oxygenation increased similarly for HBOC-201 = NS= HEX. Normotensive resuscitation: 1. MPAP trended slightly higher in HBOC-201 treated animals with highest values seen at 270 minutes, at time of first transfusion 2. Cardiac index was lower than in HEX or NS groups, but higher than in no treatment group 3. SVRI higher for HBOC-201 animals; spiked at time of first transfusion in HBOC-201 treated animals 4. Incidence of rebleeding and volume of blood lost due to rebleeding was higher in HBOC-201 treated animals

	Data from 3 animals excluded because of technical difficulties, mechanical malfunction of ventilation system.		than NS or untreated; HBOC-201 and HEX were similar. 5. Urine output lower than for HEX or NS treated animals, but higher than for untreated animals 6. DO_2lowest for HBOC-201 treated animals 7. VO_2 lowest in HBOC-201 treated animals 8. Lactate increased late in HBOC-201 animals, peaking at time of first transfusion 9. HBOC-201 decreased OER during initial bolus infusion at 30 minutes, but OER increased steadily and remained elevated during remainder of experiment. OER in HEX and NS treated animals returned to baseline faster than in HBOC-201 treated animals. 10. Transcutaneous tissue oxygenation similar for NS and HBCO-201 > HEX >> no treatment

Conditional Table 3A: Selected Clinically significant Adverse Events by Control Agent or by Age Cohort, ≤75, >75									
Selected Treatment Emergent AEs	RBC-controlled studies		Crystalloid-colloid controlled studies		Surgery studies stratified by Age				
					≤75 years old			> 75 years old	
	HBOC-201 N=531	Control N=487	HBOC-201 N=177	Control N=131	HBOC-201 N=614	Control N=529		HBOC-201 N=94	Control N=89
Death	21	13	4	1	12	9		13	5
Myocardial Infarction	7	3	7	1	8	3		6	1
Cardiac/cardiorespiratory arrest, Ventricular fibrillation	15	5	2	1	8	4		9	2
Congestive heart failure/ cardiac failure/ pulmonary edema, etc.	38	13	16	9	39	16		15	6
Pneumonia	28	19	7	3	24	13		11	9
Cerebrovascular accident/infarction	10	1	0	0	4	0		6	1
Acute renal failure	8	4	2	0	6	4		4	0
Dialysis	5	2	2	0	5	1		2	1

Conditional Table 3B: Selected Clinically significant Adverse Events by Control Agent or by Age Cohort, < 70, ≥70									
Selected Treatment Emergent AEs	RBC-controlled studies		Crystalloid-colloid controlled studies		Surgery studies stratified by Age				
					≤69 years old			> 70 years old	
	HBOC-201 N=531	Control N=487	HBOC-201 N=177	Control N=131	HBOC-201 N=476	Control N=422		HBOC-201 N=222	Control N=196
Death	21	13	4	1	9	3		16	11
Myocardial Infarction	7	3	7	1	6	2		8	2
Cardiac/cardiorespiratory arrest, Ventricular fibrillation	15	5	2	1	6	2		11	4
Congestive heart failure/ cardiac failure/ pulmonary edema, etc.	38	13	16	9	26	10		28	12
Pneumonia	28	19	7	3	16	6		19	16
Cerebrovascular accident/infarction	10	1	0	0	3	0		7	1
Acute renal failure	8	4	2	0	6	3		4	1
Dialysis	5	2	2	0	4	0		3	2

Conditional Table 4 of All Clinical Trials with HBOC-201 (Source: 06/06/06 and 06/15/06 submissions)						
	HBOC-201 N=797			Control (RBC/crystalloid/colloid) N=661		
Treatment-emergent AE	Adverse events (AEs) No. of pts	Serious adverse events (SAEs) No. of pts	Total AEs + SAEs No. of pts	Adverse events (AEs) No. of pts	Serious adverse events (SAEs) No. of pts	Total (AEs + SAEs) No. of pts
Death			**25**			**14**
Hypertension/BP increased	**166**	**2**	**168**	**60**	**0**	**60**
Pulmonary hypertension			**3**			**0**
Chest pain/pressure/tightness	**30**	**3**	**35**	**19**	**0**	**19**
Congestive heart failure, congestive cardiac failure, cardiac failure, etc.	**41**	**13**	**54**	**19**	**3**	**22**
Cardiac arrest, cardiorespiratory arrest	**2**	**15**	**17 (0115-5405) and 0107-0403**	**0**	**6**	**6**
Myocardial infarction, angina pectoris, etc	**6**	**14**	**20(0101-725)**	**7**	**4**	**11**
Cardiac arrhythmias	**143**	**12**	**155**	**91**	**11**	**102**
Hypoxia, cyanosis, saturation	**58**	**2**	**60**	**25**	**1**	**26**
Pneumonia, aspiration pneumonia	**25**	**11**	**36**	**19**	**5**	**24**
ARDS, resp. distress/fail.	**11**	**11**	**22**	**9**	**3**	**12**
CVA, TIA, cerebral infarct, etc	**7**	**9**	**16**	**0**	**3**	**3**

Conditional Table 4 (continued) of All Clinical Trials with HBOC-201(Source: 06/06/06 and 06/15/06 submissions)						
	HBOC-201 N=797			Control (RBC/crystalloid/colloid) N=661		
Treatment-emergent AE	Adverse events (AEs) No. of pts	Serious adverse events (SAEs) No. of pts	Total AEs + SAEs No. of pts	Adverse events (AEs)	Serious adverse events (SAEs)	Total (AEs + SAEs)
Oliguria, etc.	**103**	**9**	**111**	**49**	**6**	**55**
Dialysis			**7 (0115-4308)**			**2**
Hypovolemia	18	**1**	**19**	**2**	**2**	**4**
Gastrointenstinal pain, nausea, vomiting	**387**	**3**	**390**	**204**	**1**	**205**
Gallbladder	**7**	**4**	**11**	**1**	**0**	**1**
Hepatobiliary/pancreatic	**94**	**6**	**100**	**27**	**1**	**28**
Coagulation	**39**	**9**	**48**	**9**	**8**	**17**
Hemorrhage/bleeding	**91**	**17**	**108**	**45**	**10**	**55**
Sepsis/septic shock	**6**	**9**	**15**	**2**	**4**	**6**
Ischemia/ischemic events	**0**	**4**	**4**	**0**	**1**	**1**

Conditional Table 5: AEs by Study type (Normal Volunteer, non-surgery, surgery with Crystalloid/Colloid control, Surgery with RBC Control

	Normal volunteer		Non-surgical		Surgery:Crystalloid/Colloid		Surgery : RBC	
AE Category	HBOC-201	Control	HBOC-201	Control	HBOC-201	Control	HBOC-201	RBC
	N=64	N=29	N=25	N=14	N=177	N=131	N=531	N=487
HTN/ increased BP	0	0	2	1	62	17	104	42
Pulmonary hypertension								
Chest pain/pressure	3	0			9	2	19	16
Congestive heart failure,	0 (2)	0	0	0	2	0	38	13
Cardiac arrest	0	0	0	0	2	1	15	3
MI/ischemia/angina	0	0	0	1	7	2	13	8
Myocardial infarction					7	1	7	3
Cardiac arrhythmias	2	1	0	1	34	28	119	72
CVA, cerebral infarct,	0	0	0	0	0	0	16	3
Pneumonia, aspiration pneumonia	0	0	1	1	7	3	26	19
Respiratory failure/insufficiency	0	0	0	0	3	1	19	11
Oliguria, anuria, renal failure, acute renal failure	0	0	0	0	33	9	81	46
Dialysis	0	0	0	0	2	0	5	2
Hypoxia, cyanosis, saturation	0	0	0	0	22	8	54	27
Hypovolemia	0	0	0	0	4	1	15	3

Conditional Table 5 (continued): AEs by Study type (Normal Volunteer, non-surgery, surgery with Crystalloid/Colloid control, Surgery with RBC Control

	Normal volunteer		Non-surgical		Surgery:Crystallloid/Colloid		Surgery : RBC	
AE Category	HBOC-201	Control	HBOC-201	Control	HBOC-201	Control	HBOC-201	RBC
	N=64	N=29	N=25	N=14	N=177	N=131	N=531	N=487
Gastrointenstinal pain, nausea, vomiting	30	5	11	6	80	40	265	155
Gallbladder	0	0	0	0	1	0	10	1
Hepatobiliary/pancreatic	1	0	0	0	14	5	85	23
Coagulation	3	0	0	0	11	4	34	13
Hemorrhage/bleeding	0	0	0	0	23	19	85	36
Sepsis/septic shock	0	0	0	0	2	0	13	6
Ischemia/ischemic events	0	0	0	0	2	0	2	1

Conditional Table 6: AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies						
	Crystalloid-Colloid Controlled Surgeries		RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	HBOC-201 N=531	RBC N=487	Total HBOC-201	Total Control
Death	4	1	21	13		
Hypertension/BP increased/hypertensive crisis/malignant hypertension/systolic hypertension/SVR increased	**62**	**17**	**104**	**42**	166	61
Hypertension	54	15	64	32	118	47
BP increased	6	2	29	5	35	7
Hypertensive crisis			1	0	1	0
Systolic hypertension	1	0	1	0	2	0
SVR increased			1	0	1	0
Malignant hypertension			1	0	1	0
Systolic BP increased	1	0	2	1	3	1
Post-op hypertension			0	1	0	1
Hypertension-aggravated			5	4	9	4
Pulmonary hypertension	1	0	2	0	3	0
Chest pain/pressure/tightness	9	2	19	16	28	18

Conditional Table 6 (continued) : AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies						
	Crystalloid-Colloid Controlled Surgeries		RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	HBOC-201 N=531	RBC N=487	Total HBOC-201	Total Control
Congestive heart failure, congestive cardiac failure, cardiac failure, etc.	**16**	**9**	**38**	**13**	54	22
Congestive cardiac failure	4	1	11	2	15	3
Cardiac failure			2	2	2	2
Cardiorespiratory failure						
Left ventricular failure						
Pulmonary edema	6	4	16	7	22	11
Acute circulatory failure	1	1	2	0	3	1
Cardiac index decreased	2	0			2	0
Cardiac output decreased	2	0	1	0	3	1
CVP increased			2	0	2	0
Fluid overload	5	4	8	2	13	6
Cardiac arrest, cardiorespiratory arrest	**2**	**1**	**15**	**5**		
Cardiorespiratory arrest	0	0	2	0	2	0
Cardiac arrest	1	1	10	3	11	4
Ventricular fibrillation	1	0	3	1	4	1

Conditional Table 6 (continued): AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies							
	Crystalloid-Colloid Controlled Surgeries			RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131		HBOC-201 N=531	RBC N=487	Total HBOC-201 AEs	Total Control AEs
Myocardial infarction, angina pectoris, etc	**7**	**2**		**13**	**8**	20	10
Myocardial infarction	7	1		7	3		
Myocardial ischemia				4	5		
Angina pectoris	1	1					
Unstable angina	2	0					
Cardiac arrhythmias	**34**	**28**		**119**	**72**		
arrhythmia	1	0		8	5		
Atrial fibrillation	2	1		21	19		
Atrial flutter				4	1		
bradycardia	10	6		10	3		
tachycardia	4	13		67	38		
V-extrasystoles	7	3		7	4		
Supraventricular tachycardia	1	1		2	1		
V-tach	2	1		6	5		
Atrial tachycardia							
Bundle branch block/RBBB				3	0		
Sinus tachycardia	6	5		14	8		

Conditional Table 6 (continued): AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies							
	Crystalloid-Colloid Controlled Surgeries			RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	Total	HBOC-201 N=531	RBC N=487	Total HBOC-201 AEs	Total Control AEs
CVA, TIA, cerebral infarct, etc				**16**	**3**	16	3
Cerebrovascular accident				9	1	9	1
Hemiparesis				1	0	1	0
Hemiplegia				2	0	2	0
Monoparesis				1	0	1	0
Cerebral infarction				1	0	1	0
TIA				2	0	2	0
Reversible IND				0	1	0	1
Transient cerebrovasc event				1	1	1	1

Conditional Table 6 (continued): AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies							
	Crystalloid-Colloid Controlled Surgeries			RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	Total	HBOC-201 N=531	RBC N=487	Total HBOC-201 AEs	Total Control AEs
Pneumonia, aspiration pneumonia, respiratory failure/insufficiency	**7**	**3**		**28**	**19**	35	22
Pneumonia- Klebsiella				1	0	1	0
Pneumonia	5	3		19	11	24	14
Pneumonia- Pseudomonas				1	0	1	0
Pneumonia- staphylococcus				2	0	2	0
Pneumonia- aspiration				4	3	4	3
Pneumonitis	1	0				1	0
Respiratory distress.etc	**3**	**1**		**19**	**11**	22	12
ARDS				4	2	4	2
Respiratory distress	3	1		3	2	6	3
Resp failure exc neonatal				13	8	13	8

Conditional Table 6 (continued): AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies							
	Crystalloid-Colloid Controlled Surgeries			RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	Total	HBOC-201 N=531	RBC N=487	Total HBOC-201 AEs	Total Control AEs
Oliguria, etc.	**33**	**9**		**81**	**46**	114	55
Oliguria	28	9		67	33	95	42
Renal failure- acute	2	0		8	4	10	4
Renal failure-chronic				0	1	0	1
Renal failure				4	4	4	4
Renal impairment				6	5	6	5
Anuria				2	1	2	1
Dialysis	2	0		5	2	7	2
Urinary hesitation							
Urinary retention							
Hypoxia, cyanosis, saturation	**22**	**8**		**54**	**27**	76	35
Decreased oxygen sat	15	5		23	10	38	15
Hypoxia	7	3		17	7	24	10
Cyanosis				3	2	3	2
Hypovolemia	**4**	**1**		**15**	**3**	19	4
Hypovolemia	2	1		5	2	7	3
Hypovolemic shock				1	1	1	1
Dehydration	2	0		9	0	11	0

Conditional Table 6 (continued): AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies							
	Crystalloid-Colloid Controlled Surgeries			RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	Total	HBOC-201 N=531	RBC N=487	Total HBOC-201 AEs	Total Control AEs
Gastrointenstinal pain, nausea, vomiting	**80**	**40**		**265**	**155**	345	195
Abdominal pain	13	6		33	3	46	9
Abdominal pain-upper	7	1		30	11	37	9
Esophageal spasm				1	0	1	0
Vomiting	24	11		96	51	120	62
Abdominal distension	13	7		28	12	41	19
Dyspepsia	9	2		41	29	50	31
Dysphagia	4	1		32	5	36	6
Eructation	2	2		2	0	4	2
Nausea	56	24		157	90	213	114
Vomiting aggravated				1	0	1	0
Post-operative nausea	3	0				3	0
Retching	3	1		1	1	4	2

Conditional Table 6 (continued): AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies							
	Crystalloid-Colloid Controlled Surgeries			RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	Total	HBOC-201 N=531	RBC N=487	Total HBOC-201 AEs	Total Control AEs
Gallbladder	**1**	**0**		**10**	**1**	11	1
Biliary colic				1	0	1	0
Cholescystitis –acute				1	0	1	0
Cholecystitis				2	0	2	0
Cholelithiasis				5	1	5	1
Gallbladder disorder	1	0				1	0
Gallbladder edema				1	0	1	0

Conditional Table 6 (continued): AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies							
	Crystalloid-Colloid Controlled Surgeries			RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	Total	HBOC-201 N=531	RBC N=487	Total HBOC-201 AEs	Total Control AEs
Hepatobiliary/pancreatic	**14**	**5**		**85**	**23**	99	28
Pancreatitis	2	2		3	1	5	3
Hepatic failure	1	0				1	0
Hepatic disorder/abnl fn	2	0		1	0	3	0
Hepatic pain				1	0	1	0
Hepatitis				1	0	1	0
Hepatomegaly	1	0		2	0	3	0
Hepatorenal failure				1	0	1	0
Hepatorenal syndrome	1	0				1	0
ALT increased/abnl	3	2		17	8	20	10
AST increased/abnl	3	2		32	10	35	12
Blood albumin decreased	1	0		5	1	6	1
Lipase/lipase increased	7	1		41	11	48	12
LFTs abnormal	2	1		9	2	11	3
Pancreatic enz. increased				3	0	3	0

Conditional Table 6 (continued): AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies							
	Crystalloid-Colloid Controlled Surgeries			RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	Total	HBOC-201 N=531	RBC N=487	Total HBOC-201 AEs	Total Control AEs
Coagulation	**11**	**4**		**34**	**13**	45	17
Coagulation disorder	1	1		3	0	4	1
DIC				2	0	2	0
Thrombocytopenia	4	2		5	3	9	5
Thrombocythemia				4	1	4	1
aPTT prolonged				3	0	3	0
Bleeding time prolonged	1	0				1	0
Blood fibrinogen decreased				1	0	1	0
Fibrin D-dimer increased				1	0	1	0
Prothrombin level decreased				1	0	1	0
Petecchiae	2	0		2	0	4	0
Purpura	1	0		3	0	4	0
Thrombosis	1	0				1	0
Arterial thrombosis-limb	1	2				1	2
DVT				2	3	2	3
Pulmonary embolism	1	0		5	4	6	4
Thromboembolism				2	1	2	1
Thrombophlebitis-deep				0	1	0	1

Conditional Table 6 (continued) : AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies							
	Crystalloid-Colloid Controlled Surgeries			RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	Total	HBOC-201 N=531	RBC N=487	Total HBOC-201 AEs	Total Control AEs
Hemorrhage/bleeding	**23**	**19**		**85**	**36**	108	55
anemia	16	7		47	16	63	23
Duodenal ulcer hemorrhage				1	1	1	1
Gastric ulcer hemorrhage				0	1	0	1
GI hemorrhage	0	1		3	3	3	4
Rectal hemorrhage	0	1		1	3	2	3
Exsanguination	0	1				0	1
Hemorrhage				13	9	13	9
Ulcer hemorrhage				0	1	0	1
Intra-operative hemorrhage				0	1	0	1
Post-op hemorrhage	1	1		12	7	13	8
Secondary anemia	4	1		2	0	6	1
Hemoglobin decreased	2	6		10	0	12	6
Vaginal hemorrhage				1	0	1	0
Anemia aggravated	1	3		0	2	1	5

Conditional Table 6 (continued): AE subgroups in Crystalloid/Colloid Controlled and RBC-Controlled Surgery Studies							
	Crystalloid-Colloid Controlled Surgeries			RBC-Controlled Surgeries			
Treatment-emergent AEs	HBOC-201 N=177	Control N=131	Total	HBOC-201 N=531	RBC N=487	Total HBOC-201 AEs	Total Control AEs
Sepsis/septic shock	**2**	**0**		**13**	**6**	15	6
Sepsis				10	2	10	2
Septic shock				1	3	1	3
Multiple organ failure	2	0		3	3	5	3
Ischemia/ischemic events	**2**	**0**		**2**	**1**	4	1
Intestinal infarction	1	0				1	0
Intestinal ischemia				1	0	1	0
Ischemia /periph ischemia	1	0		0	1	1	1
Ischemic colitis				1	0	1	0

Conditional Table 7: Clinically Important AEs in HEM-0115 *post hoc* Subgroup Analysis							
		≤ 50 years old		51-70 years old		>70 years old	
		HBOC-201	RBC	HBOC-201	RBC	HBOC-201	RBC
		N=97	N=69	N=152	N=167	N=101	N=102
HTN, BP increased, systolic HTN, Post-op HTN, SVR, increased, etc.		11	4	31	10	18	7
CHF, LV failure, Pulm. Edema, fluid overload, etc.		3	0	7	2	11	3
Cardiac arrest, cardiopulm arrest, etc.		1	0	2 * (0115-5405	0	6	2
MI, angina, myoc. ischemia, unstable angina		2	0	3	3	3	3
Arrhythmias, tach, bradycardia, etc.		20	7	36	18	16	14
CVA, infarct, TIA, RIND, etc		0	1	2	1	5	0
Pneumonia, aspiration pneumonia,		2	0	8	1	5	5
Respiratory failure, ARDS, etc.		4	0	2	0	3	5
Oliguria, anuria, renal failure, acute renal failure, etc.		6	3	25	13	16	9
Dialysis		1	0	1	0	1	1
Hypoxia, desaturation, cyanosis, etc.		4	2	10	2	5	4
GI, abdominal .pain, dyspepsia, dysphagia, nausea, vomiting, etc.		58	21	89	50	58	40
Cholecystitis/lithiasis, GB disorder		1	0	0	1	5	0
Hepatopancreatic, pancreatitis, lipase increased, LFTs abnormal, ALT/AST increased, etc.		16	1	25	7	15	3
Coag. disorder, DVT, PE, coag, etc.		2	3	6	3	5	2
Bleeding, hemorrhage, etc.		14	3	31	12	16	10
Sepsis, septic shock, MOF		1	1	2	1	2	3

Conditional Table 8: Summary of AEs by Study Type and Age													
			Normal Volunteer Studies						Non-Surgery Studies					
			H	H	H	C	C	C	H	H	H	C	C	C
			≤ 50	51-75	>75	≤50	51-75	>75	≤50	51-75	>75	≤50	51-75	>75
			N=64	N=0	N=0	N=29	N=0	N=0	N=24	N=0	N=1	N=14	N=0	N=0
Death														
Hypertension/BP incr.			0			0			1		1	1		
Pulmonary HTN														
Chest pain/pressure/tightness.			3			1			2			0		
CHF, cardiac failure			0			0			0			0		
Cardiac /cardiopulm arrest			0			0			0			0		
MI. angina, etc			0			0			0			1		
Cardiac arrhythmias			2			1			0			1		
Hypoxia, desaturation			0			0			0			0		
Pneumonia, aspiration pneumonia, etc			0			0			1			1		
CVA/TIA/infarct			0			0			0			0		
Oliguria, ARF			6			0			0			0		
Dialysis			0						0			0		
Hypovolemia			0			0			0			0		
GI pain, N and V			30			5			11			6		
Gallbladder			0			0			0			0		
Hepatopancreatic			1			0			0			0		
Coagulation			3			0			0			0		
Hemorrhage/bleeding			0			0			0			0		
Sepsis/septic shock			0			0			0			0		
Ischemia/isch. events			0			0			0			0		

Conditional Table 8 (continued): Summary of AEs by Study Type and Age												
	Surgery: Crystalloid/Colloid Control						Surgery: RBC Control					
	H	H	H	C	C	C	H	H	H	C	C	C
	≤ 50	51-75	>75	≤50	51-75	>75	≤50	51-75	>75	≤50	51-75	>75
	N=32	N=140	N=5	N=25	N=100	N=6	N=125	N=317	N=89	N=90	N=314	N=83
Death	0	3	1	0	1	0	3	6	12	0	8	5
Hypertension/BP incr.	7	52	3	2	15	0	18	66	20	9	25	8
Pulmonary HTN												
Chest pain/pressure.	1	7	1	0	2	0	5	10	4	0	11	5
CHF, cardiac failure	2	0	0	0	0	0	4	21	13	1	6	6
Cardiac /cardiopulm arrest, V-Fib	0	2	0	1	1	0	2	4**	9**	1	2	2
MI. angina, etc	1	4	1	0	3^	0	3	5	5	0	6	2
Cardiac arrhythmias	3	29	2	7	19	2	22	75	22	8	46	18
Hypoxia, desaturation												
Pneumonia, aspiration pneumonia, etc	0	5	2	1	2	0	3	16	9	1	10	9
Resp. distress/failure	0	2	1	0	1	0	6	9	4	2	4	5
CVA/TIA/infarct	0	0	0	0	0	0	1	7	8	1	1	1
Oliguria, ARF, etc	2	27	1	1	7	1	8	57	16	4	31	11
Dialysis	0	2	0	0	0	0	1	2	2*	0	1	1
Hypovolemia	0	4	0	0	0	1	3	9	0	3	3	0
GI pain, N and V	14	66	4	5	34	2	73	151	41	29	97	27
Gallbladder	1	0	0	0	0	0	1	5	4	0	1	0
Hepatopancreatic	1	13	0	2	3	0	19	52	14	1	18	4
Coagulation	2	8	1	1	3	0	2	23	9	3	7	3
Hemorrhage/bleeding	4	17	2	5	12	2	18	53	14	6	24	6
Sepsis/septic shock	0	2	0	0	0	0	3	7	3	1	3	2
Ischemia/isch. events	0	2	0	0	0	0	0	2	0	0	0	1

Conditional Table 9: Summary of AEs by Study Type and Age													
			Normal Volunteer Studies						Non-Surgery Studies					
			H	H	H	C	C	C	H	H	H	C	C	C
			≤50	51-69	≥70	≤50	51-69	≥70	≤50	51-69	≥70	≤50	51-69	≥70
			N=64	N=0	N=0	N=29	N=0	N=0	N=24	N=0	N=1	N=14	N=0	N=0
Death														
Hypertension/BP increased			0			0			1		1	1		
Pulmonary HTN														
Chest pain/pressure/tightness.			3			1			2			0		
CHF, cardiac failure			0			0			0			0		
Cardiac /C-P arrest			0			0			0			0		
MI. angina, etc			0			0			0			1		
Cardiac arrhythmias			2			1			0			1		
Hypoxia, desaturation			0			0			0			0		
Pneumonia, aspiration pneumonia, etc			0			0			1			1		
CVA/TIA/infarct			0			0			0			0		
Oliguria, ARF			0			0			0			0		
Dialysis			0			0			0			0		
Hypovolemia			0			0			0			0		
GI pain, N and V			30			5			11			6		
Gallbladder			0			0			0			0		
Hepatopancreatic			1			0			0			0		
Coagulation			3			0			0			0		
Hemorrhage/bleeding			0			0			0			0		
Sepsis/septic shock			0			0			0			0		
Ischemia/isch. events			0			0			0			0		

Conditional Table 9 (continued): Summary of AEs by Study Type and Age												
	Surgery: Crystalloid/Colloid Control						Surgery: RBC Control					
	H	H	H	C	C	C	H	H	H	C	C	C
	< 50	51-69	≥70	< 50	51-69	≥70	< 50	51-69	≥70	< 50	51-69	≥70
	N=32	N=99	N=46	N=25	N=83	N=23	N=125	N=220	N=186	N=90	N=224	N=173
Death	0	2	1	0	1	0	3	4	15	0	2	11
Hypertension/BP incr.	7	27	28	2	12	3	18	46	40	9	14	8
Pulmonary HTN												
Chest pain/pressure.	1	3	5	0	2	0	5	8	6	0	4	12
CHF, cardiac failure	2	8	6	0	6	3	4	12	22	1	3	9
Cardiac /C-P arrest	0	1	1	0	1	0	2	3**	10**	1	0	4
MI. angina, etc	1	3	3^	0	2	0	3	4	6	0	5	3
Cardiac arrhythmias	3	18	13	7	19	2	22	48	49	8	25	39
Hypoxia, desaturation	5	9	8	2	5	1	6	15	17	2	4	2
Pneumonia, aspiration pneumonia, etc	0	3	4	1	1	1	3	10	15	1	3	6
Resp. distress/failure	0	1	2	0	1	0	6	7	6	2	1	8
CVA/TIA/infarct	0	0	0	0	0	0	1	4	11	1	1	1
Oliguria, ARF, etc	2	17	11	1	6	2	8	36	37	4	19	23
Dialysis	0	2	0	0	0	0	1	1	3*	0	0	2
Hypovolemia	0	0	4	0	0	1	3	4	8	0	2	1
GI pain, N and V	14	41	29	5	29	7	73	109	83	29	63	61
Gallbladder	1	0	0	0	0	0	1	2	7	0	1	0
Hepatopancreatic	1	10	3	2	1	2	19	34	32	1	12	10
Coagulation	2	7	2	1	3	0	2	12	20	3	5	5
Hemorrhage/bleeding	4	13	6	5	10	4	18	36	31	6	14	16
Sepsis/septic shock	0	1	1	0	0	0	3	5	5	1	2	3
Ischemia/isch. events	0	1	1	0	0	0	0	0	2	0	0	1

* Includes patient HEM-0115-4308
** Includes patients 0107-0403 and 0115-5405
^ Includes patient 0101-0725

1. Hypertension, blood pressure increased, hypertensive crisis, systolic hypertension, systemic vascular resistance (SVR) increased, malignant hypertension, postoperative hypertension, systolic blood pressure increased
2. Congestive failure- cardiac, cardiac failure, cardiorespiratory failure, left ventricular failure, pulmonary edema, acute circulatory failure, rales, cardiac index decreased, cardiac output decreased, central venous pressure (CVP) increased, fluid overload, dyspnea
3. Cardiac arrest, cardiopulmonary arrest, Ventricular fibrillation
4. Myocardial infarction, myocardial ischemia, angina pectoris, unstable angina
5. Arrhythmia, atrial fibrillation, atrial flutter, bradycardia, tachycardia, ventricular extrasystoles, supraventricular tachycardia, ventricular tachycardia, atrial tachycardia, bundle branch block, right bundle branch block, sinus tachycardia, ventricular arrhythmia, bigeminy, etc.
6. Cerebrovascular accident, cerebral infarction, hemiparesis, hemiplegia, monoparesis, transient ischemic attack, reversible ischemic neurological deficit (RIND), transient cerebrovascular event,
7. Pneumonia, pneumonia-Klebsiella, pneumonia-pseudomonas, pneumonia-staphylococcus, aspiration pneumonia, pneumonitis,,
8. ARDS, respiratory distress, respiratory failure.
9. Oliguria, anuria, acute renal failure, chronic renal failure, renal failure, renal impairment, anuria. Excludes dialysis
10. Dialysis
11. Hypoxia, decreased oxygen saturation, cyanosis
12. Gastrointestinal pain, GI pain-upper, esophageal spasm, vomiting, abdominal distension, dyspepsia, dysphagia, eructation, vomiting-aggravated, postoperative nausea, retching, abdominal pain-lower, nausea-aggravated, nausea.
13. Biliary colic, cholecystitis-acute, cholecystitis, cholelithiasis, gallbladder disorder, gallbladder edema
14. Pancreatitis, hepatic failure, hepatic pain/dysfunction, hepatic pain, hepatitis, hepatomegaly, hepatorenal syndrome, hepatorenal failure, ALT abnormal/increased, AST abnormal /increased, albumin decreased, LFTs abnormal, pancreatic enzymes increased, lipase increased, amylase increased,

15. Coagulation disorder, DIC, thrombocytopenia, thrombocythemia, aPTT prolonged, Bleeding time prolonged, fibrinogen decreased, D-dimer increased, prothrombin level decreased, petecchiae, purpura, thrombosis, arterial thrombosis-limb, deep venous thrombosis, pulmonary embolus, thromboembolism, thrombophlebitis-deep, PT change.
16. Anemia, duodenal ulcer hemorrhage, gastric ulcer hemorrhage, rectal hemorrhage, exsanguination, ulcer hemorrhage, intraoperative hemorrhage, postoperative hemorrhage, secondary anemia, hemoglobin decreased, vaginal hemorrhage, and anemia aggravated.
17. Sepsis, septic shock, multiple organ failure
18. Ischemia, ischemic events, etc.

Conditional Table 10: Clinically Important AEs in HEM-0115 *post hoc* Subgroups, number of subjects, HBOC-201 vs. Control (source: FDA review of BLA data and Biopure data tables)

Treatment Emergent AE	**Aggregate population** N=353 vs. 340	**"Trauma" subgroup** N=32 vs. 28	**<70 y/o subgroup** N=239 vs. 227	**≥70 y/o subgroup** N=111 vs. 111
Heart failure, lung edema	21 vs. 5	1 vs. 1	10 vs. 2	11 vs. 3
Cardiac arrest	9 vs. 2	1 vs. 0	3 vs. 0	6 vs. 2
↑ troponin *	18 vs. 2	0 vs. 1	9 vs. 1	9 vs. 1
CVA/TIA	7 vs. 2	0 vs. 0	2 vs. 2	5 vs. 0
HT	60 vs. 21	3 vs. 2	39 vs. 13	21 vs. 8
Medically Rx-ed	37 vs. 7			
Hypoxemia, desaturation, cyanosis	19 vs. 8	3 vs. 1	14 vs. 4	5 vs. 4
Oliguria, anuria, renal impairment	47 vs. 25	2 vs. 0	27 vs. 16	20 vs. 9
Pneumonia, pneumonitis	15 vs. 6	2 vs. 1	8 vs. 1	7 vs. 5
Dialysis	3 vs. 1		2 vs. 0	1 vs. 1
Respiratory failure/distress	9 vs. 5	4 vs. 1	6 vs. 0	3 vs. 5
Myocardial infarction	4 vs. 2	0 vs. 0	0 vs. 0	4 vs. 2

*Troponin levels were only obtained in about one-third of the subjects